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Holmlund-Suila, Elisa M.

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Original article

Iron status in early childhood is modified by diet, sex and growth: Secondary analysis of a randomized controlled vitamin D trial

Elisa M. Holmlund-Suila [a, b, *, 1], Helena H. Hauta-alus [a, b, c, d, 1], Maria Enlund-Cerullo [a, b, e], Jenni Rosendahl [a, b], Saara M. Valkama [a, b], Sture Andersson [a], Outi Mäkitie [a, b, e, f]

[a] Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Biomedicum 2 C, P.O. Box 705, 00020 HUS, Helsinki, Finland
[b] Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Biomedicum 1, P.O. Box 63, 00014, Helsinki, Finland
[c] Finnish Institute for Health and Welfare (THL), Population Health Unit, P.O. Box 30, FI-00271, Helsinki, Finland
[d] PEDEGO Research Unit, Oulu University Hospital and University of Oulu, P.O. Box 8000, FI-90014, Oulu, Finland
[e] Folkhälsan Institute of Genetics, Helsinki, Finland
[f] Department of Molecular Medicine and Surgery, Karolinska Institutet, and Clinical Genetics, Karolinska University Laboratory, Karolinska University Hospital, SE-17176, Stockholm, Sweden

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SUMMARY

Background & aims: During early childhood the risk of iron deficiency (ID) is high. Serum ferritin serves as a marker of iron status. We explored prevalence of ID and iron deficiency anemia (IDA), and identified determinants of iron status in infants and toddlers.

Methods: We performed a secondary analysis of the Vitamin D intervention in infants (VIDI) study in Finnish healthy term infants. According to study protocol, at 12- and 24-months of age iron status, growth and dietary intakes were evaluated. ID was defined as serum ferritin <10 μg/L and IDA as serum ferritin <10 μg/L and Hb <112 g/L. For the present study, altogether 766 children provided data (N = 498 infants at 12 months, N = 508 toddlers at 24 months).

Results: ID prevalence increased from 14% in infants to 20% in toddlers. IDA prevalence was 3% at both time points. In infants, ID and IDA were more common in boys than in girls (19% vs. 9%, p = 0.001 and 5% vs. 1%, p = 0.039) but no sex-difference in toddlers was observed. Of infants, 30% had daily iron intake below average requirement of 5 mg/day. Higher daily iron intake per body weight (mg/kg) independently associated with higher infant serum ferritin (B (95% CI) 0.30 (0.04, 0.56), p = 0.026). Correlation between iron intake and ferritin was stronger in infants with ID than in infants without ID. Breastfeeding was more common (63% vs. 35%, p < 0.001) among ID infants than in infants without ID. In toddlers, frequent consumption of milk products independently associated with lower ferritin (B (95% CI) −0.03 (−0.05, −0.01), p = 0.001). Consumption of meat and fish associated with better iron status. Serum ferritin at both time points associated with duration of gestation and growth. The association of growth and ferritin was age-dependent in boys, while in girls, faster growth associated consistently with lower ferritin.

Conclusions: In Northern European healthy infants and toddlers ID is common. The intake of iron remains below recommendations and food consumption and iron intake associate with iron status. Further studies are warranted to assess significance of ID on child development and clinical health outcomes. The project protocol is registered at ClinicalTrials.gov: NCT01723852.

Abbreviations: ID, iron deficiency; IDA, iron deficiency anemia; hs-CRP, high-sensitivity C-reactive protein; FFQ, food frequency questionnaire; AR, average requirement; SDS, standard deviation score; MCV, mean corpuscular volume.

* Corresponding author. Children's Hospital, Helsinki University Hospital, 00290, Helsinki, Finland.
E-mail addresses: elisa.holmlund-suila@hus.fi (E.M. Holmlund-Suila), helena.hauta-alus@helsinki.fi (H.H. Hauta-alus), maria.enlund@helsinki.fi (M. Enlund-Cerullo), jenni.rosendahl@hus.fi (J. Rosendahl), saara.valkama@hus.fi (S.M. Valkama), sture.andersson@hus.fi (S. Andersson), outi.makitie@helsinki.fi (O. Mäkitie).
[1] Equal contribution.

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1. Introduction

Iron is an essential micronutrient for many physiological functions such as red blood cell development, immune responses, brain development and energy metabolism. During infancy, iron requirements are high due to rapid growth, and in this age group iron deficiency (ID) is a major health concern worldwide. Iron deficiency may eventually lead to anemia (IDA), which is a severe form of ID. According to WHO estimates, the global prevalence of anemia in children younger than 5 years is ca. 47%, with ID described as the most common cause [1]. Based on studies conducted mostly in low- or middle-income settings, IDA associates with impaired neurocognitive and behavioral development [2,3]. The health consequences of ID without anemia are less well studied. However, ID alone may have irreversible adverse impact on neurodevelopment [3].

In term infants, iron stores are mainly built up during the third trimester of gestation and are usually sufficient for the first 6 months of life [4]. After that, infants require complementary foods that are rich in iron to maintain sufficient iron status [5]. This is especially true for breastfed infants since development of ID associates with the type of milk the child is consuming(4,6).

Serum ferritin, an iron storage protein, serves as a marker of iron status [7]. Currently, WHO recommends serum ferritin <12 μg/L as a cut-off value for ID in apparently healthy children younger than 5 years [8]. However, this cut-off value for ferritin is not based on clinical outcomes and may not be accurate for children during rapid growth, especially in infants [9,10]. Therefore, a lower cut-off value of 10 μg/L has been suggested in small children to define ID [4,11].

Being an acute-phase protein, ferritin concentration is susceptible to confounding factors e.g. inflammation and liver disease [12]. A concurrent measurement of inflammation, for example C-reactive protein (CRP), is essential when interpreting ferritin concentration as a marker of iron status [13,14].

Vitamin D Intervention in Infants (VIDI) study is a randomized controlled trial in northern Europe of healthy term-born children with normal birth weights. As a part of the VIDI study, our primary aim was to describe the prevalence of ID (serum ferritin < 10 μg/L) and IDA (serum ferritin < 10 μg/L and Hb < 112 g/L), and further examine dietary and other determinants of iron status in children aged 12 and 24 months.

2. Materials and methods

2.1. Participants and follow-up

VIDI study, a randomized, double-blind trial, was conducted in Helsinki, Finland between January 2013 and June 2016. The study comprised 987 healthy infants born at term (37 weeks and 0 days to 42 weeks and 0 days of gestation) with birth weight appropriate for gestational age. Infants were born to mothers of Northern European origin, and were randomized to receive vitamin D_3 supplementation of 10 μg (Group 10) or 30 μg (Group 30) daily doses from age 2 weeks to 24 months. The primary outcomes were bone health and incidence of infections at 24 months. The detailed study protocol and results of primary outcomes are previously reported [15,16].

The current study is a secondary analysis of iron status in the participants at 12 and 24 months of age. The study cohort includes children from both vitamin D intervention groups with available data on ferritin at 12 or 24 months and without evidence of concomitant inflammation, as determined by high-sensitivity CRP concentrations [10,17] (hs-CRP) below 1 μg/mL at 12 (N = 498) or at 24 months (N = 508) (Fig. 1). Full dietary data with iron status were available for 423 subjects at 12 months and 490 at 24 months of age (Fig. 1). Total dataset including ferritin, hs-CRP and nutritional data at 12 and 24 months were available for 266 subjects; at both time points 15 children had ID and 3 children IDA. We collected prospective data on growth parameters at birth, at 6, 12 and 24 months, and venous blood samples at 12 and 24 months.

2.2. Research ethics

Written informed consent was obtained from the parents at recruitment. The study was conducted according to the guidelines laid down in the Helsinki Declaration of 1975 as revised in 1983. The Research Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the study (107/13/03/03/2012). It was carried out with the permission of the Children's Hospital, Helsinki. The trial is registered in ClinicalTrials.gov (NCT01723852).

2.3. Biochemical analyses

Ferritin, iron, transferrin saturation, hemoglobin (Hb) and mean corpuscular volume (MCV) were analyzed from blood samples at the accredited Central Laboratory of Helsinki University Hospital (HUSLAB) using standard methodology. The laboratory-provided reference intervals were: 6–60 μg/L for ferritin, 7–28 μmol/L for iron, 12–43% for transferrin saturation (3.825 × plasma iron (μmol/L)/plasma transferrin (g/L)), 112–142 g/L for Hb and 72–85 fl for MCV. Serum 25-hydroxyvitamin D (25-OHD) was analyzed with automated IDS-iSYS immunoassay with chemiluminescence detection (Immunodiagnostic System Ltd., Bolton, UK) and hs-CRP with an enzyme-linked immunoassay (IBL international CRP high-sensitivity ELISA) in the laboratory of the Pediatric Research Center, University Helsinki according to the manufacturer's instructions. The detection limit for the hs-CRP assay was 0.02 μg/mL.

2.4. Nutritional data

Dietary intake and food consumption were collected via 3-day food record at 12 months, and at 24 months with a 47-item food frequency questionnaire (FFQ). We have previously reported in detail the used food record [18]. Shortly, food records at 12 months were filled-in by parents or daycare personnel, between January 2014 and June 2015, according to given oral and written instructions. The frequency, but not the volume, of breastmilk intake was recorded. Food records were instructed to consecutively include two weekdays and one weekend day. Food and nutrient intakes were processed with AivoDiet software (version 2.0.2.3, Aivo Oy, Turku, Finland), which utilizes Fineli Food Composition Database (version Fine68, National Institute for Health and Welfare, Helsinki, Finland, 2016). Dietary intake was calculated as a daily mean or median intake from the total food record period. Nutrient intakes chosen for analysis (energy, iron, vitamin C and calcium) were chosen on the basis of literature.

At age 24 months, we used a validated FFQ with 47 food items or food groups to measure food consumption during the previous week at home or daycare [19]. Food consumption during daycare was collected with a 2-day pre-coded food record (included pre-coded meal types but not food items) without food amounts. These data were transcribed by a trained research nurse into food frequencies and checked by another research assistant according to the FFQ food group categorizations. FFQs and pre-coded food records were filled-in between November 2014 and July 2016. Food frequencies from FFQ and food records were summed and expressed as daily means. Data on food allergies and special diets were obtained from modified International Study of Asthma and Allergies in Childhood questionnaire [20,21] and from dietary questionnaire incorporated in the FFQ. Prevalence of food allergies was in line with previous Finnish studies (e.g. Ref. [22]). We defined

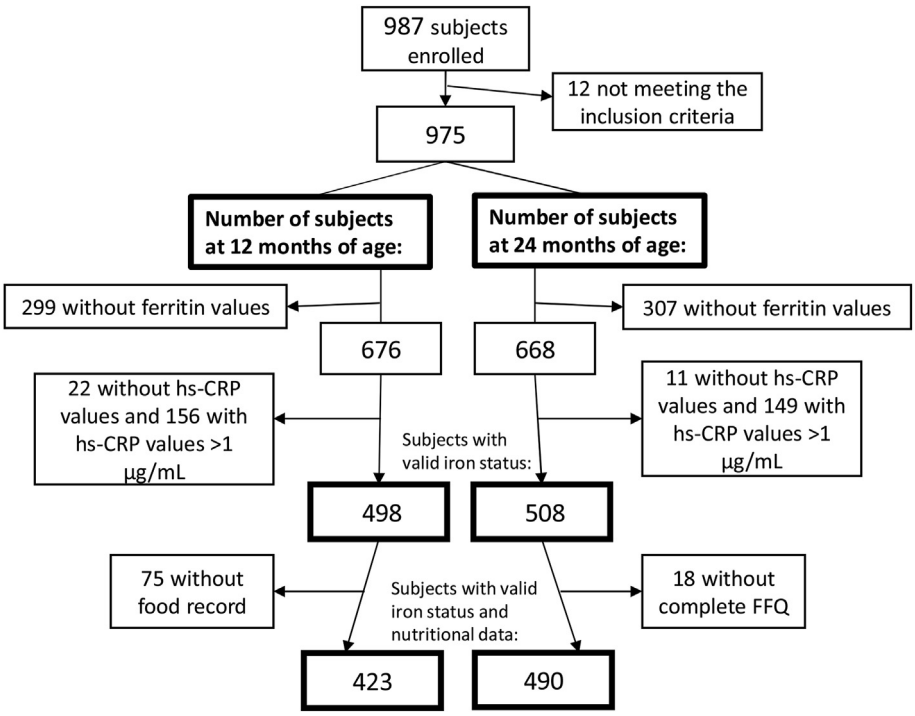

Fig. 1. Flowchart of the study participants.

adequate iron intake as 5 mg per day for 1–3 years old children, which is the average requirement (AR) of iron according to European Food Safety Authority [23].

We collected information on breastfeeding status (yes or no) prospectively with questionnaires until 24 months of age (duration of breastfeeding), and via food record at the age of 12 months. Duration of exclusive breastfeeding or the volume of breast milk ingested was not collected. At 12 months of age, all infants who received breastmilk consumed complementary foods as well [18].

2.5. Growth parameters

Birth measurements were collected from birth records, and transformed to parity-, gestational age- and sex-specific standard deviation scores (SDS) based on national reference data [24]. Previously described in detail, child weight (kg), length (cm) and head circumference (cm) were measured at 6-, 12- and 24-months' follow-up visits [25]. Weight, length, length-adjusted weight and head circumference were expressed as SDS using age- and sex-specific Finnish national references [24].

3. Statistics

We present the data as means and standard deviations (SD) or medians and interquartile ranges (IQR). The normality of the variables was visually inspected. For non-normal distributed variables logarithmic transformation was conducted when applicable. We used independent samples *t* test or Mann–Whitney *U* test to examine differences between groups. Pearson Chi-Square test was applied for categorical variables and Pearson correlation coefficients were reported.

We determined growth in weight and length, i.e. growth rate, from 6 to 12 months and from 12 to 24 months by using the standardized residuals from linear regression models indicating difference between body size SDS at 12 and 24 months compared with the predicted body size SDS based on previous growth [26]. Energy-adjustment for nutrient intakes was conducted with the residual method [27]. All dietary factors and birth parameters served as predictors and iron status as the outcome. In regards of growth, a possibility prevail that poor iron status cause stunted growth, and on the other hand, rapid growth may exhaust iron stores leading to poor iron status.

The independent determinants of serum ferritin were examined by multivariate linear regression models. Statistically significant determinants from univariate analyses served as explanatory variables in adjusted multivariate models. To avoid multicollinearity issues, the strongest variable of dietary iron intake estimation was chosen for the multivariate model. A p value of <0.05 was considered statistically significant. We used IBM SPSS Statistics 25 and 27 (IBM, Armonk, NY).

4. Results

We present subject and family characteristics in Table 1. According to the study protocol, all children included in the study were term and with normal birth weight. The average duration of breastfeeding was 11 months. Of toddlers, 7% had elimination diet due to food allergy, and 16% used dietary supplements (other than vitamin D) (Table 1). Special diets or supplement use had no relation with iron status. Parental BMI, level of education or lifestyle factors, parity, pre-eclampsia, mode of delivery or birth weight did not associate with ferritin (data not shown). Vitamin D intervention group or 25-OHD concentration did not associate with ferritin (Table 5) or ID (Supplemental Table 1).

4.1. Growth and biochemical variables

Anthropometrics and iron status markers according to sex are shown in Table 2. Ferritin concentrations ranged from 3 to 138 µg/L at 12 months and from 3 to 76 µg/L at 24 months. Boys had lower ferritin concentration than girls at both time points. At 24 months, girls presented with higher serum iron, transferrin saturation and

Table 1
Background characteristics.

	N	Mean ± SD
Family characteristics		
Maternal age, year	766	31.6 ± 4.3
Paternal age, year	755	33.4 ± 5.4
Maternal prepregnancy BMI	762	23.2 ± 3.6
Paternal BMI	742	25.8 ± 3.3
Maternal smoking, yes, %[a]	123/759	16.2
Paternal smoking, yes, %[a]	190/753	25.2
Maternal education[b]	758	5.0 ± 1.4
Paternal education[b]	750	4.5 ± 1.6
Parity, primipara, %	764	63.7
Duration of pregnancy, week	766	40.2 ± 1.1
Birth anthropometrics		
Length, cm	766	50.3 ± 1.8
Length, SDS	766	−0.13 ± 0.92
Weight, Kg	766	3.53 ± 0.39
Weight, SDS	766	−0.16 ± 0.82
Children's dietary characteristics		
Duration of breastfeeding, months	486	11.2 ± 5.7
Breastfed >6 months, %	400/486	82.3
Grain allergy, %[c]	7/490	1.4
Cow's milk allergy, %[c]	25/490	5.1
At 12 months of age[d]		
Daily frequency of breastfeeding among breastfed	165/423	4.1 ± 2.3
Dietary supplements other than vitamin D, %	45/423	10.6
Probiotics	40/423	9.5
Iron	1/423	0.2
Calcium	3/423	0.7
At 24 months of age		
Special diet, %	61/484	12.6
Food allergy diet	34/484	7.1
Vegetarian diet[e]	13/480	2.7
Dietary supplements other than vitamin D, %	76/490	15.5
Probiotics	49/490	10.0
Multivitamin or iron	11/490	2.2
Calcium	10/490	2.0

SDS, standard deviation score.
[a] Combined baseline and current smoking status.
[b] Education scale from 1 = lower secondary education to 6 = first or second stage of tertiary education.
[c] Physician-diagnosed allergy between 0 and 2 years.
[d] Data from 3-day food record.
[e] Self-reported, including all types of vegetarian diets.

MCV than boys. The observed sex-related difference in serum ferritin was not explained by more rapid growth in boys, as the results remained unchanged after adjustment with growth in length.

4.2. Iron deficiency

The prevalence of ID was 14% and 20% at age 12 and 24 months, respectively (Table 2). IDA was observed in 3% of children at both time-points. The prevalence of ID and IDA was higher in boys than in girls at 12 months but similar between sexes at 24 months. Compared with non-ID, boys with ID had slower growth in length from 6 to 12 months and faster growth in length from 12 to 24 months (Supplemental Table 1). In girls, however, ID associated with faster growth in length at both time periods, although with border-line significance at 12–24 months (Supplemental Table 1). All iron status-related markers (serum iron, transferrin saturation, Hb and MCV) were lower in ID vs. non-ID, and IDA vs. non-IDA groups (Supplemental Table 1).

4.3. Dietary factors and iron status

Daily dietary intake of iron was on average (±SD) 6.3 (±2.1) mg with 5th percentile of 2.9 mg and 95th percentile of 9.6 mg. In 30% of infants iron intake remained below the AR (5 mg/day) (Table 3). AR was exceeded by fewer infants with ID (53%) than non-ID (73%). Breastfeeding associated with higher prevalence of ID and IDA. Absolute intakes of iron, vitamin C and calcium were lower in ID and IDA infants compared with non-ID and non-IDA, but these differences attenuated after energy-adjustments (Table 3). Iron intake per body weight in kg was lower in both ID and IDA infants than in non-ID and non-IDA. Associations between iron intake and iron status attenuated after stratification by breastfeeding status (data not shown).

While iron intake (mg/d) had no correlation with serum ferritin or transferrin saturation (p for both >0.07), iron intake per body weight correlated with both ($r = 0.16$, $p = 0.010$ for ferritin and $r = 0.10$, $p = 0.038$ for transferrin saturation). Neither iron intake nor iron intake per body weight correlated with serum iron (p for both >0.07). Iron intake correlated with Hb ($r = 0.13$, $p = 0.010$) and MCV ($r = 0.14$, $p = 0.006$), and iron intake per body weight correlated with MCV ($r = 0.19$, $p < 0.001$) but not with Hb ($p > 0.16$). Figure 2 shows that iron intake per body weight correlated more strongly with ferritin in infants with ID and less so in non-ID infants. Similarly, iron intake (mg/d) correlated with ferritin in ID infants ($r = 0.35$, $p = 0.006$) but not in non-ID infants ($r = 0.03$, $p = 0.63$).

Iron status at 12 months varied with food consumption (Table 3). IDA infants consumed less milk products (infant formula excluded) than non-IDA. Similarly, compared with non-ID infants, ID infants consumed less infant formula. Further, ID infants ate less meat and fish dishes than non-ID, and a corresponding trend was observed between IDA and non-IDA infants. Stratification by breastfeeding status confirmed the associations between IDA and milk products (infant formula excluded) in breastfed infants, and between ID and meat dishes in non-breastfed infants (data not shown).

Almost half (48%, n = 234/490) of toddlers ate at daycare during the data collection period. Breastfeeding was rare (6%) at 24 months of age with no relation with iron status (Table 4). ID toddlers consumed less frequently meat and fish, including red meat, than non-ID. IDA toddlers ate fruits and berries less frequently than non-IDA.

4.4. Independent determinants of serum ferritin concentration at 12 and 24 months

At 12 months of age breastfeeding associated with lower ferritin (Table 5), and was the strongest modifying factor of ferritin. At 24 months more frequent use of milk products associated independently with lower serum ferritin, while meat and fish foods associated with higher serum ferritin (Table 5). We observed that, at both time points, female sex, and longer duration of gestation independently associated with higher ferritin while faster growth associated with lower ferritin.

5. Discussion

In this cohort of healthy term children not at known risk for ID, we observed that 14% of infants at 12 months and 20% of toddlers at 24 months were iron deficient. However, only 3% of the children had IDA. At 12 months, ID was more common in boys than in girls. Almost one third of the infants did not meet the AR of iron (5 mg per day) from food. Higher iron intake per body weight associated with better iron status. Breastfeeding was more common in infants with ID or IDA compared with infants with normal iron status. Consumption of milk products without infant formula was less in IDA infants compared with those without IDA. Equivalently, ID infants consumed less infant formula than infants with normal iron

Table 2
Anthropometric data and biochemical indicators of iron status and comparison between sexes.

	All	Boys	Girls	p value[a]
12 months				
N	498	245	253	
Anthropometric indicators of growth	Mean ± SD			
Length, cm^2	75.2 ± 2.5	76.1 ± 2.4	74.4 ± 2.4	<0.001
Length, SDS[b]	−0.57 ± 1.0	−0.54 ± 1.0	−0.61 ± 1.0	0.422
Growth in length, SD unit[b,c]	−0.06 ± 1.0	0.06 ± 1.0	−0.17 ± 1.1	0.015
Weight, Kg	9.7 ± 1.1	10.2 ± 1.1	9.3 ± 1.0	<0.001
Length-adjusted weight, SDS[b]	−0.02 ± 1.0	0.08 ± 1.0	−0.11 ± 1.0	0.040
Growth in weight, SD unit[c]	−0.04 ± 1.0	−0.01 ± 1.1	−0.07 ± 0.9	0.495
Biochemical indicators of iron status				
Ferritin, μg/L [median (IQR; 25%–75%)]	20.0 (12.0–30.0)	16.0 (11.0–25.5)	23.0 (14.0–34.0)	<0.001[d]
Fe, μmol/L[b]	12.3 ± 4.5	12.3 ± 4.4	12.4 ± 4.5	0.788
Transferrin saturation, %[b]	16.7 ± 6.4	16.3 ± 6.1	17.1 ± 6.6	0.141
Hb, g/L[e]	119 ± 8	120 ± 8	118 ± 7	0.093
MCV, fl[e]	77 ± 3	77 ± 3	77 ± 3	0.027
hs-CRP, μg/mL [median (IQR; 25%–75%)]	0.13 (0.07–0.27)	0.11 (0.06–0.24)	0.14 (0.07–0.32)	0.014[d]
Prevalence of ID[f] % (N)	13.7 (68)	18.8 (46)	8.7 (22)	0.001[g]
Prevalence of IDA[f] % (N)	2.9 (13)[h]	4.7 (10)[h]	1.3 (3)[h]	0.039[g]
24 months				
N	508	247	261	
Anthropometric indicators of growth	Mean ± SD			
Length, cm	87.7 ± 3.0	88.5 ± 2.8	86.9 ± 2.8	<0.001
Length, SDS	−0.27 ± 0.98	−0.23 ± 0.98	−0.30 ± 1.0	0.454
Growth in length, SD unit[i]	0.01 ± 1.0	−0.05 ± 0.9	0.06 ± 1.0	0.201
Weight, Kg[j]	12.5 ± 1.3	12.9 ± 1.3	12.1 ± 1.3	<0.001
Length-adjusted weight, SDS[j]	−0.09 ± 0.97	−0.02 ± 0.97	−0.16 ± 0.97	0.099
Growth in weight, SD unit[i,j]	0.01 ± 1.0	−0.07 ± 0.9	0.09 ± 1.0	0.059
Biochemical indicators of iron status				
Ferritin, μg/L [median (IQR; 25%–75%)]	16.0 (11.0–22.8)	14.0 (10.0–21.0)	17.0 (11.0–24.0)	0.011[d]
Fe, μmol/L	14.2 ± 5.0	13.6 ± 4.8	14.7 ± 5.1	0.019
Transferrin saturation, %	18.6 ± 7.1	17.5 ± 6.5	19.6 ± 7.4	0.001
Hb, g/L[k]	124 ± 8	124 ± 8	123 ± 8	0.714
MCV, fl[k]	77 ± 3	77 ± 3	78 ± 3	0.001
hs-CRP, μg/mL [median (IQR; 25%–75%)]	0.20 (0.09–0.38)	0.18 (0.09–0.33)	0.22 (0.10–0.43)	0.034[d]
Prevalence of ID[f] % (N)[f]	20.3 (103)	22.3 (55)	18.4 (48)	0.277[g]
Prevalence of IDA[f] % (N)[f]	3.2 (15)[l]	3.6 (8)[l]	2.8 (7)[l]	0.626[g]

SDS, standard deviation score; Fe, iron; MCV, mean corpuscular volume; hs-CRP, high-sensitivity C-reactive protein.
[a] Based on Independent samples *t*-test.
[b] Number of missing subject: N = 1.
[c] Growth from 6 to 12 months.
[d] Tested after logarithmic transformation, means (SDs) back transformed from logarithmic values.
[e] Number of missing subjects: N = 57, MCV mean lower in boys.
[f] ID defined as ferritin < 10 μg/L, IDA defined as ferritin < 10 μg/L and Hb < 112 g/L.
[g] Pearson Chi-Square.
[h] For boys N = 215 and for girls N = 226.
[i] Growth from 12 to 24 months.
[j] Number of missing subjects: N = 1.
[k] Number of missing subjects: N = 33.
[l] For boys N = 224 and for girls N = 251.

status. However, at 24 months frequent consumption of milk products associated with lower serum ferritin concentrations. Both non-ID infants and toddlers ate more meat and fish dishes than those with ID. In addition to sex and diet, gestational age and growth was related to ferritin.

Previously reported prevalences and definitions of ID in 12–24 month-old infants in Europe and USA show great variation and complicate comparisons. The prevalences range from 2 to 85% [6,28–30]. The definitions of ID vary from total body iron [31] to serum ferritin less than 10 or 12 μg/L up to 16 μg/L. A previous Finnish study on children aged 6–12 months, using serum ferritin <12 μg/L as a cut-off, showed 5% prevalence of ID [32]. In the current cohort, despite using a low cut-off value for ferritin (<10 μg/L), we observed notably higher prevalence of ID which increased from 14% in infants to 20% in toddlers. In contrast, the prevalence of IDA, 3% in infants and toddlers, was similar to previous studies in Europe [28].

Observation that longer duration of gestation associated with higher ferritin up to first 2 years of life, highlights the importance of iron accrual until birth. In Finland, iron supplementation in infants is mainly initiated based on birth weight (less than 2.5 kg). Whether this current policy is sufficient in late preterm infants but with a birth weight >2.5 kg could be re-evaluated. On the other hand, possible health risks related to too liberal iron supplementation have to be carefully assessed [5,33].

Our observation of lower ferritin in boys than in girls is in line with previous reports on sex-differences in infants [10,34]. Yet, association between growth and iron status has differed between previous studies [29,35]. We observed that association of growth and iron status in boys varied from infants to toddlers: in infancy, boys with ID had slower growth in length from 6 to 12 months compared with non-ID, while in toddlers the association was the opposite. Whether ID itself resulted in slower growth in infant boys requires further studies. Sex steroids were not studied, and their impact on our findings could provide more information.

Although iron from breastmilk is efficiently absorbed and breastmilk enhances the absorption of iron from other foods [36], exclusive breastfeeding for more than 4–6 months results in

Table 3
Breastfeeding and nutrient and food intake at 12 months of age according to iron deficiency (ID) and iron deficiency anemia (IDA) status. The volume and nutrient content of breastmilk is not included.

	All	ID	Non-ID	p value[a]	IDA	Non-IDA	p value[a]
N	423	60	363		11	363	
Breastfed at 12 months, % (n)	39 (165)	63 (38)	35 (127)	**<0.001**	73 (8)	39 (140)	**0.022**
Meets average requirement[b] of iron ≥5 mg, % (n)	70 (295)	53 (32)	73 (263)	**0.003**	46 (5)	70 (254)	0.083
Daily nutrient intake from food	Mean ± SD				Mean ± SD		
Energy, MJ	3.3 ± 0.9	2.9 ± 1.0	3.4 ± 0.9	**<0.001**	2.5 ± 1.1	3.3 ± 0.9	**0.002**
Energy, kcal	790 ± 214	701 ± 232	805 ± 208		593 ± 271	794 ± 213	
Iron, mg	6.3 ± 2.1	5.5 ± 2.2	6.4 ± 2.1	**0.002**	4.7 ± 2.3	6.3 ± 2.1	**0.013**
				0.37[c]			0.59[c]
Iron intake per weight, mg/kg	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	**0.001**	0.5 ± 0.2	0.7 ± 0.2	**0.021**
Vitamin C, mg	87 ± 51	76 ± 42	89 ± 52	0.066	57 ± 34	86 ± 49	0.054
				0.65[c]			0.24[c]
Calcium, mg	595 ± 310	503 ± 349	610 ± 300	**0.013**	373 ± 288	601 ± 311	**0.017**
				0.37[c]			0.63[c]
Daily food intake, g	Md (IQR)				Md (IQR)		
Vegetable foods, incl. fresh and cooked dishes	60 (90)	74 (100)	56 (90)	0.075	50 (59)	63 (94)	0.91
Fruit and berry foods	157 (108)	166 (100)	156 (113)	0.88	113 (92)	154 (108)	0.54
Cereal foods, excl. porridges	22 (34)	25 (35)	21 (34)	0.39	24 (21)	21 (36)	0.70
Porridges	243 (183)	226 (215)	245 (178)	0.36	183 (175)	249 (185)	0.16
Milk products, excl. infant formula[d]	178 (322)]	144 (229)	190 (338)	0.068	50 (136)	180 (309)	**0.014**
Infant formula	0 (217)	0 (0)	0 (252)	**0.004**[e]	0 (270)	0 (200)	0.79
Meat and fish dishes	154 (133)	114 (137)	164 (134)	**0.031**	97 (113)	161 (134)	0.054
Red meat dishes	89 (111)	78 (105)	92 (113)	0.065	72 (102)	88 (112)	0.18
Sugary products, inc. biscuits, cakes, chocolate, candy, soda	0 (3)	0 (3)	0 (3)	0.97	0 (0)	0 (3)	0.28

ID: defined as ferritin below 10 μg/L; IDA: defined as ferritin below 10 μg/L and Hb below 112 g/L.
[a] Independent samples *t*-test, Mann–Whitney *U*-test and Chi-Square test applied as appropriate. P values < 0.05 highlighted in bold.
[b] http://www.efsa.europa.eu/efsajournal.
[c] Energy-adjusted p value.
[d] Include dairy and plant-based products.
[e] Mean ranks are less in ID subjects compared with non-ID subjects.

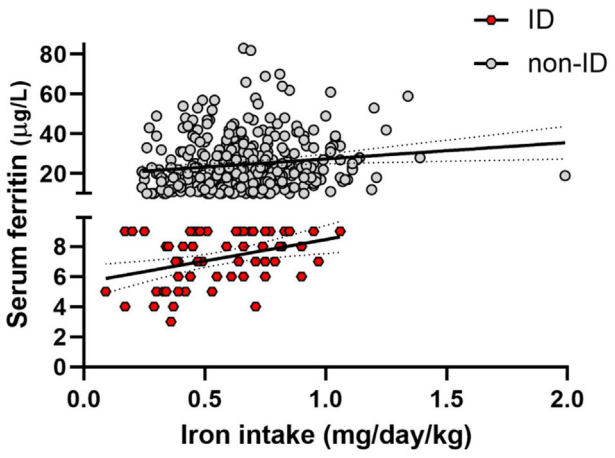

Fig. 2. Relation between daily iron intake per body weight (mg/day/kg) and serum ferritin concentration in iron deficient (ID) infants (r = 0.37, p = 0.004, n = 59) (red hexagonal), and in non-ID infants (r = 0.11, p = 0.041, n = 363) (gray circle). Line represents simple linear regression with 95% confidence bands of the best-fit line.

increased risk of ID [28,30]. The Committee of Nutrition of the American Academy of Pediatrics (AAP) recommended that those infants who are exclusively breastfed more than 4 months should receive supplemental iron 1 mg/kg daily until introduction of iron-containing complementary foods [4]. In contrast, ESPGHAN (the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition) Committee on Nutrition concluded that evidence is insufficient to support general iron supplementation of healthy infants and toddlers of normal birth weight [5]. We observed that breastfeeding at 12 months associated with ID and IDA. We have previously reported that compared with non-breastfed infants, breastfed infants in this cohort ate less those foods which were the main sources of iron [18]. Our and previous observations emphasize the importance of introducing iron-containing complementary foods at age 4–6 months, increasing the portion sizes and diversifying the diet sufficiently, especially in breastfed infants. In addition, the use of iron supplementation needs to be considered individually to ensure sufficient iron intake.

Inadequate iron intake in European children less than 36 months ranges from about 10 to 50% [37]. In our cohort, 30% of infants did not reach the AR of iron (5 mg/d). Iron intake was lower in ID and IDA infants than non-ID and non-IDA, however, this attenuated after correction for energy intake. The weak correlation between iron intake and iron status may be due to poor bioavailability of dietary iron [6,23,30]. Nevertheless, higher iron intake per body weight was an independent determinant of iron status in our study. Stronger correlation between iron intake and ferritin in ID-infants than in non-ID infants indicates dissimilar dietary iron absorption according to iron status with deficient state enhancing absorption in the intestine [38].

We observed that non-ID infants and toddlers consumed more meat and fish foods than children with ID. This is most likely related to high heme iron content in red meat with effective intestinal absorption and the enhancing effect of animal tissue on non-heme iron absorption [23,39]. In addition, other substances such as vitamin C enhance iron absorption. In line with this, we observed that fruit and berry consumption was more common in non-IDA than in IDA toddlers.

In infants, higher intake of infant formula was a protective factor against ID. In toddlers, however, more frequent intake of milk products associated with lower ferritin concentrations, in line with previous studies [40,41]. ESPGHAN Committee states that unmodified cow's milk should be limited to <500 mL/day in toddlers [5], whereas Finnish health authorities recommend ≥400 mL/day of liquid milk products. This generates a quite narrow window for optimal nutritional status in early childhood; ensuring adequate

Table 4
Daily food group frequencies at 24 months of age according to iron deficiency (ID) and iron deficiency anemia (IDA) status.

	All	ID	Non-ID	p value[a]	IDA	Non-IDA	p value[a]
N	490	99	391		14	457	
Breastfed > 23 mo, % (n)	6 (28)	6 (6)	6 (22)	0.87	7 (1)	6 (27)	0.88
	Md (IQR)				Md (IQR)		
Fresh and cooked vegetables, incl. pulse[b]	3.0 (2.0)	3.0 (2.1)	3.0 (1.9)	0.27	3.3 (3.0)	3.1 (1.9)	0.71
Fruits and berries, incl. mass-produced baby foods, smoothies	2.3 (1.4)	2.4 (1.6)	2.3 (1.4)	0.62	1.7 (1.0)	2.4 (1.4)	**0.032**
Cereal foods, incl. bread, rice, pasta, porridge[c]	4.3 (3.0)	4.0 (3.1)	4.3 (2.9)	0.65	4.1 (2.4)	4.3 (2.9)	0.92
Dairy and plant-based products, incl. cow's milk, yoghurt, plant-based milk[c]	6.5 (3.1)	7.0 (2.7)	6.4 (3.3)	0.12	7.5 (4.7)	6.4 (3.2)	0.059
All dairy and plant-based milk drinks[c]	4.6 (2.7)	5.0 (2.6)	4.6 (2.6)	0.26	5.1 (4.6)	4.6 (2.6)	0.41
Meat and fish[d]	2.1 (1.3)	1.9 (1.0)	2.1 (1.1)	**0.010**	2.1 (1.4)	2.1 (1.2)	0.30
Red meat, incl. sausages[e]	0.9 (0.6)	0.9 (0.6)	0.9 (0.6)	**0.033**[f]	0.6 (1.1)	0.9 (0.6)	0.051
Sugary products, incl. biscuits, chocolate, candy, soda[c]	1.0 (1.3)	1.0 (1.3)	1.0 (1.3)	0.73	0.9 (1.5)	1.0 (1.3)	0.97

ID: defined as ferritin below 10 μg/L; IDA: defined as ferritin below 10 μg/L and Hb below 112 g/L.
[a] Kruskal–Wallis test. P values < 0.05 highlighted in bold.
[b] Number of missing subject: N = 1.
[c] Number of missing subjects: N = 2.
[d] Number of missing subjects: N = 25.
[e] Number of missing subjects: N = 4.
[f] Mean ranks are less in ID subjects compared with non-ID subjects.

Table 5
Modifying factors of serum ferritin at 12 and 24 months.

	LnFerritin			p value
	B	95% CI	Beta	
Multivariate model (at 12 months, N = 423)				
Breastfeeding (no vs. yes)	−0.31	−0.44 to −0.18	−0.24	**<0.001**
Sex (boy vs. girl)	0.27	0.16–0.38	0.22	**<0.001**
Duration of gestation (day)	0.01	0.01–0.02	0.16	**<0.001**
Growth in weight (SD unit)	−0.10	−0.16 to −0.03	−0.15	**0.004**
Iron intake per weight in kilograms (mg/kg)	0.33	0.05–0.60	0.12	**0.020**
Meat and fish dishes (g/day)	>0.00	<0.00 to >0.00	0.09	0.059
Growth in length (SD unit)	−0.05	−0.11 to 0.01	−0.08	0.125
Infant formula (g/day)	>0.00	<0.00 to >0.00	0.04	0.393
Multivariate model (at 24 months, N = 464)				
Duration of gestation (day)	0.01	0.01–0.02	0.17	**<0.001**
Meat and fish foods (times/day)	0.09	0.04–0.14	0.18	**<0.001**
Dairy and plant-based products (times/day)	−0.03	−0.05 to −0.01	−0.16	**0.001**
Sex (boy vs. girl)	0.14	0.04–0.24	0.13	**0.005**
Growth in length (SD unit)	−0.06	−0.12 to −0.01	−0.11	**0.026**
Growth in weight (SD unit)	−0.05	−0.11 to 0.01	−0.09	0.0**75**

Results are based on linear regression model, using enter method; dependent variable was ferritin after logarithmic transformation and statistically significant determinants from univariate analyses served as explanatory variables. P values < 0.05 highlighted in bold.
SD, standard deviation; 25-OHD, 25-hydroxyvitamin D.

intake of calcium and iodine may potentially create a challenge for optimal iron absorption in some children.

Our study has several strengths and also some limitations. The VIDI cohort comprises a large and unique population of healthy term infants with high parental socioeconomic status. Approximately half of the original cohort sample were included in the current study, because we included subjects with full data only and excluded those with inflammation, thus possibility for drop-out bias exists. According to study protocol, susceptible infants for ID, such as preterm, low-birth weight or newborns with growth restriction were excluded. A concurrent measurement of soluble transferrin receptor would have validated our definition of ID [4,42], however, we observed other iron status markers to be consistently lower in ID children than non-ID. Although usual intakes of iron were not estimated [43,44] we calculated a mean value of iron intake from the 3-day food record. Iron intake correlated with iron status confirming the validity of our measure. We applied two different dietary assessment methods; food record at 12 months and FFQ at 24 months, thus comparison of food consumption data between 12 and 24 months is limited. Limitations prevail in nutrient intakes in breastfed infants as we lacked information on the amount of breast milk consumed.

6. Conclusions

Iron deficiency, defined as serum ferritin <10 μg/L, is common in healthy term-born infants and toddlers, and the intake of iron remains below recommendations. Intake of iron fortified infant formula at 12 months of age supports sufficient iron status but later in childhood excessive regular milk intake can increase the risk for ID. Consumption of meat and fish foods as well as fruits and berries associate with better iron status. The significance of ID without anemia on clinical health outcomes such as neurodevelopment needs to be explored in future studies.

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Authors' contributions

EHS, HHa, SA and OM designed research; EHS, HHa, MEC, JR and SV conducted the study; EHS and HHa analyzed data and had primary responsibility for final content. All authors contributed to the study, and read and approved the final manuscript.

Conflict of interest

Authors have nothing to declare.

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Appendix A. Supplementary data

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