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Iron status, body size and growth in the first two years of life

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EKM and MEK designed the study and drafted the manuscript. CnÍC carried out data collection.

DMM is the overall principal investigator (PI) of the Cork BASELINE Birth Cohort Study and JOBH,

LCK, ADI and MEK are co-PIs and specialist leads. LCK is the PI of the SCOPE Ireland pregnancy

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1 **ABSTRACT**

2 Rapid growth in infancy has been shown to adversely affect iron status up to one year; the effect of
3 growth on iron status in the second year of life has been largely unexplored. We aimed to investigate
4 the impact of growth and body size in the first two years on iron status at two years. In the
5 prospective, maternal-infant Cork BASELINE Birth Cohort Study, infant weight and length were
6 measured at birth, 2, 6, 12 and 24 months and absolute weight (kg) and length (cm) gain from 0-2, 0-
7 6, 0-12, 6-12, 12-24 and 0-24 months was calculated. At two years ($n=704$), haemoglobin, mean
8 corpuscular volume and serum ferritin (umbilical cord concentrations also) were measured. At two
9 years, 5% had iron deficiency (ferritin $<12\mu\text{g/L}$) and 1% had iron deficiency anaemia (haemoglobin
10 $<110\text{g/L} + \text{ferritin} <12\mu\text{g/L}$). Weight gain from 6-12, 0-24 and 12-24 months were all inversely
11 associated with ferritin concentrations at two years but only the association with weight gain from 12-
12 24 months was robust after adjustment for potential confounders including cord ferritin (adj. estimate
13 [95% CI]: $-4.40 [-8.43, -0.37] \mu\text{g/L}$, $P=0.033$). Length gain from 0-24 months was positively
14 associated with haemoglobin at two years ($0.42 [0.07, 0.76] \text{g/L}$, $P=0.019$), prior to further adjustment
15 for cord ferritin. To conclude, weight gain in the second year was inversely associated with iron
16 stores at two years, even after accounting for iron status at birth. Further examinations of iron
17 requirements, dietary intakes and growth patterns in children in the second year in high-resource
18 settings are warranted.

19 **Keywords** birth cohort, iron status, serum ferritin, weight gain, length gain, body size

20 INTRODUCTION

21 Iron deficiency is the most common micronutrient deficiency worldwide (World Health Organisation,
22 2008). Infants and young children are at particular risk of iron deficiency and subsequent anaemia,
23 with iron requirements per kilogram of body weight higher during this period than any other time of
24 life (Domellof *et al.*, 2014). Globally, it is estimated that 25% of preschool-age children have iron
25 deficiency anaemia (McLean *et al.*, 2009). In Europe, the prevalence of iron deficiency ranges from
26 3-48% and most investigators have reported the prevalence of iron deficiency anaemia below 5%
27 (Eussen *et al.*, 2015). Worryingly, iron deficiency with and without anaemia in early childhood has
28 been shown to have long-term consequences for cognitive, motor and behavioural development
29 (Georgieff, 2011).

30 Term infants are born with iron reserves that can last for about the first six months of life, after which
31 the infant relies heavily on iron intakes to meet the high iron requirements for growth (Agostoni *et al.*,
32 2008). In the first year of life, a number of dietary factors can influence iron status, including the
33 delayed introduction of appropriate complementary foods beyond six months (Chantry *et al.*, 2007,
34 Agostoni *et al.*, 2008, Maguire *et al.*, 2013, Wang *et al.*, 2016). While in the second year,
35 consumption of unmodified cows' milk as a beverage and low consumption of iron-fortified products
36 has been associated with an increased risk of low iron status and iron deficiency (Gunnarsson *et al.*,
37 2004, Uijterschout *et al.*, 2014, McCarthy *et al.*, 2016). The dietary transition, including replacing
38 breast milk or infant formula with unmodified cows' milk as an important beverage, in conjunction
39 with the rapid growth associated with this period, makes the second year of life an especially
40 vulnerable period.

41 Associations between iron status, body size and growth in the first year of life have been well
42 documented. In high-resource settings, birth weight was positively associated with iron status at one
43 year of age (Persson *et al.*, 1998, Sherriff *et al.*, 1999), while weight gain in infancy has been
44 inversely associated with iron status at one year (Morton *et al.*, 1988, Michaelsen *et al.*, 1995,
45 Thorsdottir *et al.*, 2003). However, apart from a small Icelandic study (Gunnarsson *et al.*, 2004), the

46 relationship between iron status, body size and growth in the second year of life in healthy children
47 has been largely unexplored. Therefore, the aim of the current study was to investigate the influence
48 of body size and growth in the first two years of life, with a particular focus on the second year, on
49 iron status at two years in apparently healthy children from a prospective birth cohort in Ireland. As
50 iron status at birth has been shown to track through to early childhood (Georgieff *et al.*, 2002, Hay *et*
51 *al.*, 2007), a secondary, novel aim of this study was to explore the effect of iron status at birth on
52 associations between iron status, body size and growth over the first two years of life.

53 **MATERIALS AND METHODS**

54 **Study design and participants**

55 Participants were recruited from the Cork BASELINE (Babies after SCOPE: Evaluating the
56 Longitudinal Impact using Neurological and Nutritional Endpoints) Birth Cohort Study, which
57 followed infants born to mothers in the SCOPE (Screening for Pregnancy Endpoints) Ireland
58 pregnancy cohort. In SCOPE, low risk, nulliparous women with a singleton pregnancy were recruited
59 before 15 weeks' gestation from Cork University Maternity Hospital, as part of an international
60 multicentre pregnancy cohort study aimed at investigating early indicators of pregnancy
61 complications (Kenny *et al.*, 2014).

62 Written informed consent to the Cork BASELINE Birth Cohort Study was provided by the parents of
63 participants; 1537 infants recruited from the SCOPE study at 15 weeks' gestation and 600 recruited at
64 birth through the postnatal wards of Cork University Maternity Hospital (from 2008 to 2011).

65 Participants were followed prospectively from birth, with assessments at day 2 and at 2, 6, 12 and 24
66 months. Study assessments at five years of age were completed in December 2016. Information was
67 gathered by interviewer-led questionnaires and clinical assessments performed by trained researchers
68 in accordance with the Declaration of Helsinki, with further information on study design and
69 procedures reported previously (O'Donovan *et al.*, 2015). Ethical approval for the Cork BASELINE
70 Birth Cohort Study was granted by the Clinical Research Ethics Committee of the Cork teaching

71 hospitals (ECM 5(9) 01/07/2008) and it is registered at the National Institutes of Health Clinical Trials
72 Registry (www.clinicaltrials.gov NCT01498965).

73 Detailed dietary information was collected for all participants in assessments at age 2, 6 and 12
74 months, including information on early feeding methods and complementary feeding. In this study,
75 predominant breastfeeding refers to breast milk as the main source of nutrition but infants may have
76 received infant formula ‘top-ups’ at some stage (post-delivery awaiting the increase in milk volume or
77 while mothers were on medication). At the 24-month assessment, food and nutrient intake data were
78 collected in the form of a two-day weighed food diary in a subgroup of the cohort. Parents were
79 instructed to record detailed information about the amount and types of all foods, beverages and
80 supplements consumed during the diary period. Consumption data were converted to nutrient intake
81 data using the nutritional analysis software Weighed Intake Software Package WISP© (Tinuviel
82 Software, Anglesey, UK), as previously described (McCarthy *et al.*, 2016).

83 **Anthropometric measures**

84 Naked body weight was measured at birth and 2 months to the nearest 0.01 kg and 6, 12 and 24
85 months to the nearest 0.1 kg using a digital scales (seca 384, seca, Birmingham, United Kingdom).
86 Supine length correct to the nearest 0.1 cm was measured at birth, 2, 6 and 12 months (seca 210) and
87 at two years, standing height was measured using a wall mounted stadiometer (seca 206). Body mass
88 index (BMI) at two years was calculated; dividing weight (kg) by height (m) squared. Age- and sex-
89 specific weight, length and BMI standard deviation scores (SDS) were generated using LMS growth
90 software and the UK-WHO 0-4 year growth reference data (Pan and Cole, 2007, Scientific Advisory
91 Committee on Nutrition/Royal College of Paediatrics and Child Health, 2012). Absolute weight (kg)
92 and length (cm) gain from 0-2, 0-6, 0-12 and 0-24 months was calculated as the difference between
93 weight/length at each time-point and birth weight/length and weight and length gain from 6-12 and
94 12-24 months was calculated as the difference between the two time-points. Overweight and obesity
95 at two years were defined using the UK-WHO age- and sex-specific BMI charts; overweight was

96 defined as a BMI >91st and ≤98th percentile and obesity as a BMI >98th percentile (Scientific Advisory
97 Committee on Nutrition/Royal College of Paediatrics and Child Health, 2012).

98 **Biological samples**

99 Umbilical cord blood was collected at birth in infants recruited from the SCOPE pregnancy cohort
100 study and venous blood was collected from all BASELINE Study participants at the 24-month
101 assessment, whose parents provided consent. Ferritin and C-reactive protein (CRP, assessed using a
102 high sensitivity CRP assay) were analysed in umbilical cord and 24-month serum samples in the
103 laboratory of the Cork Centre for Vitamin D and Nutrition Research, University College Cork, by
104 immunoturbidimetric assay using the RX Monaco Clinical Chemistry Analyser (Randox Laboratories
105 Ltd., Co. Antrim, UK). Haemoglobin and mean corpuscular volume (MCV) were measured in whole
106 blood collected at the 24-month assessment by the Haematology Laboratory of Cork University
107 Hospital on the Sysmex XE 2100 Automated Hematology System (Sysmex America Inc., IL, USA).
108 Participants with potential infections/inflammation as indicated by an elevated CRP (>5 mg/L) were
109 excluded from analyses.

110 **Statistical analysis**

111 Data were analysed using IBM SPSS® for Windows™ version 21 (IBM Corp., Armonk, NY, USA).
112 Descriptive statistics were generated and normal distribution of the data was examined by
113 skewness/kurtosis. Comparisons between categorical variables were made using Chi square (χ^2) tests,
114 while independent t-tests or non-parametric tests were employed for continuous variables, depending
115 on their distribution. Univariate and multivariate adjusted linear regression models were developed to
116 estimate the influence of growth (weight/length gain) and body size (weight/length/BMI) variables on
117 concentrations of haematological indices at two years. Factors identified in the univariate models as
118 significant at the 10% ($P < 0.1$) level were retained in the final multivariate models. Potential
119 confounders included in the final models were infant gender, birth weight, maternal age at delivery,
120 education level, obstetric mode of delivery, duration of (any) breastfeeding (months) and mean daily
121 iron intake (mg/day) at 24 months. As iron intakes were only available for a subgroup ($n = 278$),

122 regression models were first adjusted for potential confounders without iron intakes and then iron
123 intakes were included. Final adjusted results presented are from the models including iron intakes as
124 a potential confounder as the results were similar both including and excluding iron intakes. Other
125 early feeding methods, complementary feeding and maternal health characteristics during pregnancy
126 (obesity/smoking/iron status) were not associated with any of the haematological indices at two years.
127 To explore the effect of iron status at birth on associations between body size/growth and iron status
128 at two years, final regression models were subsequently adjusted for cord ferritin concentrations,
129 which reflect iron stores at birth (Siimes *et al.*, 1974, MacPhail *et al.*, 1980). The residuals of the final
130 models were normally distributed and associations were expressed as unadjusted/adjusted estimates
131 and 95% confidence intervals (CI). $P < 0.05$ was considered significant in final models.

132 **RESULTS**

133 **Participants**

134 Of those initially recruited to the Cork BASELINE Birth Cohort Study, 1537 children attended the
135 24-month assessment and 47% ($n = 729$) of those provided a blood sample. Children born premature
136 (<37 weeks' gestation, $n = 25$) were excluded for this analysis, giving a final sample size of 704. The
137 children included in this study (**Table 1**) did not differ in any principal characteristics from the rest of
138 the BASELINE Study cohort that attended the 24-month assessment but did not provide a blood
139 sample.

140 The distributions of the haematological indices assessed at birth (only those recruited from the
141 SCOPE Ireland study) and two years in study participants are presented in **Table 2**. Serum ferritin
142 concentrations were positively correlated with MCV at two years ($r = 0.282$, $P < 0.0001$), but no
143 significant correlations with haemoglobin concentrations were observed. Using World Health
144 Organisation definitions, iron deficiency (ferritin $< 12 \mu\text{g/L}$) was observed in 5% ($n = 31$) of children
145 and five children (1%) had iron deficiency anaemia (haemoglobin $< 110 \text{ g/L}$ + ferritin $< 12 \mu\text{g/L}$) at
146 two years. Using other commonly used thresholds for serum ferritin, 12 children (2%) had

147 concentrations <10 µg/L (Bates *et al.*, 2014) and 136 (21%) had concentrations <15 µg/L (Hay *et al.*,
148 2004, Capozzi *et al.*, 2010) at two years.

149 **Body size**

150 There were no significant differences in measures of body size (weight/length/BMI) at any time-point
151 between those with and without iron deficiency, iron deficiency anaemia or with ferritin
152 concentrations <10 or 15 µg/L at two years. The unadjusted associations between serum ferritin,
153 haemoglobin and MCV at two years and weight and length SDS from birth to two years from the
154 univariate linear regression models are presented in **Table 3** (associations with absolute weight and
155 length are presented in Supplemental Table 1). Body size at birth, 2, 6 or 12 months was not
156 associated with any haematological indices at two years. Weight and BMI at two years were
157 inversely associated with serum ferritin, however only the association with weight SDS was
158 significant (adjusted estimate [95% CI]: -2.84 [-3.58, -0.11] µg/L, $P = 0.041$) following adjustment
159 for infant gender, birth weight, maternal age at delivery, education level, obstetric mode of delivery,
160 duration of breastfeeding and mean daily iron intake at 24 months in the final regression model.
161 Weight and height at two years were positively associated with haemoglobin, although only the
162 association with height (0.39 [0.07, 0.72] g/L, $P = 0.018$) and height SDS (1.54 [0.45, 2.63] g/L, $P =$
163 0.006) remained significant in the final adjusted models. To account for the effect of iron status at
164 birth on the observed associations with body size measures, the final models were subsequently
165 adjusted for cord ferritin concentrations. After this adjustment, none of the previously observed
166 associations remained significant.

167 There were no significant differences in median [IQR] haemoglobin concentrations (120.0 [114.0,
168 124.0] vs. 120.0 [116.0, 125.0] g/L, $P = 0.187$), MCV (75.7 [74.3, 77.8] vs. 76.3 [74.1, 78.4] fL, $P =$
169 0.159) or ferritin concentrations (18.7 [15.1, 25.1] vs. 20.4 [15.5, 27.5] µg/L, $P = 0.077$) between
170 those that were overweight or obese ($n = 149$) at two years and those that were not. There were also
171 no significant differences in haematological indices when overweight and obesity were separated into
172 two categories.

173 **Growth**

174 Growth was assessed by absolute weight (kg) and length (cm) gain from 0-2, 0-6, 0-12, 0-24, 6-12
175 and 12-24 months. There were no significant differences in any growth measures between those with
176 and without iron deficiency, iron deficiency anaemia or with ferritin concentrations <10 or 15 µg/L at
177 two years. Associations between serum ferritin, haemoglobin and MCV at two years and growth
178 measures from birth to two years from unadjusted linear regression models are depicted in **Table 4**.
179 Weight gain from 6-12, 0-24 and 12-24 months was inversely associated with ferritin and positively
180 associated with haemoglobin at two years. Weight gain from 12-24 months was also inversely
181 associated with MCV. Following adjustment for confounding factors (infant gender, birth weight,
182 maternal age at delivery, education level, obstetric mode of delivery, duration of breastfeeding and
183 mean daily iron intake at 24 months), only the inverse association between weight gain from 12-24
184 months and ferritin concentrations remained robust (-4.33 [-7.36, -1.30] µg/L, $P = 0.005$). Length
185 gain from 0-24 months and 12-24 months was positively associated with haemoglobin concentrations
186 at two years and the association with length gain from 0-24 months remained robust (0.42 [0.07, 0.76]
187 g/L, $P = 0.019$) following adjustment. However, after subsequent adjustment for cord ferritin
188 concentrations, only the inverse association between weight gain from 12-24 months and ferritin
189 concentrations at two years (-4.40 [-8.43, -0.37] µg/L, $P = 0.033$) remained significant. When the
190 children with serum ferritin concentrations <12 µg/L were excluded, this association remained
191 significant in the children with normal iron stores only (-4.26 [-8.35, -0.18] µg/L, $P = 0.041$).

192 **DISCUSSION**

193 This study has described associations between iron status, body size and growth in the first two years
194 of life in a large sample of healthy children from a well-characterised maternal-infant cohort with
195 concomitant dietary, growth and biomarker data collected prospectively throughout the first two
196 years. The influence of growth and body size on iron status in the first year of life has been
197 documented previously (Sherriff *et al.*, 1999, Thorsdottir *et al.*, 2003), however explorations in the
198 second year of life have been limited to a small ($n = 71$) Icelandic study that observed an inverse

199 association between weight gain from birth to two years and serum ferritin concentrations at two years
200 (Gunnarsson *et al.*, 2004). To our knowledge, our data are the first to highlight the importance of the
201 second year specifically, for iron status, with an inverse association between weight gain from 12 to
202 24 months and serum ferritin at two years observed in this healthy cohort. This observed inverse
203 association is not unanticipated; the high growth rate, often combined with inadequate dietary iron
204 intakes during this period, results in iron being transferred from the storage sites to support
205 erythropoiesis and provide the iron necessary for growth (World Health Organisation, 2001, Domellof
206 *et al.*, 2014).

207 This is also the first study to show that the inverse association between weight gain in the second year
208 and ferritin concentrations was robust after accounting for iron status at birth. The iron endowment at
209 birth has been suggested to provide the iron necessary for growth in the first months of life, therefore
210 the larger the iron stores at birth, the greater the protection an infant has from the iron burden
211 associated with growth in infancy and early childhood (Ziegler *et al.*, 2014). Our novel findings
212 suggest that while large iron stores at birth have a protective effect against low iron stores in infancy,
213 this protection may not extend into the second year of life. This reduced endogenous protection, in
214 combination with the dietary transition from breast milk or infant formula to unmodified cows' milk
215 as an important beverage, a product known to adversely affect iron status (Uijterschout *et al.*, 2014,
216 McCarthy *et al.*, 2016), further consolidates the importance of closely examining dietary requirements
217 and nutritional status in children in the second year of life. This examination is necessary to ensure
218 that iron requirements in the second year are adequate to avoid deficiency and suboptimal or low iron
219 status, yet not excessive, given the reported adverse consequences of excess iron for growth and
220 infection risk in subgroups of the population (Iannotti *et al.*, 2006, Domellof *et al.*, 2014).

221 We observed positive associations between height at two years and length gain in the first two years
222 and haemoglobin concentrations at two years, prior to accounting for iron status at birth. This
223 association appears to be biologically plausible given that to support the high iron requirements and
224 expanding blood volume during growth in infancy, iron is taken from storage sites and prioritised
225 towards erythropoiesis and the production of haemoglobin (Domellof *et al.*, 2014). This is a potential

226 explanation for the contrast in the positive associations with haemoglobin compared to the inverse
227 associations with ferritin observed in this study; however further research is required to fully clarify
228 the relationship with haemoglobin concentrations. Associations between height/linear growth and
229 iron status have been reported previously, with iron deficiency implicated as a cause of stunting in
230 children in low-resource settings (Bougle *et al.*, 2000). Adults with hereditary hemochromatosis, an
231 autosomal recessive iron-overload disorder (Pietrangelo, 2004), have been reported to be taller than
232 the healthy population, with some authors suggesting that people with the condition may benefit in
233 their first two decades from constantly enhanced iron absorption, providing a sufficient supply of iron
234 for linear growth (Cippà and Krayenbuehl 2013).

235 Weight status has previously been shown to influence iron status, with overweight children almost
236 twice as likely to be iron deficient than children that were not overweight (Nead *et al.*, 2004, Brotanek
237 *et al.*, 2007). Potential explanations for this have included genetic influences, an inadequate diet or
238 physical inactivity, while animal studies have suggested altered iron metabolism and tissue
239 distribution in overweight and obesity (Failla *et al.*, 1988, Nead *et al.*, 2004). In contrast to studies in
240 older children and adolescents, we observed no significant differences in concentrations of
241 haematological indices at two years in those overweight or obese at two years. However, given the
242 rising prevalence of overweight and obesity in young children worldwide, the potential adverse
243 effects of overweight and obesity on iron status in early childhood warrant further investigation.

244 The prospective, longitudinal design of the Cork BASELINE Birth Cohort Study, with multiple
245 anthropometric measurements throughout infancy and early childhood, has enabled this detailed
246 exploration of associations between iron status, body size and growth in early childhood. The
247 generalizability of our results may be limited, given the region-based recruitment of the cohort;
248 however, findings are generalizable to other healthy, low risk maternal-infant populations.

249 Exploration of associations between iron deficiency anaemia and body size and growth were
250 somewhat limited by the small number of children with iron deficiency anaemia, however in this
251 high-resource setting, our purpose was to investigate associations between individual haematological
252 indices and growth indicators, as opposed to investigating malnutrition *per se*.

253 To conclude, in this low risk, high-resource setting, weight gain in the second year of life was
254 inversely associated with iron stores at two years in apparently healthy children, even after accounting
255 for iron status at birth. This novel finding suggests that while large iron stores at birth have a
256 protective effect against low iron stores later in infancy, this effect does not extend beyond the first
257 year of life. Therefore public health policies and dietary strategies aimed at preventing iron
258 deficiency, but also suboptimal or low iron status in the second year of life are highly pertinent.
259 Furthermore, a specific examination of iron requirements, adequacy of dietary intakes and analysis of
260 growth patterns in children in the second year of life in high-resource settings is warranted.

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KEY MESSAGES

- The dietary transition, including replacing breast milk or infant formula with unmodified cows' milk as an important beverage, in conjunction with the rapid growth associated with this period, makes the second year of life an especially vulnerable period for iron deficiency.
- In this low risk, high-resource setting, weight gain in the second year of life was inversely associated with iron stores at two years in apparently healthy children, even after accounting for iron status at birth.
- A specific examination of iron requirements, adequacy of dietary intakes and analysis of growth patterns in children in the second year of life in high-resource settings is warranted.

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Table 1 Principal characteristics of participants of the Cork BASELINE Birth Cohort Study with haematological indices measured at two years ($n = 704$)

| | Median [IQR] or % |
|---|-------------------|
| Maternal¹ | |
| Caucasian | 99 |
| Attended university/third level education | 85 |
| Relationship status, single | 5 |
| Mode of delivery, vaginal | 71 |
| Age at delivery (years) | 32.0 [29.0, 34.8] |
| Child | |
| Gender, male | 54 |
| Birth weight (kg) | 3.6 [3.3, 3.8] |
| Gestational age (weeks) | 40.3 [39.3, 41.0] |
| Predominantly breastfed at hospital discharge | 72 |
| Predominantly breastfed at 2 months | 32 |
| Started complementary feeding (17-26 weeks) | 78 |
| <i>24-month assessment</i> | |
| Age (years) | 2.1 [2.1, 2.2] |
| Weight (kg) | 12.9 [12.0, 13.9] |
| Height (cm) | 88.1 [86.2, 90.3] |
| BMI (kg/m ²) | 16.7 [15.9, 17.6] |
| Mean daily iron intake (mg/day) ² | 6.2 [4.9, 7.8] |
| UK-WHO ³ – overweight | 15 |
| UK-WHO ³ – obese | 7 |

BMI: body mass index; IQR: interquartile range; WHO: World Health Organisation.

¹ Maternal data collected at 15 weeks' gestation unless otherwise stated.

² Data available in 278 participants.

³ Scientific Advisory Committee on Nutrition/Royal College of Paediatrics and Child Health 2012.

Table 2 Distribution of haematological indices measured at birth and two years in participants of the Cork BASELINE Birth Cohort Study

| | <i>n</i> | Mean | SD | Median | 10th centile | 25th centile | 75th centile | 90th centile |
|-----------------------|----------|-------|-------|--------|--------------|--------------|--------------|--------------|
| Haemoglobin (g/L) | 588 | 120.4 | 7.1 | 120.0 | 112.0 | 116.0 | 125.0 | 129.0 |
| MCV (fL) | 588 | 76.0 | 3.9 | 76.1 | 72.0 | 74.1 | 78.3 | 79.8 |
| Serum ferritin (µg/L) | | | | | | | | |
| Birth | 379 | 238.8 | 136.4 | 187.5 | 84.6 | 133.4 | 387.1 | 429.3 |
| Two years | 647 | 24.6 | 16.7 | 19.9 | 13.4 | 15.4 | 27.2 | 39.4 |

MCV: mean corpuscular volume; SD: standard deviation.

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Table 3 Unadjusted associations between serum ferritin, haemoglobin and MCV at two years and weight, length and BMI standard deviation scores (SDS) from birth through to 24 months of age

| | Serum ferritin ($\mu\text{g/L}$) | | Haemoglobin (g/L) | | MCV (fL) | |
|-----------------|------------------------------------|---------|---------------------|---------|---------------------|---------|
| | Estimate [95% CI] | p value | Estimate [95% CI] | p value | Estimate [95% CI] | p value |
| Birth weight | -0.25 [-1.50, 1.00] | 0.697 | -0.46 [-1.02, 0.11] | 0.116 | 0.22 [-0.09, 0.53] | 0.165 |
| Birth length | -1.04 [-2.30, 0.23] | 0.107 | -0.21 [-0.76, 0.33] | 0.448 | 0.28 [-0.02, 0.58] | 0.065 |
| 2 month weight | 0.54 [-0.92, 2.00] | 0.468 | -0.47 [-1.14, 0.20] | 0.170 | 0.36 [-0.02, 0.73] | 0.063 |
| 2 month length | 0.57 [-0.67, 1.82] | 0.365 | -0.12 [-0.68, 0.44] | 0.673 | 0.42 [-0.11, 0.57] | 0.089 |
| 6 month weight | 0.28 [-1.07, 1.62] | 0.688 | -0.23 [-0.84, 0.38] | 0.462 | 0.23 [-0.11, 0.57] | 0.181 |
| 6 month length | -0.15 [-1.38, 1.09] | 0.817 | 0.14 [-0.41, 0.69] | 0.625 | 0.35 [-0.05, 0.65] | 0.122 |
| 12 month weight | -0.04 [-1.49, 1.42] | 0.959 | 0.16 [-0.51, 0.82] | 0.646 | 0.16 [-0.21, 0.53] | 0.386 |
| 12 month length | 0.06 [-1.17, 1.28] | 0.929 | 0.21 [-0.35, 0.76] | 0.463 | 0.39 [-0.08, 0.69] | 0.113 |
| 24 month weight | -1.46 [-2.86, -0.06] | 0.041 | 0.78 [0.14, 1.42] | 0.017 | -0.09 [-0.45, 0.25] | 0.582 |
| 24 month length | -0.35 [-1.66, 0.97] | 0.605 | 0.92 [0.34, 1.51] | 0.002 | 0.06 [-0.26, 0.39] | 0.703 |
| 24 month BMI | -1.33 [-2.74, 0.08] | 0.055 | 0.03 [-0.62, 0.67] | 0.933 | -0.20 [-0.55, 0.16] | 0.280 |

CI: confidence interval; MCV: mean corpuscular volume.

Data presented as unadjusted estimates [95% CI] from univariate linear regression analysis where 1 SDS was the unit of change.

Table 4 Unadjusted associations between serum ferritin, haemoglobin and MCV at two years and weight/length gain from birth to two years

| | Serum ferritin ($\mu\text{g/L}$) | | Haemoglobin (g/L) | | MCV (fL) | |
|-------------------------|------------------------------------|---------|--------------------|---------|----------------------|---------|
| | Estimate [95% CI] | p value | Estimate [95% CI] | p value | Estimate [95% CI] | p value |
| Weight gain (kg) | | | | | | |
| 0-2 months | 0.38 [-1.92, 2.68] | 0.744 | 0.43 [-0.57, 1.44] | 0.397 | -0.15 [-0.71, 0.40] | 0.592 |
| 0-6 months | -0.10 [-1.61, 1.40] | 0.894 | 0.22 [-0.44, 0.88] | 0.515 | -0.18 [-0.54, 0.18] | 0.333 |
| 0-12 months | -0.42 [-1.69, 0.84] | 0.510 | 0.58 [0.01, 1.15] | 0.049 | -0.23 [-0.55, 0.09] | 0.154 |
| 0-24 months | -1.16 [-2.06, -0.26] | 0.011 | 0.61 [0.19, 1.02] | 0.004 | -0.22 [-0.44, 0.01] | 0.059 |
| 6-12 months | -1.53 [-2.71, -0.34] | 0.012 | 1.04 [0.48, 1.59] | <0.0001 | -0.25 [-0.55, 0.06] | 0.112 |
| 12-24 months | -2.92 [-4.62, -1.22] | 0.001 | 1.03 [0.25, 1.80] | 0.010 | -0.43 [-0.86, -0.01] | 0.049 |
| Length gain (cm) | | | | | | |
| 0-2 months | 0.82 [-0.13, 1.50] | 0.089 | 0.15 [-0.15, 0.46] | 0.328 | 0.08 [-0.09, 0.25] | 0.351 |
| 0-6 months | 0.13 [-0.47, 0.73] | 0.667 | 0.22 [-0.05, 0.48] | 0.107 | -0.02 [-0.16, 0.13] | 0.840 |
| 0-12 months | 0.04 [-0.46, 0.55] | 0.873 | 0.19 [-0.03, 0.41] | 0.091 | 0.02 [-0.10, 0.14] | 0.767 |
| 0-24 months | 0.08 [-0.33, 0.49] | 0.698 | 0.32 [0.14, 0.50] | 0.001 | -0.03 [-0.14, 0.07] | 0.510 |
| 6-12 months | -0.19 [-0.88, 0.49] | 0.572 | 0.06 [-0.24, 0.37] | 0.692 | 0.02 [-0.15, 0.19] | 0.791 |
| 12-24 months | 0.15 [-0.51, 0.82] | 0.653 | 0.44 [0.15, 0.74] | 0.003 | -0.14 [-0.31, 0.02] | 0.094 |

CI: confidence interval; MCV: mean corpuscular volume.

Data presented as unadjusted estimates [95% CI] from univariate linear regression analysis.

Supplemental Table 1 Unadjusted associations between serum ferritin, haemoglobin and MCV at two years and weight, length and BMI from birth through to 24 months of age

| | Serum ferritin ($\mu\text{g/L}$) | | Haemoglobin (g/L) | | MCV (fL) | |
|----------------------------------|------------------------------------|---------|---------------------|---------|---------------------|---------|
| | Estimate [95% CI] | p value | Estimate [95% CI] | p value | Estimate [95% CI] | p value |
| Birth weight (kg) | -0.84 [-3.51, 1.83] | 0.538 | -1.26 [-2.47, 0.06] | 0.102 | 0.27 [-0.38, 0.93] | 0.413 |
| Birth length (cm) | -0.65 [-1.27, 0.02] | 0.104 | -0.11 [-0.38, 0.16] | 0.438 | 0.06 [-0.09, 0.21] | 0.453 |
| 2 month weight (kg) | 0.07 [-1.87, 2.00] | 0.947 | -0.28 [-1.13, 0.57] | 0.520 | 0.05 [-0.42, 0.52] | 0.843 |
| 2 month length (cm) | 0.15 [-0.43, 0.72] | 0.610 | 0.05 [-0.20, 0.30] | 0.702 | 0.09 [-0.05, 0.23] | 0.191 |
| 6 month weight (kg) | -0.24 [-1.62, 1.14] | 0.733 | -0.16 [-0.76, 0.44] | 0.597 | -0.09 [-0.42, 0.24] | 0.582 |
| 6 month length (cm) | -0.27 [-0.81, 0.28] | 0.334 | 0.10 [-0.14, 0.33] | 0.423 | 0.02 [-0.11, 0.14] | 0.809 |
| 12 month weight (kg) | -0.53 [-1.72, 0.65] | 0.377 | 0.26 [-0.27, 0.79] | 0.328 | -0.14 [-0.43, 0.16] | 0.358 |
| 12 month length (cm) | -0.31 [-0.78, 0.15] | 0.188 | 0.12 [-0.08, 0.33] | 0.241 | 0.04 [-0.08, 0.15] | 0.511 |
| 24 month weight (kg) | -1.11 [-1.96, -0.27] | 0.010 | 0.40 [0.01, 0.79] | 0.043 | -0.16 [-0.37, 0.05] | 0.135 |
| 24 month length (cm) | -0.19 [-0.57, 0.20] | 0.347 | 0.25 [0.08, 0.42] | 0.005 | -0.02 [-0.12, 0.08] | 0.693 |
| 24 month BMI (kg/m^2) | -1.04 [-2.02, -0.05] | 0.040 | -0.04 [-0.48, 0.41] | 0.875 | -0.20 [-0.45, 0.04] | 0.106 |

CI: confidence interval; MCV: mean corpuscular volume.

Data presented as unadjusted estimates [95% CI] from univariate linear regression analysis.