

Development of iron-deficiency anaemia at six months of age in Jordanian infants exclusively breastfed for four to six months

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Editorial comment

This paper has two particularly interesting findings: (1) The rate of iron-deficiency anaemia at four months of age was rather high, which challenges the current thinking that there are adequate iron stores to protect against iron deficiency up to six months of age. (2) Longer exclusive breastfeeding had a protective effect against iron-deficiency anaemia. It is somewhat surprising to see the rather large difference in iron status between those with four months of exclusive breastfeeding and those with five months. It should be noted that this is an observational study, not a controlled study. It is possible that infants with a shorter period of exclusive breastfeeding differ from those with a longer period of exclusive breastfeeding in other factors. For example, the iron status of the mother during pregnancy will affect iron stores at birth. Ideally, some important factors such as birthweight can be used as covariates to control for the potential difference among the three groups compared, since low-birthweight infants have lower iron stores. Other factors might be infection in the infant and the effects of deficiencies in other haematopoietic nutrients. This is an interesting and provocative study, which may help to promote longer periods of exclusive breastfeeding. Given the nature of the study, with its relatively small sample size, and the potential significance of the study, larger studies repeated elsewhere are needed.

Abstract

The iron status at six months of age for a total of 66 three-month-old, full-term, normal-birthweight Jordanian

infants who were exclusively breastfed for four, five, or six months postpartum is reported here. Three groups of infants were identified who were exclusively breastfed for four months (n = 14), five months (n = 15), and six months (n = 37). Iron status was evaluated by haemoglobin, plasma ferritin, and mean corpuscular volume. The prevalences of nutritional anaemia (haemoglobin < 10.5 g/dl), iron-deficiency anaemia (haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml), and depletion of iron stores (plasma ferritin < 12 ng/ml) were significantly higher (p < .05) in infants exclusively breastfed for four months than in the other two groups. Significant differences were not observed between the latter two groups in the prevalence of any of these indices. Under the conditions of this study, which was carried out in a community of low to medium socio-economic status where iron-fortified weaning foods were not used, the data strongly suggest that exclusive breastfeeding for five or six months is protective against the development of nutritional anaemia or iron-deficiency anaemia at six months of age. The weaning foods used in this community have low iron content and bioavailability.

Introduction

Iron-deficiency anaemia is the most common nutritional disorder in the world [1, 2]. From a public health point of view, the importance of iron-deficiency anaemia prevention arises from the seriousness of its consequences for the health of infants, children, and women of reproductive age, requiring highly coordinated efforts to prevent its development during such sensitive periods of life [3, 4].

At birth the average amount of total body iron in full-term infants is about 75 mg per kilogram of body weight [5, 6]. These iron stores are normally adequate to meet the infant's needs for about the first four months of life [7], or for a rather longer period of about six months in breastfed infants, as stated by Fairweather-Tait in 1992 [8].

Whereas iron-deficiency anaemia is specific to iron

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deficiency, nutritional anaemia can be precipitated by the deficiency not only of iron but also of any of several other nutrients, including vitamin B₆, vitamin C, vitamin E, folic acid, copper, and protein [9, 10], singly or in combination. In developing countries, however, it is generally accepted that the major cause of anaemia is iron deficiency [1].

Pisacane et al. observed that the prevalence of anaemia (haemoglobin < 11 g/dl) was significantly lower among 12-month-old Italian infants who were exclusively breastfed for 6.5 months than among those exclusively breastfed for 5.5 months [11]. In contrast, Dewey et al. found that exclusive breastfeeding did not prevent the development of anaemia at four or six months of life: 25% of the four-month-old exclusively breastfed infants ($n = 22$) developed anaemia (haemoglobin < 10.3 g/dl), a proportion which was not significantly different from a prevalence of 32% observed in exclusively breastfed infants at six months [12].

In this paper, we report the prevalence of nutritional anaemia and iron-deficiency anaemia for infants at six months of age as affected by the duration of exclusive breastfeeding for the first four, five, or six months of life.

Subjects and methods

Subjects

One hundred seventeen full-term, appropriate-for-gestational-age infants (63 boys and 54 girls) who were born to mothers willing to continue exclusive breastfeeding for at least the first four months after birth participated in the study between August 1994 and October 1995. The participants represented a random sample of mothers who attended the training at the Maternal and Child Health Centre at Wadi Srour in Amman. Mothers were informed about the study protocol, including the need for drawing four blood samples from the infants at 3, 6, 9, and 12 months of age. The mother's consent was required for participation. The selected group of infants formed the basis for a nine-month longitudinal study (3–12 months of age) that was conducted to test the effectiveness of applying certain intervention strategies at six months of age for the prevention of iron-deficiency anaemia [13, 14]. Only 66 infants (32 boys and 34 girls) completed the study. Forty-nine dropped out for one reason or another, and two three-month-old infants (one boy and one girl) were excluded because their haemoglobin levels were below 9.5 g/dl at three months of age.

Diet

All infants were exclusively breastfed for the first four, five, or six months, beyond which solid foods were

introduced according to the desire of their mothers. When the infants were six months old, the mothers were asked if iron-fortified cereals or formulas were given. Solid food was introduced at four, five, or six months of age for 21%, 23%, and 56% of the study infants, respectively.

Anthropometry

The infants' body weight, length, and head circumference were measured monthly. Body weight was measured to the nearest 0.01 kg with a paediatric scale (Seca, Germany). Recumbent length was measured to the nearest millimetre with a wooden measuring board (Shorr, USA). Head circumference was measured to the nearest millimetre with a narrow, flexible, and non-stretchable tape made of fibreglass. Measurements were compared with the respective median National Center for Health Statistics (NCHS) references [15]. Growth was assessed by weight-for-age, length-for-age, and weight-for-length, in addition to growth velocity expressed as gain in body weight per day.

Iron status

Under medical supervision, a 2-ml blood sample was drawn by qualified personnel from a superficial vein of the forearm of the infant, using sterile butterfly-gauge 23 syringes (Betcon Dickinson, Ontario, Canada). Blood collected in sterile vacuum tubes with EDTA was analysed for complete blood count using a Coulter counter (Seorno, Japan). A portion of blood was used for obtaining plasma which was kept frozen at -18°C until further analysis for the determination of plasma ferritin by radioimmunoassay with a commercial kit (Incstar, USA).

Infants at three or six months of age were considered to have anaemia if their haemoglobin fell below 9.5 or 10.5 g/dl, respectively [5]. The combined cut-off points that indicate iron-deficiency anaemia were based on the infant's age. At three months the cut-off points were haemoglobin < 9.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml. At six months they were haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml.

Statistical analysis

A two-tailed Student's t test, analysis of variance (ANOVA), and a two-sample binomial test were used to detect significant differences between the study groups.

Results

The haematological findings at six months of age are

presented in table 1. All red blood cell indices tended to be higher for female infants than for male infants, with statistically significant differences only for haemoglobin (11.5 ± 0.8 vs. 10.9 ± 1.0 g/dl) and mean corpuscular haemoglobin concentration (33.6 ± 1.0 vs. 32.8 ± 1.4 ; $p < .01$).

Table 2 shows that at six months of age, irrespective of sex, markedly more infants exclusively breastfed for four months were anaemic (haemoglobin < 10.5 g/dl) than those exclusively breastfed for five or six months (57%, 20%, and 11% of infants, respectively). Although differences between the latter two groups were not statistically significant, there is a clear tendency in favour of exclusive breastfeeding for six months rather than five months. However, even this tendency disappears

TABLE 1. Haematological values (mean \pm SD) at six months of age for infants exclusively breastfed for four to six months, according to sex

Value ^a	Boys ($n = 32$)	Girls ($n = 34$)
Hb (g/dl)	10.9 ± 1.02	11.5 ± 0.79^b
Hct (%)	33.1 ± 2.77	34.0 ± 2.56
MCV (fl)	74.9 ± 7.15	76.1 ± 5.01
MCH (pg/dl)	24.6 ± 2.71	25.5 ± 1.68
MCHC (%)	32.8 ± 1.38	33.6 ± 1.03^c
PF (ng/ml)	42.3 ± 40.69	53.2 ± 59.36
RBC (10^6 /ml)	4.4 ± 0.44	4.5 ± 0.37

a. Hb, Haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PF, plasma ferritin; RBC, red blood cell count.

b. $t = 2.6$, $p < .01$.

c. $t = 2.5$, $p < .01$.

TABLE 2. Percentage of exclusively breastfed infants who developed nutritional anaemia, iron-deficiency anaemia, and depletion of iron stores at six months of age, according to the duration of exclusive breastfeeding

Condition ^b	Duration of exclusive breastfeeding (mo) ^a					
	4 ($n = 14$)		5 ($n = 15$)		6 ($n = 37$)	
	No.	%	No.	%	No.	%
Nutritional anaemia	8	57 ^c	3	20 ^d	4	11 ^d
Iron-deficiency anaemia	4	29 ^c	0	0 ^d	0	0 ^d
Depletion of iron stores	6	43 ^c	0	0 ^d	3	8 ^d

a. Rows with different superscript letters differ significantly at $p < .005$.

b. Nutritional anaemia is defined by haemoglobin < 10.5 g/dl; iron-deficiency anaemia by haemoglobin < 10.5 g/dl, mean corpuscular volume < 70 fl, and plasma ferritin < 12 ng/ml; and depletion of iron stores by plasma ferritin < 12 ng/ml.

in the case of iron-deficiency anaemia, since none of the infants exclusively breastfed for five or six months developed iron-deficiency anaemia.

Table 2 also shows the prevalence of depleted iron stores (plasma ferritin < 12 ng/ml) according to the duration of exclusive breastfeeding. None of the infants exclusively breastfed up to five months and only 8% of those exclusively breastfed up to six months had depleted iron stores. However, a markedly higher percentage (43%) of infants exclusively breastfed for four months had depleted iron stores ($p < .05$).

Table 2 also shows that at six months of age, the prevalence of nutritional anaemia was markedly higher than that of iron-deficiency anaemia among infants who were exclusively breastfed for four months (57% vs. 29%; $p < .05$). Similarly, the prevalence of nutritional anaemia after five months (20%) or six months (11%) of exclusive breastfeeding tended to be higher than that of iron-deficiency anaemia, which disappeared completely (0%) after five or six months of exclusive breastfeeding.

Table 3 shows the mean values of haemoglobin, plasma ferritin, and mean corpuscular volume at six months of age for infants exclusively breastfed for four to six months according to the duration of exclusive breastfeeding and iron status.

Growth performance, based on the limited sample size, was not adversely affected by nutritional anaemia or iron-deficiency anaemia. Indeed, none of the study infants were underweight, stunted, or wasted (data not shown).

Better iron status with regard to nutritional anaemia and iron-deficiency anaemia in female infants has also been observed in an ongoing study by Faqih and co-workers of 9- to 36-month-old Jordanian infants whose mothers attend the same Maternal and Child Health Centre at Wadi Srour. Of the 51 girls in the study, 9.8% had nutritional anaemia and 7.8% had iron-deficiency anaemia, as compared with 21.7% and 17.4% of the 46 boys (unpublished data). None of the infants have participated in any other studies.

Discussion

The results presented in table 2 strongly suggest that in Jordan exclusive breastfeeding for five to six months has a better protective effect against the development of nutritional anaemia and iron-deficiency anaemia than a shorter period of four months of exclusive breastfeeding. About 29% of Jordanian infants who were exclusively breastfed for four months developed iron deficiency at six months of age, as compared with none of the infants who were exclusively breastfed for five or six months. The high bioavailability of iron in breastmilk, ranging from 50% to 70% [16] may partly explain these results. In addition, the earlier introduction of

TABLE 3. Haemoglobin, plasma ferritin, and mean corpuscular volume in infants exclusively breastfed for four to six months, according to the duration of exclusive breastfeeding and iron status^a

Duration (mo)	Iron status	No.	Hb(g/dl)		PF(ng/ml)		MCV(fl)	
			Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
4 (n = 14)	NA	8 (4 with IDA)	9.7 \pm 0.9	8.4–10.4	35.8 \pm 47.0	5.0–161.3	71.0 \pm 8.3	59.5–80.5
	ID	2	12.1 \pm 1.15	10.9–13.2	8.0 \pm 2.15	5.8–10.1	76.7 \pm 3.55	73.2–80.3
	No NA or ID	4	11.6 \pm 0.4	11.0–11.8	63.0 \pm 34.8	30.5–111.3	80.7 \pm 4.7	75.1–86.5
5 (n = 15)	NA	3 (0 with IDA)	10.0 \pm 0.6	9.3–10.4	72.0 \pm 71.5	17.5–153	70.5 \pm 5.2	64.7–74.7
	ID	0						
	No NA or ID	12	11.6 \pm 0.7	10.5–13.2	64.6 \pm 73.8	12.1–277.0	79.5 \pm 4.4	73.4–86.9
6 (n = 37)	NA	4 (0 with IDA)	10.2 \pm 0.1	10.1–10.4	61.2 \pm 61.3	26.4–152.9	74.6 \pm 3.0	72.0–77.6
	ID	3	11.5 \pm 0.8	10.7–12.2	8.2 \pm 2.3	5.7–10.1	71.3 \pm 3.6	67.4–74.5
	No NA or ID	30	11.5 \pm 0.6	10.5–13.3	54.6 \pm 48.9	13.7–187.2	77.4 \pm 5.2	67.6–88.3

a. Hb, Haemoglobin; PF, plasma ferritin; MCV, mean corpuscular volume; NA, nutritional anaemia (Hb < 10.5 g/dl); IDA, iron-deficiency anaemia (Hb < 10.5 g/dl, MCV < 70 fl, PF < 12 ng/ml); ID, iron deficiency (depletion of iron stores [PF < 12 ng/ml] but Hb > 10.5 g/dl).

solid foods associated with the shorter period of exclusive breastfeeding (four vs. five or six months) necessitates the ingestion of certain food factors that inhibit iron absorption, such as phytates in cereals and tannins in tea [2]. Bread and tea are consumed as weaning foods by Jordanian infants at quite an early age [17, 18]. In addition, according to their mothers, iron-fortified baby foods were not given to the study infants. Thus, in Jordan, where iron-fortified formula or cereals are not generally given to weanlings, especially in families of low to medium economic status, breastfeeding is the main preventive measure against iron-deficiency anaemia.

Upon further analysis, Dewey et al. [12] observed that none of the infants with birthweights over 3,000 g had depleted iron stores (plasma ferritin < 12 μ g/L) at six months of age, whether they were exclusively breastfed for six months or whether they were exclusively breastfed for four months, then given continued breastfeeding in addition to iron-fortified foods. Only 5.3% (1/19) of the exclusively breastfed infants had anaemia (haemoglobin < 10.3 g/dl), as compared with 18% (4/22) of those who were exclusively breastfed for four months, then given breastmilk plus iron-fortified foods. This suggests that exclusive breastfeeding for six months was protective against the development of anaemia in infants with birthweights over 3,000 g, irrespective of the fact that iron-fortified food was given to the other

group between four and six months of age. The results are consistent with our study, although we did not include low-birthweight infants.

Excluding the intake of iron-fortified weaning foods, which were not used in this study, typical weaning foods consumed by the Jordanian infants up to six months of age were conducive to iron deficiency at that age. This is further supported by the results of national [18] and regional [19] studies. Meat, poultry, or fish are given seldom, if at all, to Jordanian infants at six months of age, especially by poor families. For example, during the period from 8 to 24 months of age, about 54% of the infants never received any meat, and about 80% never received any infant formula [19].

Meat provides haem iron, which has a high bioavailability besides its enhancing effect on dietary non-haem iron [20]. In the UNICEF national study in Jordan, rice pudding was introduced in the second month; cooked rice, milk, yoghurt, and *labneh* (concentrated yoghurt with about 70% water) were introduced in the fourth month; and pasta and cereal grains were introduced in the sixth month at a rate of one to two servings per week. Lentils, broad beans, and chickpeas were introduced mainly in the fifth month.

More strikingly, Khatib and Hijazi [19] observed that the proportion of infants who had received the following foods during the fifth and sixth months (and during

the third and fourth months as given in parentheses) were: meats, 8.5% (1.6%); rice, 69% (56.2%); bread and biscuits 78.1% (52.4%); legumes, 43.5% (11.3%); eggs, 51.6% (33.3%); cow's milk and yoghurt, 65.0% (45.8%); vegetables, 47.6% (14%); tea, 2.0% (15.5%); juices, 62.4% (42.6%); and family dishes, 17.5% (6.2%).

In Jordan bread, biscuits, or crackers are soaked in tea and are eaten together as a weaning food [17]. The strong inhibitory effect of tea on iron absorption is well documented [9, 20–22]. This effect is expected to increase when tea is consumed with bread, which has a high phytate content. Currently, locally available wheat flour is not fortified with iron. Two types of wheat flour are produced: the “unified” type, with a low extraction rate of about 72%, and the “brown” type, with a higher extraction rate of about 80%. The brown flour is subsidized by the government and is sold at a lower price and consumed at a higher rate than the “unified” flour. This means a higher phytate intake, with more hindrance to iron absorption.

The high phytate content of lentils and the high polyphenol content of spinach, lentils, and eggplants is associated with poor absorption of non-haem iron [23]. Data on weaning foods in Jordan, in addition to our own experience, indicate that solid weaning foods introduced to infants between four and six months of age are also based on cow's milk: pudding, yoghurt, and *labneh*. Cow's milk is a poor source of iron. Eggs are also given as weaning foods in Jordan. They not only have little bioavailable iron but also reduce the bioavailability of food non-haem iron [20]. However, fruit juices and certain other vegetables, especially tomatoes, are available in Jordan at affordable prices. They enhance non-haem iron absorption due to their vitamin C content and thus ameliorate the inhibitory effect of the above-mentioned weaning foods. Otherwise, the prevalence of iron deficiency among the study infants might have been higher.

In infancy the main determinants of iron requirements are iron stores at birth, growth requirements, and the need to replace iron losses [7]. Dewey et al. [12] concluded that birthweight was a critical risk factor in the development of anaemia (low haemoglobin) and in the depletion of iron stores (low plasma ferritin). In our study, iron stores at birth were assumed to be the same among the study groups, since all infants were full term and of normal birthweight (> 2,500 g). Also, it was assumed that the iron status of their mothers during pregnancy was not low enough to have influenced the infants' iron stores at birth. This is presumably due to the fact that all pregnant women attending Jordanian Maternal and Child Health Centres, in particular the Wadi Srou Centre, routinely receive free iron sulphate and folic acid tablets and dietary counselling, starting during the first trimester. Furthermore, mothers who are diagnosed as anaemic during pregnancy are treated with iron and folic acid. The rela-

tion between maternal and infant iron status seems to be controversial when, at least, mothers are not severely anaemic during pregnancy. For example, a cross-sectional study of 47 paired samples at term was carried out in urban mothers of low social class in northern Jordan [24]. No correlation between maternal and cord serum ferritin was observed, even when two cut-off points for low maternal ferritin at the 50th and the 25th percentiles were examined. This is in agreement with the observations of Bhargara et al. [25] in low- to medium-income urban Indian mothers and with certain other studies [7, 26], but it is in conflict with others [27, 28]. Stekel [7] concluded that “It is only under unusual circumstances of severe iron deficiency in the mother that iron endowment of the newborn may be affected.” A correlation between the iron status of mothers and their infants during the first year of life was found in certain prospective studies [29–33], but not in others [34, 35].

The prevalence of anaemia among the mothers attending the Maternal and Child Health Centres in 1990 and 1991 was about 25% [36], a figure not much different from the national average of 29% for Jordanian women of child-bearing age in 1994 [37]. These figures suggest that Jordanian women have better iron status than women worldwide (an average of 37% are anaemic), African women (44% anaemic), or Asian women (45% anaemic) [38]. However, neither anthropometric measurements nor further in-depth analysis of the data from the Jordanian national study was carried out.

At birth the infant of normal weight has iron stores, in the form of 75 mg of ferritin per kilogram of body weight, that supply most of the requirements for iron during the first four to six months of life. Daily iron requirements to satisfy infant growth and basal losses are estimated at 0.5 mg of iron absorbed up to 6 months of age and at 0.9 mg of iron during the subsequent 6 to 12 months [7]. In this study, the growth velocity of infants three to six months of age was within the normal range of 15 to 20 g/day and did not differ significantly among the study groups. Therefore growth velocity should not be considered as a variable that might have confounded the results. Iron losses are mainly in the stools and due to occult blood provoked by cow's milk [39], enteric infection, and parasitic infestation (mainly hookworm and schistosoma, and to lesser degree giardia [40]). In Jordan the main parasites related to iron status are *Entamoeba coli*, giardia, and pinworms, but not hookworms, schistosoma, or malaria. In a 1994 UNICEF national study of 8- to 11-year-old schoolchildren, the prevalences of giardia, ascariis, *Hymenolepis nana*, and enterobius were 8.4%, 1.5%, 1.3%, and 1.9%, respectively [41]. *Entamoeba coli* was the most common, with a prevalence of 14.3%. Equally important, anaemia was not considered to be related to the presence of intestinal parasites. Likewise, parasites were not considered an important aetiological factor in the high

prevalence (65.5%) of anaemia (haemoglobin < 11 g/dl) that was observed in 6- to 12-month-old infants among the underprivileged Palestinians living in Jordan and served by the United Nations Relief and Works Agency [42]. We did not study intestinal parasites, but the results obtained suggest that exclusive breastfeeding until six months of age was, if anything, important against the negative effects that might have influenced the infant's iron status.

The fact that the prevalence of nutritional anaemia was always higher than that of iron-deficiency anaemia after four, five, or six months of exclusive breastfeeding strongly suggests that nutritional anaemia is caused by a deficiency of haematopoietic nutrients other than iron, such as folic acid, vitamin A, pyridoxine, vitamin B₁₂, copper, or protein [9, 10]. Further work is needed to determine which deficiencies are relevant under the local conditions of this study, which was limited to the region served by the Maternal and Child Health Centre of Wadi Srou in Amman.

Conclusions

The data presented in this study strongly suggest that exclusive breastfeeding for five or six months rather

than for four months is protective against the development of nutritional anaemia or iron-deficiency anaemia up to at least six months of age.

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