

Are weaning foods causing impaired iron and zinc status in 1-year-old Swedish infants? A cohort study

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We analysed whether 12-month-old Swedish infants who have been fed iron-fortified and relatively zinc-rich foods, according to current recommendations, have adequate iron and zinc status. A cohort of 76 healthy, full term Swedish infants was followed regarding feeding habits and growth from birth to 12 months of age, when haemoglobin, iron and zinc status were evaluated. Twenty-six percent of the infants had low (<12 µg/l) s-ferritin values, indicative of iron depletion, and 36% of the infants had low s-zinc concentrations (<10.7 µmol/l). Only two infants had both low haemoglobin and low s-ferritin values. s-Zinc and s-iron were positively correlated, and s-zinc and s-transferrin receptor values were negatively correlated. Lower birthweight was associated with lower s-ferritin levels at 12 months in boys, and with increased s-transferrin receptor values in girls. Feeding habits during infancy were relatively homogenous, dominated by breastfeeding, iron-fortified milk- and cereal-based follow-on formula. No clear association between feeding pattern and iron and zinc status was found. The results indicate that in a group of healthy, well growing 12-month-old Swedish infants one-quarter is iron-depleted, although iron deficiency anaemia is rare, and one-third may be zinc-depleted. The high cereal intake of Swedish infants from 6 months of age may have limited the bioavailability of both iron and zinc from the diet. □ *Anaemia, bioavailability, depletion, feeding, infants, iron, zinc*

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Iron stores decrease during the first 6 months of life, and the estimated iron requirement during the second half of infancy is higher than before and after this period (1). In affluent societies the nutritional status of infants exclusively breastfed or fed iron-fortified formulas is adequate up to 6 months of age (2–4). However, we have previously found that serum transferrin receptor (s-TfR) concentrations tend to be higher in breastfed infants than in infants fed iron-fortified infant formulas at 6 months of age (3). As elevated s-TfR levels indicate an increased cellular need for iron, this may be an early warning sign of depleted iron stores, even if s-ferritin levels did not indicate iron deficiency. Similarly, a recent study on Danish infants showed a decline in s-zinc levels at 6 months of age, although no negative effect on growth was observed (5). To assess if the situation improves during the second half-year of life, when iron-fortified foods become common and relatively zinc-rich foods such as meat and cereals are being introduced, we evaluated iron and zinc status at 12 months of age in a cohort of apparently healthy term infants.

Subjects and methods

As part of a European infant feeding and growth study (6), a cohort of newborn infants from the city of Umeå, Sweden

was recruited from September 1991 to August 1992. Twelve healthy newborns per month in one Child Health Centre were invited to participate. The following exclusion criteria were used: gestational age <37 or > 42 weeks, birthweight <2500 g, unknown gestational age, congenital malformation, inherited metabolic disease, neonatal disease with hospitalization >7 d, chronic disease and twins. Twenty-four parents declined to enter the study, mainly owing to forthcoming migration from the area. At 1 y of age, 97 infants (80%) remained in the study. Parents accepted that a blood sample was taken in 76 of those 97 infants (62%). At the time of blood sampling, no infant suffered from acute infections. The infants not participating in the haematological evaluation did not differ from the entire group with respect to anthropometry at birth, mother's age or level of education.

Information on feeding pattern was obtained through monthly interviews at the Child Health Centre during the first 6 months and then at 9 and 12 months of age. Introduction of various foods was registered in a qualitative manner. Weight (naked) and length were measured during the same visits, and had also been measured at birth. Maternal educational background was classified as primary (9 y of school), secondary (12 y) or university/similar (>12 y).

In this study, infants were considered breastfed if fed by the breast on a daily basis, disregarding frequency, volume

or introduction of fluids or solid foods. Formula includes different types of iron-fortified cows' milk-based infant formulas (typically containing 4–9 mg iron/l) and, in a few cases, iron-fortified milk protein hydrolysate formula (12 mg iron/l) or soy formula (7 mg iron/l). From 6 months of age, formulas extensively used in Sweden are iron-fortified cows' milk- and cereal-based follow-on formulas containing 12 mg iron/l as FeSO_4 .

Haemoglobin concentration (Hb) was analysed by the cyanomethemoglobin method (3), mean corpuscular volume (MCV) by Coulter Counter, s-iron and total iron binding capacity (TIBC) by commercial kits (Boehringer Mannheim, Indianapolis, IN, USA), s-ferritin and s-transferrin receptor by immunoradiometric assays (R&D Systems, Minneapolis, MN, USA). For both s-ferritin and s-transferrin receptor assays, control sera provided by the manufacturers were included in duplicate within each assay. Analyses were considered valid if control values fell within the established range. s-Zinc was analysed by atomic absorption spectrophotometry as described earlier (7). Volumes of serum samples were sufficient for analysis only in 56/76 infants.

If samples were normally distributed and variances were homogenous ANOVA results were used, else the non-parametrical Kruskal–Wallis analysis of variance was employed and the Kruskal–Wallis H and *p* value were calculated.

The study was approved by the research ethics committee of the Medical Faculty, Umeå University.

Results

Mean Hb concentrations were 117 and 115 g/l for boys and girls, respectively. There was no significant difference in Hb distribution between sexes. Thirteen percent (95% CI: 7–23%) of the infants had Hb <110 g/l, 8% <105 g/l and 1% <100 g/l (Table 1).

Means of s-ferritin values differed significantly ($p = 0.04$) between boys and girls (Table 1). Twenty-six percent (95% CI: 17–38%) of the infants had values <12 $\mu\text{g/l}$ (boys 32%, girls 21%, $p = 0.36$), indicating low iron stores. When applying multiple criteria as suggested by INACG (8), s-ferritin <10 $\mu\text{g/l}$, s-Fe <10 $\mu\text{mol/l}$, MCV <73 fl, none of the infants had iron deficiency anaemia. Interestingly, there was no association between low s-ferritin (<12 $\mu\text{g/l}$) and low Hb values (<110 g/l, $\text{Chi}^2 = 0.08$, $p = 0.78$). Only two of the infants with low Hb had low s-ferritin values, i.e. what could be expected by chance. In fact, there was a negative correlation between Hb and s-ferritin values ($r = -0.25$, Table 2). It should be noted that blood samples were not taken if the parent reported a current infection of the infant.

The mean s-TfR value was 3.8 mg/l, with a range almost entirely above 3.0 mg/l, the suggested cut-off value for adults according to the manufacturer. There was no correlation between Hb and the ratio between s-TfR and s-ferritin. s-Ferritin levels were negatively associated

with TIBC ($r = -0.24$), while there was no significant correlation between s-ferritin and s-TfR values.

The mean s-zinc concentration was 11.5 μmol , with 35.7% of the values <10.7 μmol , the cut-off value commonly used (9). s-Zinc values were positively correlated to s-Fe ($r = 0.38$, $p = 0.01$), TIBC ($r = 0.27$, $p = 0.04$), negatively to s-TfR ($r = -0.29$, $p = 0.03$), but not significantly correlated to Hb ($r = 0.26$, $p = 0.06$) or MCV ($r = 0.26$, $p = 0.06$). s-Zinc and s-ferritin or s-zinc and s-TfR/s-ferritin ratios were not significantly correlated to each other. There was no difference in average levels of Hb, s-ferritin, s-TfR or s-zinc between groups of infants of mothers with different educational background.

At 6 months, 61% of the infants were breastfed, and 22% exclusively breastfed. Among 1-year old infants, 16% were still partially breastfed, while 91% received iron-fortified cereal- and milk-based follow-on formula, 42% received cows' milk and 87% were given meat, fish or poultry every day. Seventy-four percent of the 1-y-old infants had a diet dominated by follow-on formula plus solids, including meat, fish and poultry, while 14% got formula but no meat, fish or poultry. Breastfed, but not formula-fed, infants at 12 months (12%) all received solids with meat, fish or poultry. Boys receiving follow-on formula without additional meat, fish or poultry had a significantly lower median haemoglobin (110 g/l) than the others (118 g/l, $p = 0.007$). This difference was not shown for girls. No significant associations were found between these feeding groups and s-ferritin, s-TfR or TfR/ferritin ratio. Daily intake of cows' milk was not associated with Hb concentration or other iron status measurements. No differences were found in s-zinc values between feeding groups at 12 months of age.

Although a birthweight <2500 g or gestational age <37 weeks served as exclusion criteria there was a significant positive correlation between birthweight and s-ferritin among boys (0.36, $p = 0.03$) and a negative correlation between birthweight and s-TfR among girls (-0.33, $p = 0.04$), Table 3. In girls, birthweight was also negatively correlated to the ratio between s-TfR and s-ferritin (-0.43, $p = 0.02$). Weight at 12 months was positively correlated to Hb in girls, but not in boys. Birth length, weight or length velocities 9–12 months or length at 12 months were not significantly correlated to any of these haematological or biochemical measurements. No correlation was demonstrated between these measurements of growth and s-zinc values at 12 months, and there was no difference in daily weight gain from 9 to 12 months of age between infants that at 12 months of age had low or normal s-zinc values (10.8 g/d and 10.6 g/d, respectively, $p = 0.90$). The corresponding values for linear growth were 4.0 and 4.2 mm/d, respectively ($p = 0.61$).

Discussion

A normal term infant is believed to be born with sufficient body iron stores to satisfy physiological requirements until

Table 1. Iron and zinc status among 12-month-old Swedish infants (haematology and iron parameters on 37M, 39F; zinc values on 28M and 28F, respectively).

	Boys	Girls	Total	<i>p</i>
Haemoglobin (g/l)				
Mean	117.1	115.0	116.0	0.30
SD	6.6	7.5	7.2	
Median	118	116	117	0.74
Range	104–133	89–128	89–133	
<110	10.8%	15.4%	13.2%	0.22
<105	2.7%	12.8%	7.9%	
MCV (fL)				
Mean	77.7	77.2	77.5	0.65
SD	3.6	4.6	4.1	
Median	78	78	78	0.22
Range	67–84	58–85	58–85	
s-Ferritin (µg/l)				
Mean	21.1	30.6	26.0	0.04
SD	17.6	25.0	22.1	
Median	17.0	25.7	20.8	0.36
Range	2.7–89.2	1.6–131.6	1.6–131.6	
<12	32.4%	20.5%	26.3%	0.20
<10	24.3%	12.8%	18.4%	
s-Iron (µmol/l)				
Mean	11.2	10.1	10.6	0.30
SD	6.7	8.2	7.5	
Median	11.1	8.5	9.0	0.30
Range	2.1–27.3	0.2–47.8	0.2–47.8	
TIBC (µmol/l)				
Mean	56.8	54.2	55.4	0.05
SD	7.7	8.9	8.4	
Median	57.1	53.3	55.4	0.05
Range	40.2–73.8	36.8–87.7	36.8–87.7	
s-Transferrin receptor (mg/l)				
Mean	3.9	3.7	3.8	0.26
SD	0.6	0.6	0.6	
Median	3.9	3.7	3.7	0.26
Range	2.5–5.7	2.6–5.5	2.5–5.7	
s-Zinc (µmol/l)				
Mean	11.6	11.4	11.5	0.42
SD	1.6	1.7	1.6	
Median	11.6	11.1	11.3	0.40
Range	9.0–15.4	9.6–17.4	9.0–17.4	
<10.7	28.6%	42.9%	35.7%	

at least 4 months of age (1, 10, 11). Hence, iron deficiency is rare in term infants during the first 6 months of life unless they are fed infant formula that has not been fortified with iron (2,12). We recently showed that at 6 months of age, Hb and s-ferritin values were indistinguishable between

exclusively breastfed infants and infants fed a whey-pre-dominant, cows' milk-based formula containing either 4 or 7 mg iron/l (3). None of the infants, regardless of feeding group, had haematological values indicative of iron deficiency. These results are in agreement with those of Siimes

Table 2. Pearson correlation coefficients between iron status parameters in 12-month-old Swedish infants (*n* = 71), *p* values are given in parentheses.

	Haemoglobin	MCV	Ferritin	s-iron	TIBC	Transferrin receptors
Haemoglobin	1.0	0.45 (0.00)	-0.25 (0.03)	0.23 (0.05)	0.14 (0.25)	-0.07 (0.58)
MCV		1.0	-0.11 (0.37)	0.37 (0.00)	0.04 (0.76)	-0.21 (0.07)
Ferritin			1.0	-0.08 (0.50)	-0.24 (0.04)	-0.13 (0.29)
s-Iron				1.0	0.58 (0.00)	-0.28 (0.02)
TIBC					1.0	0.12 (0.31)
Transferrin receptors						1.0

Table 3. Correlations between measurements of infant growth (birthweight and weight at 12 months) and haemoglobin (Hb) and measurements of iron status [s-ferritin, s-transferrin receptors (s-TfR) and ratio between s-TfR and s-ferritin] of boys ($n = 37$) and girls ($n = 39$) at 12 months of age. Pearson correlation coefficients, p values in parentheses.

	Boys		Girls	
	Birthweight	Weight at 12 months	Birthweight	Weight at 12 months
Hb	-0.15 (0.38)	0.24 (0.19)	0.16 (0.34)	0.36 (0.05)
s-Ferritin	0.36 (0.03)	0.07 (0.71)	0.22 (0.18)	-0.02 (0.89)
s-TfR	-0.08 (0.67)	0.33 (0.08)	-0.33 (0.04)	-0.15 (0.46)
TfR/ferritin	-0.25 (0.19)	-0.26 (0.18)	-0.43 (0.02)	-0.25 (0.19)

et al. (2), who found that although human milk is low in iron (0.2–0.4 mg iron/l), iron deficiency, with or without anaemia, was not seen in exclusively breastfed term infants during the first 6 months of life; iron deficiency was in fact rare even at 9 months of age.

After finding satisfactory iron status at 6 months of age we had expected this situation to be similar at 12 months, even if iron deficiency is common during late infancy and early childhood in both developing countries and affluent societies (10). The Swedish population is relatively homogenous with small differences in infant feeding practices between socioeconomic groups (13), and infant foods are universally iron fortified. In spite of this we found that one-quarter of the infants had s-ferritin levels $<12 \mu\text{g/l}$, which, according to an internationally accepted cut-off (14), is indicative of severely depleted iron stores. Thirteen percent of the infants had Hb $<110 \text{ g/l}$ indicating anaemia (9, 10, 14), although not combined with low s-ferritin, indicating depleted iron stores. There was a negative association between s-ferritin and Hb, which illustrates the difficulties in representing iron status in late infancy with available indicators. We cannot exclude that the shown negative association is related to recent infections, although blood samples were not taken when the parents reported current infections. The frequency of infants having low Hb values is comparable to recent data from several European countries (6, 11, 15, 16).

From 6 until at least 12 months of age, milk- and cereal-based follow-on formula (13 mg iron/l) and/or porridge (2.5 mg iron/serving) will constitute on average two meals/d, and an earlier study showed that 80% of iron intake in non-breastfed infants at 6 months came from follow-on formula and other cereals (17). From 6–8 months of age, canned or home-prepared baby-foods containing meat products are also introduced. Iron intake at 6 and 12 months of age was in earlier Swedish infant studies found to be 10–12 mg/d (17, 18). Hence, through fortification of infant formulas (7–9 mg iron/l) and follow-on formulas (10–13 mg iron/l) the recommended intake of iron is met by the vast majority of Swedish infants during the first 12 months of age.

The positive association between birthweight and s-ferritin found in 12 months old boys and the negative correlation between birthweight and s-TfR in girls, may reflect that those with higher needs did not receive or did

not absorb sufficient amounts of iron. A low birthweight may represent a lower gestational age and lower iron stores at birth. Early introduction of whole cows' milk has been shown to result in impaired iron status in infants up to 12 months of age owing to low iron content and possibly gastrointestinal blood loss (12, 19, 20). We found no association between s-ferritin values and daily cows' milk feeding at 12 months; however, the average energy intake from milk and milk products in this age group of Swedish infants is, according to an earlier study, relatively limited, only 17% (17). A second possibility is lower bioavailability of iron from cereals than from cows' milk based formula (21, 22, 23). Low iron bioavailability from cereals is due to the presence of phytate that forms insoluble complexes with divalent cations such as iron and zinc (24). Reducing the phytate level of infant cereals has recently been shown to improve iron absorption (25). The possible causal association between high cereal intake and low s-ferritin is also supported by findings from a Danish study, where bread intake at 6–9 months of age had a negative effect on change in s-ferritin (26).

A substantial proportion of the infants (36%) was found to have low s-zinc concentrations. s-Zinc is not an ideal indicator of zinc status as it can be affected by infection, growth rate, post-prandial time, etc. (27). However, in our cohort, none of the infants had an acute infection at the time of examination and they were growing normally. Cereals and the milk- and cereal-based follow-on formula in combination provide a diet high in phytate, which also has an inhibitory effect on zinc bioavailability. Studies in human adults have shown that zinc absorption from such formula was 18% as compared to 28% and 31% from cows' milk and milk formula, respectively (28). Another possible explanation for the low s-zinc values is the high protein intake during this period of life (29). We have previously shown that protein intake of infants from this area at 6 and 12 months of age was 3.3 g/kg/d and 4.2 g/kg/d, respectively (17). Lönnerdal and Chen showed in a study on 5–8 months old infants that a high protein level in formula can lead to low s-zinc values (30).

In conclusion, we surprisingly found several indicators suggesting suboptimal iron and zinc status at 12 months of age. We believe that the high cereal intake of Swedish infants is a likely explanation for the low iron status. We are currently conducting a long-term prospective study of

iron and zinc intake in relation to various levels of cereal intake in which detailed information on feeding patterns and dietary intakes is obtained. Such information is needed in combination with a critical examination of the validity of various indicators of iron and zinc status as well as the age-appropriate cut-off values to be used.

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