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Iron deficiency in healthy 18-month-old Danish children is associated with no oral iron supplementation in infancy and prolonged exclusive breast-feeding

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Abstract

Fe deficiency (ID) defined as plasma ferritin <12 µg/l is associated with delayed cognitive development in early childhood and increased incidence of infections; however, the longitudinal association between early-life factors and ID in 18-month-old children in Denmark is unknown. The present study aimed to determine the prevalence of ID and to describe risk factors associated with ID in healthy 18-month-old Danish children. Blood samples, anthropometric measurements and self-reported questionnaire data had been obtained in the birth cohort, Odense Child Cohort. The questionnaires were modified from those used in the Danish National Birth Cohort. Plasma ferritin and C-reactive protein in venous, non-fasting samples were analysed in the final sample size of 370 children after exclusion of seventy-nine children due to chronic disease, acute infection, C-reactive protein >10 mg/l, twin birth or prematurity. Associations with ID were analysed by logistic regression, adjusting for sex, maternal education, duration of partial breast-feeding and current intake of milk, fish and meat. Overall, fifty-six children had ID (15.1 %). Factors associated with increased risk were exclusive breast-feeding beyond 4 months (OR 5.97; 95 % CI 1.63, 21.86) and no intake of oral Fe supplements from 6 to 12 months (OR 3.99, 95 % CI 1.33, 11.97). Duration of partial breast-feeding and current diet was not associated with ID. In conclusion, the ID prevalence was 15.1 %, and both exclusive breast-feeding beyond 4 months and no intake of oral Fe supplements from 6 to 12 months were associated with increased risk of ID in 18-month-old children.

Key words: Odense Child Cohort: Iron-deficiency prevalence: Oral iron supplementation: Duration of exclusive breast-feeding

The WHO defines Fe deficiency (ID) as serum ferritin under 12 µg/l⁽¹⁾. ID is one of the most common micronutrient deficiencies worldwide and is a main cause of anaemia along with infections and parasitic infestations^(1–3). In 2015, ID was reported in Western Europe, with a prevalence of 11.8 % in a study of 12- to 36-month-old children from Germany, the Netherlands and the UK⁽³⁾.

ID affects myelination and basal ganglia function and is associated with impaired neurodevelopment^(4–8). ID has been associated with delayed cognitive development during the rapid growth spurt of the brain in early childhood^(6,7), leading to potential longitudinal effects on cognition later in life, which are exemplified by study findings and positive effects of Fe supplementation for non-anaemic ID on cognition, learning capacity and memory^(5,6,8,9).

ID may also increase the risk of infection as Fe is required for normal immune function as a critical component of peroxide- and nitrous oxide-generating cellular enzymes used in the bactericidal activity of macrophages and because Fe affects T-cell number and function^(10,11,12).

Infant's need for Fe is greater than that of an adult because of its rapid growth and development^(12,13,14). A negative association was found between knee-heel-growth velocity and serum ferritin levels over the age of 2–6 months among healthy Danish infants, indicating that Fe stores are used during growth⁽¹²⁾. The size of Fe stores at birth depends on several factors. Preterm infants have lower Fe stores as Fe is mainly transferred from the maternal circulation to the fetus after 30 weeks of gestation, while delayed cord-clamping postpartum has a positive effect on Fe stores in full-term infants^(4,15).

Abbreviations: CBP, Clinical Biochemistry and Pharmacology; CRP, C-reactive protein; ID, Fe deficiency; OCC, Odense Child Cohort.

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Regardless of the size of Fe stores at birth, they are depleted during approximately the first 6 months of life^(2,3,13). Dietary Fe intake is thus needed from 4 to 6 months onwards as Fe absorption from infant formula is poor and Fe content in human milk is very low (0.2–0.9 mg/l)^(16–18). Fe or Hb status of healthy mothers does not correlate with Fe content in their breast milk 6 months postpartum, so increasing the Fe intake in the diet of a healthy mother will not increase the Fe content of her milk^(16–18).

Dietary Fe is either haem Fe from myoglobin and Hb from meats and fish, or non-haem Fe from vegetable sources. The bio-availability of haem Fe is generally better than that of non-haem Fe. Furthermore, the composition of the diet is important as, for example, vitamin C and haem Fe enhance absorption of non-haem Fe, whereas Ca in cows' milk may inhibit Fe absorption^(19,20).

Little is known about the prevalence of ID and its associated early-life factors in Scandinavian 18-month-old children. This population is significant for investigating possible longitudinal associations between ID and early-life factors and recommendations on Fe supplementation in the first year of life. A study among Danish 9-month-olds found that ferritin levels were positively associated with compliance to oral Fe supplementation or daily intake of ≥ 400 ml Fe-fortified infant formula from 6 months of age and that intake of meat and fish was not significantly associated with ferritin levels⁽¹⁴⁾. Until 2015, the Danish recommendations were that full-term infants should receive Fe supplementation (about 8 mg/d) or at least 400 ml Fe-fortified formula or gruel from age 6 to 12 months. Since 2015 oral Fe supplementation is no longer recommended. Parents are now recommended not to offer cows' milk, but only breast milk or infant formula throughout the first year of life, and they are recommended to serve Fe-rich food such as fish and meat from 6 months of age^(14,21). However, in Denmark only 60 % of children are exclusively breast-fed until 12 weeks of age and only 12 % of children are exclusively breast-fed until 6 months of age, meaning that most children are introduced to solids before 6 months of age^(22,23). In this cohort of children born in 2010–2013, we, therefore, have the possibility to examine the importance of Fe supplementation within the first year of life for Fe status in later childhood. The aims of the present study were to determine the prevalence of ID and to describe factors associated with ID longitudinally in healthy 18-month-old Danish children.

Materials and methods

The present study is a subproject of the Odense Child Cohort (OCC), a birth cohort of 2550 children enrolled during the years 2010–2013 from the municipality of Odense, Denmark. Blood samples, anthropometric and clinical measures as well as questionnaire data on, for example, supplementation, diet and breast-feeding, were collected at predetermined ages. Further details on the OCC are previously published⁽²⁴⁾. The present study uses data collected at birth and at 18 months of age.

The blood samples we used from the OCC had been randomly selected and analysed for plasma ferritin and C-reactive

protein (CRP) in a separate study of normal reference levels of ferritin in healthy children. The present study was conducted by the Department of Clinical Biochemistry and Pharmacology (CBP) at the Odense University Hospital and it is referred to as the CBP study, which is not yet published.

Blood samples

Samples of venous blood were collected at Hans Christian Andersen Children's Hospital, Odense University Hospital, by trained paediatric phlebotomists. Non-fasting venous blood samples were collected between 08.00 and 17.00 hours. Samples for values of ferritin and CRP were collected in BD Vacutainer® PST™ Gel Separator Tubes with Li heparin. They were kept at room temperature, centrifuged and analysed within 4 h of collection.

Ferritin values were analysed by chemiluminescence microparticle immunoanalysis technology (Ferritin 7K59; Abbott Laboratories) using Architect Instruments (Abbott Laboratories). CRP concentrations were measured by immunoturbidimetric assay (CRP Vario 6K26; Abbott Laboratories) using Architect Instruments (Abbott Laboratories). The inter-assay CV were <6 % for ferritin and <3 % for CRP.

As ferritin is an acute-phase reactant, children with CRP levels above 10 mg/l were excluded from the study. In addition, children born prematurely or twins, children who had contact with the paediatrics department at the Odense University Hospital for infection within 7 d before or after blood sampling and had chronic disease were excluded from the study.

Questionnaire data

The questionnaires used in the Danish National Birth Cohort⁽²⁵⁾ were modified and used for this study. The present study used parent-reported background data such as birth date, birth weight and birth length, socio-economic status of parents, etc. from time of enrolment as well as parent-reported questionnaire data at 18 months of age on breast-feeding, Fe supplementation from 6 to 12 months, infant formula and current intake of cows' milk, meat and fish.

Parents reported the number of weeks the child had been exclusively breast-fed (defined as only breast milk and water) and partially breast-fed (defined as any breast milk concurrent with other foods or drinks besides water), the child's age when breast-feeding ceased, the age at which the child was fed infant formula for the first time and the brand that was used and whether the child was still fed infant formula. However, data on the volume of infant formula were not obtained, thus excluding the possibility of analysing the intake of infant formula. Exclusive breast-feeding beyond 4 months of age was analysed as a binary variable with a cut-off at 17 weeks, since this is a common practice among breast-feeding parents in Denmark, even though national guidelines recommend exclusive breast-feeding for 6 months⁽²²⁾.

Parents also reported whether they chose to give their children the recommended 8 mg/d of oral Fe supplementation and the age at which supplementation was started and stopped. The most common form of supplementation was Fe drops⁽¹⁴⁾. Only children receiving 8 mg/d of oral Fe supplementation

continuously in the period from 6 to 12 months of age were allocated to the 'yes' group of oral Fe supplementation. Children who did not receive oral Fe supplementation continuously in this period were allocated to the 'no' group of Fe supplementation.

The average intake of fish and meat per d, week or month was reported as children's spoonful (equivalent to 15 g), half a Danish meatball or one slice of meat or fish on bread. Parents were given the choice to report 'My child is almost never served meat/fish'. Current intake of unmodified cows' milk, both as drinks and as yogurt, was reported in units of 100 ml/d or 'uncertain because of the intake in day care' or 'does not tolerate cows' milk'. Ca and Fe were not quantified using the questionnaire, as the variety of different foods in these categories would make such an estimation inaccurate. Instead we only focused on the overall intake of fish, meats and dairy products.

The child's weight was measured in kg. The child's length was measured as crown-heel length in cm at 18 months. Weight velocity (kg/month) and length velocity (cm/month) were measured from time of birth to exact age at measurement. For children aged 0-5 years, the *z* scores of weight and length for age were calculated using the WHO growth standards⁽²⁶⁾. Anthropometric measurements from birth were collected routinely by midwives at birth, whereas measurements at 18 months of age were collected by four technicians with a minimum of 10 years' experience in paediatrics at the OCC⁽²⁴⁾.

Statistical analyses

A power calculation showed that the 370 samples in the present study provided a sufficient sample size to determine the prevalence of ID at a confidence level of $\pm 3\%$, assuming a known prevalence of ID in Western Europe of 11.8 %⁽³⁾. *P* values <0.05 were thus considered significant.

ID was defined as a binary outcome at the plasma ferritin cut-off value $<12 \mu\text{g/l}$. Baseline differences between the two groups, ID and non-ID, were tested by independent *t* tests.

The association between ID and our two main variables (exclusive breast-feeding beyond 4 months and intake of oral Fe supplementation) was analysed using both crude and adjusted logistic regression models. The crude model only tested the direct association between each of the main exposures individually to the outcome. The adjusted model included three variables that were chosen after stepwise backward elimination analyses with the elimination cut-off value $P > 0.20$ (i.e. current intake of unmodified cows' milk/yogurt, fish and meat) as well as three clinically relevant, albeit non-significant, variables (duration of partial breast-feeding, maternal education and sex) and basic characteristics and anthropometric measures (see Tables 2 and 3).

The final adjusted logistic regression model was validated in a nested likelihood ratio test against the crude logistic model with each explanatory variable in turn. The adjusted logistic regression model showed better goodness of fit than the crude model.

To deal with the missing values of the explanatory variables, the crude models were tested in a smaller sample consisting of complete cases and thus not influenced by missing values of the explanatory variables. Missing values for duration of exclusive breast-feeding were assumed to be missing at random, so we

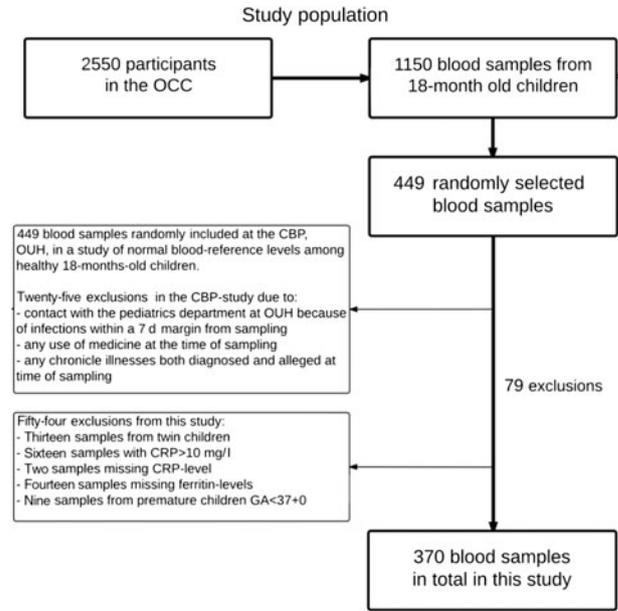


Fig. 1. Flow chart of study population. OCC, Odense Child Cohort; CBP, Clinical Biochemistry and Pharmacology; OUH, Odense University Hospital; CRP, C-reactive protein; GA, gestational age.

used the iterative chained equations approach. The adjusted regression model was repeated with multiple imputations on exclusive breast-feeding to test the impact of missing values on our adjusted model. The findings with multiple imputations did not differ from the findings of the adjusted regression model and are thus not discussed here. All statistical analyses were done in STATA 14.1 statistical software (StataCorp LP).

Ethics

The OCC and the normal-reference study at CBP were approved by the Regional Scientific Ethical Committees for Southern Denmark under the project IDs S-20090130 and S-20130159, respectively. Both projects are approved by the Danish Data Protection Agency. Written consent was collected from all parents participating in the OCC. The Regional Scientific Ethical Committees of Southern Denmark defined the present study as a database study not requiring further approval because we used data from blood samples already obtained and analysed by the OCC and the CBP study.

Results

Of the 2550 children enrolled in the OCC, blood samples were taken from 1150 children at their 18-month-old examination. Most of the missing blood samples were due to either the parents declining, or the child crying or moving too much, hindering the collection of blood samples. Some venepunctures were unsuccessful, and the phlebotomists routinely only punctured a child once. Of the 1150 blood samples collected, the CBP randomly included 449 samples for their study on normal reference levels in healthy children, while seventy-nine were excluded due to various reasons (Fig. 1).

Table 1. Basic characteristics of all participants in the Odense Child Cohort (OCC), excluded participants and the final study participants (Numbers of participants and percentages)

	OCC		Excluded participants		Study participants	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Participants	2101	100.0	79	100.0	370	100.0
Sex						
Male	1088	51.8	44	55.7	202	54.6
Female	1013	48.2	35	44.3	168	45.4
Birth weight						
≤ 2999 g	359	17.1	35	44.3	39	10.5
3000–3999 g	1379	65.6	36	45.6	261	70.5
≥ 4000 g	352	16.8	8	10.1	68	18.4
Birth length						
≤ 50 cm	598	28.5	40	50.6	74	20
51–54 cm	1233	58.7	28	35.4	241	65.1
≥ 55 cm	250	11.9	9	11.4	49	13.2
Gestational age						
< 37 weeks	113	5.4	23	29.1	0	0
37–39 weeks	832	39.6	28	35.4	149	40.3
> 40 weeks	1151	54.8	28	35.4	220	59.5
Mode of delivery						
Vaginal	1610	76.6	56	70.9	291	78.6
Elective caesarean section	275	13.1	13	16.5	49	13.2
Acute caesarean section	211	10	10	12.7	29	7.8
Maternal smoking						
No	1988	94.6	77	97.5	353	95.4
Yes	106	5	2	2.5	16	4.3
Maternal education						
Lower: HS or less	554	26.4	23	29.1	95	25.7
Intermediate: HS + 1–3 years	641	30.5	25	31.6	101	27.3
Higher: HS + 4 years or more	335	15.9	10	12.7	73	19.7
Maternal ethnic background						
Danish	1844	87.8	68	86.1	348	94.1
Western	102	4.9	3	3.8	13	3.5
Not Western	111	5.3	1	1.3	9	2.4

HS, high school.

The final study population of 370 participants was similar to the excluded OCC participants with regard to sex, mode of delivery, maternal smoking, educational level and ethnic background. Both groups had a slight overweight of males, the majority were delivered by vaginal birth, the vast majority of mothers were non-smokers and Danish by ethnicity (Table 1). The participants in our study did differ from the general OCC with regard to birth weight, birth length and gestational age as a natural consequence of excluding premature children, twins and children with chronic diseases (Table 1). With regard to all background data, no significant differences were found between the ID and non-ID groups. There were low numbers of missing data, and they were evenly distributed between groups. Potential interactions between all variables were tested individually and found non-significant.

Iron deficiency prevalence

The overall prevalence of ID in our study population was 15.1%, as fifty-six out of 370 children had ferritin values <12 µg/l. Only one child in our study had ID anaemia. Children with ID did not differ from non-ID children with regard to sex, gestational age, birth weight and length, mode of delivery, exact age at blood sampling, maternal smoking or ethnicity. More mothers in the

ID group were highly educated (Table 2), but this was a non-significant difference as the OR for ID in children of highly educated mothers was 1.92 (95% CI 0.62, 5.92) compared with intermediate and low educated mothers.

Anthropometrics

Children in the ID and non-ID groups were not significantly different in absolute weight/length, weight/length velocity or weight/length *z* scores. For 0 to 5-year-old children, all *z* scores were within the normal range of WHO growth standards (Table 3).

Factors associated with iron deficiency

Exclusive breast-feeding for longer than 4 months was significantly associated with ID (OR 5.97; 95% CI 1.63, 21.86), while no intake of oral Fe supplementation from the age of 6–12 months was also significantly associated with ID at 18 months of age (OR 3.99, 95% CI 1.33, 11.97; Table 4).

The following variables were not significantly associated with ID: sex of the child (boy OR 0.80, 95% CI 0.29, 2.18), duration of partial breast-feeding (measured continuously per week, OR 1.00, 95% CI 0.97, 1.04), current intake of milk or other dairy

Table 2. Basic characteristics of the 370 study participants grouped in non-iron-deficient (non-ID) and iron-deficient (ID) participants (Numbers of participants and percentages)

	Non-ID		ID*	
	n	%	n	%
Participants	314	100.0	56	100.0
Sex				
Male	168	53.5	34	60.7
Female	146	46.5	22	39.3
Birth weight				
≤ 2999 g	32	10.2	7	12.5
3000–3999 g	221	70.4	37	66.1
≥ 4000 g	58	18.5	10	17.9
Birth length				
≤ 50 cm	62	19.7	11	19.6
51–54 cm	200	63.7	38	67.9
≥ 55 cm	43	13.7	5	8.9
Gestational age				
37–39 weeks	125	39.8	24	42.9
40–42 weeks	189	60.2	31	55.4
Mode of delivery				
Vaginal	244	77.7	43	76.8
Elective caesarean section	39	12.4	9	16.1
Acute caesarean section	27	8.6	2	3.6
Maternal smoking				
No	298	94.9	51	91.1
Yes	13	4.1	3	5.4
Maternal education				
Lower: HS or less	61	19.4	12	21.4
Intermediate: HS + 1–3 years	93	29.6	8	14.3
Higher: HS + 4 years or more	69	22	26	46.4
Maternal ethnic background				
Danish	298	94.9	50	89.3
Western	9	2.9	4	7.1
Not Western	7	2.2	2	3.6
Age at blood sample and questionnaire				
17–19 months	122	38.9	22	39.3
20–23 months	154	49	27	48.2

HS, high school.

* ID defined by plasma ferritin <12 µg/l.

products (over 500 ml/d, OR 0.82, 95 % CI 0.27, 2.50), fish intake (in g/week, OR 1.00, 95 % CI 0.99, 1.01) and meat intake (in g/week, OR 1.00, 95 % CI 1.00, 1.00).

Discussion

Iron deficiency prevalence

In the present study of 18-month-old Danish children, we found an ID prevalence of 15.1 %. This is in line with studies of children in Germany, the Netherlands and the UK, where the ID prevalence was on average 11.8 %⁽³⁾. It is also comparable to studies of 18- to 36-month-old children in Northern Israel with an ID prevalence of 19.9 %⁽²⁷⁾ and several American studies on toddlers, where the ID prevalence varied between 7 and 20.5 %⁽²⁸⁾.

Until 2015, the recommendation in Denmark was that infants aged 6–12 months should receive either 8 mg Fe as a daily supplement or at least 400 ml of Fe-fortified formula daily⁽¹⁴⁾. This recommendation was valid during our study period. In 2015, new recommendations similar to most other Western countries were published by the Danish health authorities in accordance with the 2014 European Society for Paediatric Gastroenterology

Table 3. Anthropometric characteristics of the 370 study participants grouped in non-iron-deficient (non-ID) and iron-deficient (ID) participants (Mean values and standard deviations)

	Non-ID		ID*		P†
	Mean	sd	Mean	sd	
Age (months)	19	0.8	19	0.9	1.00
Weight (kg)	11.7	1.2	12.2	1.3	1.00
z score	0.6	0.8	1.0	0.9	1.00
Length (cm)	84.2	3.0	85.1	3.1	0.98
z score	0.5	1.0	0.9	1.0	0.99
Weight velocity‡ (kg/month)	0.4	0.1	0.5	0.1	1.00
Length velocity‡ (cm/month)	1.7	0.1	1.7	0.2	0.99

* ID defined by plasma ferritin <12 µg/l.

† Independent t tests between non-ID and ID values.

‡ Velocity, monthly average from birth to exact age.

Table 4. Risk for iron deficiency (ID; plasma ferritin <12 µg/l) (Odds ratios and 95 % confidence intervals)

	OR	95 % CI	P
Exclusive breast-feeding beyond 4 months			
Crude	2.47	1.16, 5.25	0.02
Adjusted	5.97*	1.63, 21.86	0.01
No intake of Fe supplements from 6 to 12 months of age			
Crude	1.41	0.71, 2.77	0.32
Adjusted	3.99*	1.33, 11.97	0.01

* Adjusted for sex, maternal education, duration of partial breast-feeding and current intake of milk, fish and meat. None of these was significantly associated with ID.

Hepatology and Nutrition (ESPGHAN) position paper on Fe requirements of infants and toddlers^(13,21). Infants aged 6–12 months with normal birth weight and born at full-term are no longer recommended Fe supplements. Parents are instead encouraged to offer Fe-rich food such as meat and fish from 6 months of age, advised not to offer unmodified cows' milk and yogurt before the age of 12 months and thereafter to limit the intake of unmodified cows' milk to under 500 ml/d⁽²¹⁾.

Oral iron supplementation

We found a significant association between no oral Fe supplementation between 6 and 12 months of age and an increased risk of ID at 18 months of age. Our results are similar to those of Gondolf *et al.* who found that Fe supplementation in the 9-month-olds (either as drops or fortified formula) was associated with higher ferritin levels, while meat and fish intake was not associated with ferritin levels⁽¹⁴⁾. The key difference from our study is that the study by Gondolf *et al.* was carried out while the children were still at the age of receiving Fe supplementation. Our finding of an association between no Fe supplementation from 6 to 12 months of age and higher risk for ID at 18 months of age (6 months after Fe supplementation ceased) indicates not only an immediate association between supplementation and ferritin levels but also a long-term association.

Our findings may stand in contrast to the new Danish recommendations of no Fe supplementation for children born at full

term⁽²¹⁾. However, as studies have found association between higher risk of ID and the intake of unmodified cows' milk⁽³⁾, which inhibits Fe absorption and contains very little Fe in itself, the useful recommendations are avoiding the intake of unmodified cows' milk before 12 months of age and encouraging the intake of Fe-rich foods from 6 months of age.

Furthermore, it is important to remember that oral Fe supplementation of Fe-sufficient children may have adverse effects as found in one Swedish study where Fe-sufficient children who received oral Fe supplementation had reduced growth in length⁽²⁹⁾. This is a relevant consideration as the vast majority of our study population was Fe sufficient.

It would be relevant to conduct a new study among 18-month-old Danish children to evaluate whether the new recommendations have had any influence on the ID prevalence at this age.

Exclusive breast-feeding beyond 4 months

We found an association between exclusive breast-feeding beyond 4 months and increased risk of ID at 18 months of age. This is in line with other studies^(3,13), and the American Academy of Paediatrics has suggested that all exclusively breast-fed infants should receive Fe supplements of 1 mg/kg/d from 4 months of age (and even if they are only partially breast-fed from 4 months but receive more than half of their daily feeds as human milk). Our findings may support this recommendation, and it may be relevant to examine whether Fe supplementation from 4 to 6 months of age would be an attractive alternative to introducing Fe-rich solids at 4–6 months of age. A systematic review from 2013 on Nordic children concluded that exclusive breast-feeding beyond 4 months is associated with better outcomes, for example, less overweight and fewer infections⁽²²⁾.

Current diet

We found no association between current diet and ID at 18 months of age. This is in accordance with the study of 9-month-old children by Gondolf *et al.*⁽¹⁴⁾, indicating that current diet is not significantly associated with Fe status. However, both studies were conducted when Fe-rich food such as fish and meat from 6 months of age were not emphasised in food recommendations and unmodified cows' milk was offered before 12 months of age.

We also have no information on cord-clamping at birth or intake of fruit, vegetables and legumes, all of which could influence the child's current Fe status^(14,15,19,20). Future studies of dietary factors associated with ID in Scandinavian toddlers should include these aspects.

Growth

We did not find associations between anthropometric measurements and the risk for ID. A previous Danish study found negative associations between knee–heel growth velocity and serum ferritin levels in younger infants from 2 to 6 months⁽¹²⁾. We used crown–heel length for our length measurements.

Educational level of mothers

This factor seemed to be associated with ID as the proportion of highly educated mothers in the ID group was more than twice compared with the non-ID group. However, education was not significantly associated with ID after adjustment for breast-feeding. The initially observed difference in ID prevalence among children of highly educated mothers *v.* those of intermediate and low education was not controlled for breast-feeding, and the proportion of mothers who breast-fed exclusively for more than 4 months was 58.9 % (43/73) among highly educated women, 42.6 % (43/101) among mothers with intermediate education and 41.1 % (39/95) among mothers with low education. The explanatory factor is thus likely to be the duration of exclusive breast-feeding beyond 4 months rather than educational level *per se*.

The OCC consists of more highly educated, predominantly ethnic Danish non-smoking parents compared with the general Danish and Scandinavian populations⁽²³⁾. The prevalence of ID has been linked to lower socio-economic groups, for example, in India⁽³⁰⁾. In the present study, the higher proportion of children with ID in the group of highly educated mothers may be explained by the strong association between exclusive breast-feeding above 4 months and the increased risk of ID. More of the highly educated mothers breast-fed exclusively beyond 4 months compared with mothers with intermediate and lower education. Therefore, there is a risk of overrepresentation of ID in the OCC in comparison with the Danish population even though our prevalence data are in line with other studies from similar countries.

Questionnaire data were collected at 18 months of age and thus up to 1 year after the actual exposure (e.g. the duration of exclusive breast-feeding and the start of Fe supplementation). This leads to less accurate data recordings and possibly weaker associations. However, we have no reason to believe that these inaccuracies caused systematic biases in the reporting of Fe status as parents were unaware of the outcome (ID or non-ID).

Ferritin can be measured either in plasma collected in Li–heparin tubes, as in the present study, or in serum collected in serum separator tubes. However, a clinical biochemistry study on the stability of routine tests found that only ferritin showed a minor bias in results after 10 h of storage, when comparing samples from Li–heparin tubes with those from serum tubes and using similar analytical methods as in our study. Even with this minor bias, the study concludes that whole blood in either Li–heparin or serum tubes stored for 10 h at 20–22°C can be used for routine analysis without restrictions⁽³¹⁾. The blood samples in our study were analysed within 4 h of collection, so we assume that the plasma ferritin values and ID prevalence are comparable to those of other studies that measure serum ferritin.

Conclusion

The prevalence of ID among healthy Danish 18-month-old children was 15.1 %. We found a higher risk of ID among children exclusively breast-fed beyond 4 months of age and among children with no Fe supplementation from 6 to 12 months of age. We found no association between ID and maternal educational

level, duration of partial breast-feeding or the child's current intake of milk, fish and meat.

Further prevalence studies should determine whether the new Danish recommendations of no Fe supplements and encouragement of Fe-rich complementary foods increase the prevalence of ID among healthy full-term children. If this is the case, randomised controlled trials are needed to investigate the efficacy, safety and long-term effects of oral Fe supplementation in infancy for prevention of ID in Scandinavian children. Further studies exploring dietary factors that contribute to healthy Fe status among Scandinavian children are also needed.

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A. T. N. A. is the main author of this article and carried out the study. S. H. is the senior investigator of the Odense Child Cohort, who collected all the data used in the present study. He participated in the design of the study, its research questions and in the interpretation of the analyses and also edited the manuscript. C. M. participated in the design of the study, its research questions and in the interpretation of the analyses and also edited the manuscript. S. D. S. participated in the design of the study, its research questions, in data analysis and in the interpretation of the analyses and also edited the manuscript. H. B. K. participated in the sorting of data and in the methodical execution of the study. M. B. S. analysed the blood samples and participated in the writing of the methodological section of this article. All authors approved the final manuscript.

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References

- World Health Organization (2001) Iron deficiency anaemia: assessment, prevention and control. http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en2001 (accessed May 2016).
- McLean E, Cogswell M, Egli I, *et al.* (2009) Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutr* **12**, 444–454.
- Akkermans MD, van der Horst-Graat JM, Eussen SR, *et al.* (2016) Iron and vitamin D deficiency in healthy young children in Western Europe despite current nutritional recommendations. *J Pediatr Gastroenterol Nutr* **62**, 635–642.
- Bakoyiannis I, Gkioka E, Daskalopoulou A, *et al.* (2015) An explanation of the pathophysiology of adverse neurodevelopmental outcomes in iron deficiency. *Rev Neurosci* **26**, 479–488.
- Lukowski AF, Koss M, Burden MJ, *et al.* (2010) Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. *Nutr Neurosci* **13**, 54–70.
- Lozoff B, Beard J, Connor J, *et al.* (2006) Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev* **64**, S34–S43; discussion S72–S91.
- Jauregui-Lobera I (2014) Iron deficiency and cognitive functions. *Neuropsychiatr Dis Treat* **10**, 2087–2095.
- Bruner AB, Joffe A, Duggan AK, *et al.* (1996) Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* **348**, 4.
- Abdullah K, Kendzerska T, Shah P, *et al.* (2013) Efficacy of oral iron in improving the developmental outcome of pre-school children with non-anaemic iron deficiency: a systematic review. *Public Health Nutr* **16**, 1497–1506.
- Jonker FA & Hensbroek BM (2014) Anaemia, iron deficiency and susceptibility to infections. *J Infect* **69**, Suppl. 1, S23–S27.
- Beard JL (2001) Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* **131**, 120.
- Michaelsen KF, Milman N & Samuelson G (1995) A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. *Acta Paediatr* **84**, 1035–1044.
- Domellof M, Braegger C, Campoy C, *et al.* (2014) Iron requirements of infants and toddlers. *J Pediatr Gastroenterol Nutr* **58**, 119–129.
- Gondolf UH, Tetens I, Michaelsen KF, *et al.* (2013) Iron supplementation is positively associated with increased serum ferritin levels in 9-month-old Danish infants. *Br J Nutr* **109**, 103–110.
- Oliveira Fde C, Assis KF, Martins MC, *et al.* (2014) Timing of clamping and factors associated with iron stores in full-term newborns. *Rev Saude Publica* **48**, 10–18.
- Mastroeni SS, Okada IA, Rondo PH, *et al.* (2006) Concentrations of Fe, K, Na, Ca, P, Zn and Mg in maternal colostrum and mature milk. *J Trop Pediatr* **52**, 272–275.
- Emmett PM & Rogers IS (1997) Properties of human milk and their relationship with maternal nutrition. *Early Hum Dev* **49**, Suppl., S7–S28.
- Shashiraj, Faridi MM, Singh O, *et al.* (2006) Mother's iron status, breastmilk iron and lactoferrin – are they related? *Eur J Clin Nutr* **60**, 903–908.
- Teucher B, Olivares M & Cori H (2004) Enhancers of iron absorption: ascorbic acid and other organic acids. *Int J Vitam Nutr Res* **74**, 403–419.
- Hallberg L, Rossander-Hultén L, Brune M, *et al.* (1992) Bioavailability in man of iron in human milk and cow's milk in relation to their calcium contents. *Pediatr Res* **31**, 524–527.
- The Danish Health Authority (2015) 03/17/15–04:31: New advice on infant food. <http://danish228.rssing.com/channel-26035462/latest.php?q=new+advice+on+infant+food&site=rssing.com&styp=rssing> (accessed August 2019).
- Hörnell A, Lagström H, Lande B, *et al.* (2013) Breastfeeding, introduction of other foods and effects on health: a systematic literature review for the 5th Nordic Nutrition Recommendations. *Food Nutr Res* **57**, 10.3402/fnr.v57i0.20823.
- Bruun S, Buhl S, Husby S, *et al.* (2017) Breastfeeding, infant formula, and introduction to complementary foods-comparing data obtained by questionnaires and health visitors' reports to weekly short message service text messages. *Breastfeed Med* **12**, 554–560. (Epublication ahead of print version 23 August 2017).
- Kyhl HB, Jensen TK, Barington T, *et al.* (2015) The Odense Child Cohort: aims, design, and cohort profile. *Paediatr Perinat Epidemiol* **29**, 250–258.
- Olsen J, Melbye M, Olsen SF, *et al.* (2001) The Danish National Birth Cohort – its background, structure and aim. *Scand J Public Health* **29**, 300–307.
- WHO Multicentre Growth Reference Study Group (2006) WHO Child Growth Standards based on length/length, weight and age. *Acta Paediatr Suppl.* **450**, 76–85.
- Levin C, Harpaz S, Muklashi I, *et al.* (2016) Iron deficiency and iron-deficiency anemia in toddlers ages 18 to 36 months: a prospective study. *J Pediatr Hematol Oncol* **38**, 205–209.



28. Edena AN & Sandoval C (2012) Iron deficiency in infants and toddlers in the United States. *Pediatr Hematol Oncol* **29**, 5.
29. Dewey KG, Domellof M, Cohen RJ, *et al.* (2002) Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. *J Nutr* **132**, 3249–3255.
30. Bharati S, Pal M, Chakrabarty S, *et al.* (2015) Socioeconomic determinants of iron-deficiency anemia among children aged 6 to 59 months in India. *Asia Pac J Public Health* **27**, 11.
31. Henriksen LO, Faber NR, Moller MF, *et al.* (2014) Stability of 35 biochemical and immunological routine tests after 10 hours storage and transport of human whole blood at 21 degrees C. *Scand J Clin Lab Invest* **74**, 603–610.