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

Elaine K McCarthy,[1,2] Carol ní Chaoimh,[1,2] Louise C Kenny,[2,3] Jonathan O'B Hourihane,[2,4] Alan D Irvine,[5-7] Deirdre M Murray,[2,4] Mairead E Kiely[1,2*]

[1]Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences [2]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), [3]Department of Obstetrics and Gynaecology, and [4]Department of Paediatrics and Child Health, University College Cork, Ireland; [5]Department of Clinical Medicine, Trinity College, Dublin, Ireland; [6]Department of Paediatric Dermatology, Our Lady's Children's Hospital, Dublin, Ireland; [7]National Children's Research Centre, Dublin, Ireland

*__Corresponding author__ Prof. Mairead Kiely, Cork Centre for Vitamin D and Nutrition Research, Room 127, Food Science Building, University College Cork, Cork, Ireland. Email: m.kiely@ucc.ie

Phone +353214903394

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ABSTRACT

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

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

INTRODUCTION

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

Term infants are born with iron reserves that can last for about the first six months of life, after which the infant relies heavily on iron intakes to meet the high iron requirements for growth (Agostoni *et al.*, 2008). In the first year of life, a number of dietary factors can influence iron status, including the delayed introduction of appropriate complementary foods beyond six months (Chantry *et al.*, 2007, Agostoni *et al.*, 2008, Maguire *et al.*, 2013, Wang *et al.*, 2016). While in the second year, consumption of unmodified cows' milk as a beverage and low consumption of iron-fortified products has been associated with an increased risk of low iron status and iron deficiency (Gunnarsson *et al.*, 2004, Uijterschout *et al.*, 2014, McCarthy *et al.*, 2016). 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.

Associations between iron status, body size and growth in the first year of life have been well documented. In high-resource settings, birth weight was positively associated with iron status at one year of age (Persson *et al.*, 1998, Sherriff *et al.*, 1999), while weight gain in infancy has been inversely associated with iron status at one year (Morton *et al.*, 1988, Michaelsen *et al.*, 1995, Thorsdottir *et al.*, 2003). However, apart from a small Icelandic study (Gunnarsson *et al.*, 2004), the relationship between iron status, body size and growth in the second year of life in healthy children has been largely unexplored. Therefore, the aim of the current study was to investigate the influence of body size and growth in the first two years of life, with a particular focus on the second year, on iron status at two years in apparently healthy children from a prospective birth cohort in Ireland. As iron status at birth has been shown to track through to early childhood (Georgieff *et al.*, 2002, Hay *et al.*, 2007), a secondary, novel aim of this study was to explore the effect of iron status at birth on associations between iron status, body size and growth over the first two years of life.

MATERIALS AND METHODS

Study design and participants

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

Written informed consent to the Cork BASELINE Birth Cohort Study was provided by the parents of participants; 1537 infants recruited from the SCOPE study at 15 weeks' gestation and 600 recruited at birth through the postnatal wards of Cork University Maternity Hospital (from 2008 to 2011). Participants were followed prospectively from birth, with assessments at day 2 and at 2, 6, 12 and 24 months. Study assessments at five years of age were completed in December 2016. Information was gathered by interviewer-led questionnaires and clinical assessments performed by trained researchers in accordance with the Declaration of Helsinki, with further information on study design and procedures reported previously (O'Donovan *et al.*, 2015). Ethical approval for the Cork BASELINE Birth Cohort Study was granted by the Clinical Research Ethics Committee of the Cork teaching hospitals (ECM 5(9) 01/07/2008) and it is registered at the National Institutes of Health Clinical Trials Registry (www.clinicaltrials.gov NCT01498965).

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

Anthropometric measures

Naked body weight was measured at birth and 2 months to the nearest 0.01 kg and 6, 12 and 24 months to the nearest 0.1 kg using a digital scales (seca 384, seca, Birmingham, United Kingdom). Supine length correct to the nearest 0.1 cm was measured at birth, 2, 6 and 12 months (seca 210) and at two years, standing height was measured using a wall mounted stadiometer (seca 206). Body mass index (BMI) at two years was calculated; dividing weight (kg) by height (m) squared. Age- and sex-specific weight, length and BMI standard deviation scores (SDS) were generated using LMS growth software and the UK-WHO 0-4 year growth reference data (Pan and Cole, 2007, Scientific Advisory Committee on Nutrition/Royal College of Paediatrics and Child Health, 2012). Absolute weight (kg) and length (cm) gain from 0-2, 0-6, 0-12 and 0-24 months was calculated as the difference between weight/length at each time-point and birth weight/length and weight and length gain from 6-12 and 12-24 months was calculated as the difference between the two time-points. Overweight and obesity at two years were defined using the UK-WHO age- and sex-specific BMI charts; overweight was defined as a BMI >91st and ≤98th percentile and obesity as a BMI >98th percentile (Scientific Advisory Committee on Nutrition/Royal College of Paediatrics and Child Health, 2012).

Biological samples

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

Statistical analysis

Data were analysed using IBM SPSS® for Windows™ version 21 (IBM Corp., Armonk, NY, USA). Descriptive statistics were generated and normal distribution of the data was examined by skewness/kurtosis. Comparisons between categorical variables were made using Chi square (χ^2) tests, while independent t-tests or non-parametric tests were employed for continuous variables, depending on their distribution. Univariate and multivariate adjusted linear regression models were developed to estimate the influence of growth (weight/length gain) and body size (weight/length/BMI) variables on concentrations of haematological indices at two years. Factors identified in the univariate models as significant at the 10% (P <0.1) level were retained in the final multivariate models. Potential confounders included in the final models were infant gender, birth weight, maternal age at delivery, education level, obstetric mode of delivery, duration of (any) breastfeeding (months) and mean daily iron intake (mg/day) at 24 months. As iron intakes were only available for a subgroup (n = 278), regression models were first adjusted for potential confounders without iron intakes and then iron intakes were included. Final adjusted results presented are from the models including iron intakes as a potential confounder as the results were similar both including and excluding iron intakes. Other early feeding methods, complementary feeding and maternal health characteristics during pregnancy (obesity/smoking/iron status) were not associated with any of the haematological indices at two years. To explore the effect of iron status at birth on associations between body size/growth and iron status at two years, final regression models were subsequently adjusted for cord ferritin concentrations, which reflect iron stores at birth (Siimes *et al.*, 1974, MacPhail *et al.*, 1980). The residuals of the final models were normally distributed and associations were expressed as unadjusted/adjusted estimates and 95% confidence intervals (CI). P <0.05 was considered significant in final models.

RESULTS

Participants

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

The distributions of the haematological indices assessed at birth (only those recruited from the SCOPE Ireland study) and two years in study participants are presented in **Table 2**. Serum ferritin concentrations were positively correlated with MCV at two years (r = 0.282, P <0.0001), but no significant correlations with haemoglobin concentrations were observed. Using World Health Organisation definitions, iron deficiency (ferritin <12 µg/L) was observed in 5% (n = 31) of children and five children (1%) had iron deficiency anaemia (haemoglobin <110 g/L + ferritin <12 µg/L) at two years. Using other commonly used thresholds for serum ferritin, 12 children (2%) had concentrations <10 µg/L (Bates *et al.*, 2014) and 136 (21%) had concentrations <15 µg/L (Hay *et al.*, 2004, Capozzi *et al.*, 2010) at two years.

Body size

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

There were no significant differences in median [IQR] haemoglobin concentrations (120.0 [114.0, 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 = 0.159) or ferritin concentrations (18.7 [15.1, 25.1] vs. 20.4 [15.5, 27.5] µg/L, P = 0.077) between those that were overweight or obese (n = 149) at two years and those that were not. There were also no significant differences in haematological indices when overweight and obesity were separated into two categories.

Growth

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

DISCUSSION

This study has described associations between iron status, body size and growth in the first two years of life in a large sample of healthy children from a well-characterised maternal-infant cohort with concomitant dietary, growth and biomarker data collected prospectively throughout the first two years. The influence of growth and body size on iron status in the first year of life has been documented previously (Sherriff *et al.*, 1999, Thorsdottir *et al.*, 2003), however explorations in the second year of life have been limited to a small ($n = 71$) Icelandic study that observed an inverse association between weight gain from birth to two years and serum ferritin concentrations at two years (Gunnarsson *et al.*, 2004). To our knowledge, our data are the first to highlight the importance of the second year specifically, for iron status, with an inverse association between weight gain from 12 to 24 months and serum ferritin at two years observed in this healthy cohort. This observed inverse association is not unanticipated; the high growth rate, often combined with inadequate dietary iron intakes during this period, results in iron being transferred from the storage sites to support erythropoiesis and provide the iron necessary for growth (World Health Organisation, 2001, Domellof *et al.*, 2014).

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

We observed positive associations between height at two years and length gain in the first two years and haemoglobin concentrations at two years, prior to accounting for iron status at birth. This association appears to be biologically plausible given that to support the high iron requirements and expanding blood volume during growth in infancy, iron is taken from storage sites and prioritised towards erythropoiesis and the production of haemoglobin (Domellof *et al.*, 2014). This is a potential explanation for the contrast in the positive associations with haemoglobin compared to the inverse associations with ferritin observed in this study; however further research is required to fully clarify the relationship with haemoglobin concentrations. Associations between height/linear growth and iron status have been reported previously, with iron deficiency implicated as a cause of stunting in children in low-resource settings (Bougle *et al.*, 2000). Adults with hereditary hemochromatosis, an autosomal recessive iron-overload disorder (Pietrangelo, 2004), have been reported to be taller than the healthy population, with some authors suggesting that people with the condition may benefit in their first two decades from constantly enhanced iron absorption, providing a sufficient supply of iron for linear growth (Cippà and Krayenbuehl 2013).

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

The prospective, longitudinal design of the Cork BASELINE Birth Cohort Study, with multiple anthropometric measurements throughout infancy and early childhood, has enabled this detailed exploration of associations between iron status, body size and growth in early childhood. The generalizability of our results may be limited, given the region-based recruitment of the cohort; however, findings are generalizable to other healthy, low risk maternal-infant populations. Exploration of associations between iron deficiency anaemia and body size and growth were somewhat limited by the small number of children with iron deficiency anaemia, however in this high-resource setting, our purpose was to investigate associations between individual haematological indices and growth indicators, as opposed to investigating malnutrition *per se*.

To conclude, 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. This novel finding suggests that while large iron stores at birth have a protective effect against low iron stores later in infancy, this effect does not extend beyond the first year of life. Therefore public health policies and dietary strategies aimed at preventing iron deficiency, but also suboptimal or low iron status in the second year of life are highly pertinent. Furthermore, 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.

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 %
Maternal[1]	
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
24-month assessment	
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

	n	**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.

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 (μg/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]	0463	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 (μg/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 (µg/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.