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Title

Iron intakes and status of two year old children in the Cork BASELINE Birth Cohort Study

Running Title

Iron intakes and status of two year olds

Authors and Institutional Addresses

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Conflict of Interest

The authors declare no conflict of interest.

Author's contributions

E.K.M. carried out data collection, database construction and data analysis. M.K. designed the study and E.K.M. and M.K. drafted the manuscript. M.K. had responsibility for the final content. C.ní C. carried out data collection. D.M.M. is the overall PI of the Cork BASELINE Birth Cohort Study and J.O'B.H., L.C.K., A.D.I. and M.K. are co-PIs and specialist leads. L.C.K. is the PI of the SCOPE Ireland pregnancy cohort study. All authors reviewed and approved the final submission.

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1 **Abstract [248 words]**

2 Young children are at risk of iron deficiency and subsequent anaemia, resulting in long-term
3 consequences for cognitive, motor and behavioural development. This study aimed to describe the iron
4 intakes, status and determinants of status in two year old children. Data were collected prospectively
5 in the mother-child Cork BASELINE Birth Cohort Study from 15 weeks' gestation throughout early
6 childhood. At the 24-month assessment, serum ferritin, haemoglobin and mean corpuscular volume
7 were measured and food/nutrient intake data were collected using a two-day weighed food diary. Iron
8 status was assessed in 729 children (median [IQR] age: 2.1 [2.1, 2.2] years) and 468 completed a food
9 diary. From the food diary, mean (SD) iron intakes were 6.8 (2.6) mg/day and 30% had intakes < UK
10 Estimated Average Requirement (5.3 mg/day). Using WHO definitions, iron deficiency was observed
11 in 4.6% ($n=31$) and iron deficiency anaemia in five children (1.0%). Following an iron series workup,
12 five more children were diagnosed with iron deficiency anaemia. 21% had ferritin concentrations
13 $<15\mu\text{g/l}$. Inadequate iron intakes (OR [95% CI]: 1.94 [1.09, 3.48]) and unmodified cows' milk intakes
14 $\geq 400\text{ml/day}$ (1.95 [1.07, 3.56]) increased the risk of low iron status. Iron-fortified formula
15 consumption was associated with decreased risk (0.21 [0.11, 0.41] $P<0.05$). In this, the largest study
16 in Europe, a lower prevalence of low iron status was observed than in previous reports. Compliance
17 with dietary recommendations to limit cows' milk intakes in young children and consumption of iron-
18 fortified products appears to have contributed to improved iron status at two years.

19 **Keywords**

20 Birth cohort, iron deficiency, anaemia, iron intakes, food fortification, cows' milk consumption.

21

22 **Introduction**

23 Anaemia is estimated to affect 1.62 billion people worldwide, with roughly 50% of cases attributed to
24 iron deficiency (World Health Organisation, 2008). Infants and young children (aged 6-24 months) are
25 at particular risk of iron deficiency and subsequent anaemia due to the increased requirements
26 associated with this period of rapid growth. Term infants are born with iron reserves that can last for
27 the first 4-6 months of life; however after this the infant relies on dietary intakes. Iron deficiency and
28 iron deficiency anaemia in early childhood can have long-term consequences for cognitive, motor and
29 behavioural development (Lozoff *et al.*, 2006, Georgieff, 2011).

30 Risk factors for iron deficiency and iron deficiency anaemia include low birth weight, low
31 socioeconomic status, presence of infection/illness and dietary factors (Burke *et al.*, 2014). Dietary
32 factors include adequate dietary iron intakes, the form of iron ingested (haem/non-haem) and the
33 presence of inhibitors and enhancers of iron absorption in meals. In particular, low intakes of iron-rich
34 foods, such as meat and the early introduction/high intakes of unmodified cows' milk can be detrimental
35 to iron status (Thane *et al.*, 2000, Gunnarsson *et al.*, 2007).

36 There is wide variability in diagnostic criteria for both iron deficiency and iron deficiency anaemia in
37 young children (Domellof *et al.*, 2002a), but it is estimated that approximately 25% of preschool-age
38 children have iron deficiency anaemia worldwide (McLean *et al.*, 2009). However, there is significant
39 variability in prevalence rates. In developing countries, rates are very high; in rural India, 61.9% of 12-
40 23 month olds had iron deficiency (serum ferritin < 12 $\mu\text{g/l}$) and 73% of these were anaemic
41 (haemoglobin < 110 g/l) (Pasricha *et al.*, 2010). The prevalence of iron deficiency ranged from 3-48%
42 in young European children, while iron deficiency anaemia rates were typically below 5%, although
43 increased rates of up to 40% have been reported in Eastern Europe (Eussen *et al.*, 2015). 35.1% of
44 British children aged 1.5-3 years had iron deficiency and 5.2% had iron deficiency anaemia (Bates *et*
45 *al.*, 2014). Comparatively better iron status in the USA and iron deficiency of 8-9% and iron deficiency
46 anaemia of 3% (Brotanek *et al.*, 2007) could be a consequence of public health efforts to tackle poor

47 iron status through food fortification and supplementation policies, targeted at vulnerable minority
48 groups in particular.

49 The iron status of young children in Ireland was last explored in the late 1990s; 50% of two year olds
50 had serum ferritin concentrations $< 10 \mu\text{g/l}$ (Freeman *et al.*, 1998). Since then, dietary recommendations
51 have stipulated a 12-month threshold prior to the introduction of unmodified cow's milk as a main drink
52 (Food Safety Authority of Ireland, 2011). Therefore, the aim of the current study was to determine the
53 iron intakes (including dietary sources) and status of two year old children from a large prospective
54 birth cohort study in Ireland and to identify the important determinants of iron status.

55 **Materials and Methods**

56 *Participants*

57 The Cork BASELINE (Babies after SCOPE: Evaluating the Longitudinal Impact using Neurological
58 and Nutritional Endpoints) Birth Cohort Study is a prospective birth cohort study, established in 2008
59 to investigate links between early nutrition and perinatal outcomes and physical and mental growth and
60 development during childhood. The birth cohort is a follow-on from the SCOPE (Screening for
61 Pregnancy Endpoints) Ireland pregnancy study, an international multicentre study aimed at
62 investigating early indicators of pregnancy complications. Detailed demographic, socioeconomic,
63 anthropometric and clinical data and biological samples were collected for all SCOPE participants
64 (North *et al.*, 2011).

65 Written informed consent to the Cork BASELINE Birth Cohort Study was provided by parents; 1,537
66 recruited from the SCOPE study and an additional 600 recruited at birth through the postnatal wards of
67 the Cork University Maternity Hospital (recruitment concluded November 2011). Participants were
68 followed prospectively, beginning at day 2 and at 2, 6, 12 and 24 months, with assessments at five years
69 on-going. Detailed information on early life environment, diet, lifestyle, health, growth and
70 development was gathered by interviewer-led questionnaires and clinical assessments and entered at
71 the time of appointment into an internet-based, secure database developed by Medical Science Online

72 (MedSciNet, Sweden), compliant with the US Food and Drug Administration and the Health Insurance
73 Portability Accountability Act. A detailed methodology of the Cork BASELINE Birth Cohort Study
74 has been provided by O'Donovan *et al.* (2014).

75 Research objectives and measurements in the Cork BASELINE Birth Cohort Study were conducted
76 according to the guidelines laid down by the Declaration of Helsinki and ethical approval was granted
77 by the Clinical Research Ethics Committee of the Cork teaching hospitals, ref ECM 5(9) 01/07/2008
78 and is registered at the National Institutes of Health Clinical Trials Registry
79 (<http://www.clinicaltrials.gov>), ID: NCT01498965. The SCOPE study is registered at the Australian,
80 New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>), ID: ACTRN12607000551493.

81 *Dietary assessment*

82 At the 24-month assessment, food and nutrient intake data were collected in the form of a two-day
83 weighed food diary, carried out over non-consecutive days. Parents were instructed to record detailed
84 information about the amount and types of all foods, beverages and supplements consumed during the
85 diary period. Information on cooking methods, brand names of products, details of recipes and any
86 leftovers were also recorded.

87 Consumption data were converted to nutrient intake data using the nutritional analysis software
88 Weighed Intake Software Package WISP[®] (Tinuviel Software, Anglesey, UK), using food composition
89 data from McCance and Widdowson's *The Composition of Foods* sixth (Food Standards Agency, 2002)
90 and fifth (Holland *et al.*, 1995) editions. The food database was updated to incorporate composite dish
91 recipes, nutritional supplements, fortified foods, new food products and food items specific to infants
92 and young children. Food composition data from the Irish Food Composition Database (Black *et al.*,
93 2011) and modifications made during the National Preschool Nutrition Survey (NPNS) (Irish
94 Universities Nutrition Alliance 2012) were also incorporated. Each food code was manually checked
95 to ensure that it accurately represented the amount of iron in products.

96 Energy under-reporting was identified using minimum energy intake cut-off points, calculated as
97 multiples of Basal Metabolic Rate (BMR), with individuals below a certain cut-off point classified as

98 under-reporters. BMR was calculated for all participants using equations developed by Schofield and
99 colleagues (1985). The appropriate age-specific cut-off of 1.28 (based on principles of fundamental
100 energy physiology) for children aged 1-5 years as suggested by Torun and colleagues (Torun *et al.*,
101 1996) was used. Data were analysed both including and excluding under-reporters.

102 All food and drinks consumed by participants were assigned into 73 food groups, which were then
103 aggregated into subgroups depending on the analysis. Mean daily intakes of iron for participants were
104 calculated by averaging their intake over the diary recording period. The percentage contributions of
105 food groups to iron intake were calculated using the population proportion method, by summing the
106 amount of iron from a particular food group for all participants and dividing this by the sum of iron
107 from all foods for all participants (Krebs-Smith *et al.*, 1989). Mean daily intakes were also calculated
108 using the mean proportion method, taking an arithmetic mean of all individual mean intakes. For the
109 analysis, participants were subdivided into consumer groups. Iron-fortified products were identified by
110 the presence of iron in the manufactured item that would have much less or no iron in it naturally,
111 including infant formula and breakfast cereals. Participants that consumed at least one fortified product
112 over the diary period were considered an iron-fortified product consumer, while those that did not were
113 non-consumers. Participants that consumed any infant formula or growing-up milk (GUM) products
114 during the diary period were classified as formula consumers.

115 GUM products are described as formula/milk tailored to meet the nutritional requirements of 1-3 year
116 old children, with two 150 ml beakers per day of the product recommended by manufacturers. In this
117 study, GUM products are referred to as iron-fortified formula to account for differences in terminology
118 used for these products globally.

119 *Additional data collected*

120 At earlier study assessments at 2, 6 and 12 months, data on feeding and nutrition were collected,
121 including early feeding methods, duration of breastfeeding (if applicable), formula use and timing of
122 complementary feeding, which was used in the current analysis.

123 At the 24-month assessment, all parents were asked if their child currently consumed any GUM
124 products or if they were currently or had in the previous four weeks taken any iron supplements. These
125 questions were in addition to information provided by the food diary if completed.

126 *Biological samples and analytical methods*

127 Venous blood was collected from all BASELINE study participants at the 24-month assessment, with
128 parental consent. After blood collection, samples were immediately stored at 5°C and were then
129 processed to serum for storage at -80°C.

130 Whole blood collected at the 24-month assessment was sent immediately to the Haematology
131 Laboratory of Cork University Hospital for determination of haemoglobin concentrations and mean
132 corpuscular volume (MCV) on the Sysmex XE 2100 Automated Hematology System (Sysmex America
133 Inc., IL, USA). Ferritin and high sensitivity C-reactive protein (CRP) were analysed in the laboratory
134 of the Cork Centre for Vitamin D and Nutrition Research, University College Cork, by
135 immunoturbidimetric assay on the RX Monaco Clinical Chemistry Analyser (Randox Laboratories Ltd.,
136 Co. Antrim, UK). As serum ferritin is an acute phase reactant, children with potential infections as
137 indicated by an elevated CRP (CRP > 5 mg/l) were subsequently excluded from all analyses. An iron
138 series workup (serum iron [abnormal < 10 µmol/l] , serum transferrin [acceptable range: 1.8-3.2 g/l],
139 transferrin saturation [abnormal < 10%]) was performed by the Biochemistry Laboratory of Cork
140 University Hospital on selected participants' samples using the AU5800 Chemistry Analyser (Beckman
141 Coulter Inc., CA, USA).

142 *Statistical analysis*

143 Data were analysed using IBM SPSS® for Windows™ version 21 (IBM Corp., Armonk, NY, USA).
144 Data are presented using descriptive statistics, including means, standard deviations (SD), medians,
145 interquartile ranges (IQR) and percentages, where appropriate. Comparisons between categorical
146 variables were made using Chi square (χ^2) or Fisher's exact tests, while independent t-tests or non-
147 parametric tests (Mann-Whitney U) were employed for continuous variables, depending on their
148 distribution.

149 Due to the low numbers of participants with iron deficiency and iron deficiency anaemia, determinants
150 of each iron status indicator (ferritin, MCV and haemoglobin) were explored individually. Serum
151 ferritin concentrations were divided into quartiles. Logistic regression analysis was performed to model
152 the associations of maternal and child characteristics (including gender, gestational age, mode of
153 delivery, early feeding methods, dietary factors at two years and maternal age, relationship status,
154 income, profession, education level, smoking status, body mass index (BMI), iron status and
155 supplement use) with the risk of being in the lowest quartile of serum ferritin concentrations (≤ 15.5
156 $\mu\text{g/l}$; other three quartiles used as reference). From the univariate analysis, variables identified as
157 significant at the 10% ($P < 0.1$) level were included in the adjusted multivariate analysis, with
158 associations expressed as odds ratios (OR) and 95% confidence intervals (CI). Numbers of participants
159 in models varied depending on data available for each participant and method was repeated with
160 haemoglobin concentrations $< 110 \text{ g/l}$ and $\text{MCV} < 74 \text{ fl}$ as the dependent variables in models.

161 **Results**

162 *Participants*

163 Of the 2,137 infants initially recruited to the study, 1,537 attended the 24-month assessment. Of those,
164 729 (47%) gave blood and 468 (30%) completed a two-day weighed food diary. A subgroup of 286
165 children had both a food diary and blood collected at two years of age. There were no significant
166 differences in socio-demographic characteristics between those with a completed food diary and blood
167 sample and those with only a blood sample and the participants with a completed food diary were
168 representative of the entire 24-month cohort. Characteristics of the study participants that completed a
169 food diary and/or gave blood for iron status analysis are depicted in **Table 1**.

170 *Dietary intakes*

171 The mean percentage and actual (mg/day) contribution of food groups to iron intakes in those with a
172 completed food diary are presented in **Table 2**. The mean (SD) daily iron intake of the total population
173 was 6.8 (2.6) mg and 30% had intakes below the Estimated Average Requirement (EAR, UK EAR of
174 5.3 mg/day) (Department of Health, 1991, Scientific Advisory Committee on Nutrition, 2010). Twenty-

175 one percent consumed any formula products during the two-day diary period, with a mean (SD) daily
176 iron intake of 9.3 (2.8) mg. Iron-fortified formula contributed to 40% (3.7 mg) of iron intakes in
177 consumers. Ninety percent of children consumed iron-fortified products during the diary period.
178 Consumers had mean daily iron intakes 2.0 mg higher than non-consumers, while only 26% of
179 consumers had inadequate intakes compared to 65% of non-consumers ($P < 0.001$). Intakes at the 97.5th
180 centile in formula consumers and iron-fortified product consumers were 17.1 and 12.7 mg/day,
181 respectively. The prevalence of iron supplement use was low with seven participants (1%) using them
182 during the diary period. Stratification of participants into food consumer groups depicted in **Figure**
183 **1(a)** illustrated a clear difference in mean daily iron intakes between base diet (no iron-fortified
184 products) consumers and formula consumers especially.

185 The main food groups contributing to iron intakes were breakfast cereals (31%), breads and grains
186 (14%), iron-fortified formula (11%) and meat and meat products/dishes (10%). Iron intakes from the
187 base diet ranged from 0.3-0.7 mg/day. Ninety-three percent of total iron intakes were attributed to non-
188 haem iron sources and iron intakes at the 97.5th centile in the total population were 12.5 mg/day. The
189 median [IQR] daily intakes of meat and fish (including from composite dishes) were 39.8 [22.9, 61.3]
190 and 20.7 [11.6, 29.8] g, respectively. Of the 468 children with completed food diaries, 36% ($n = 167$)
191 were classified as energy under-reporters. With energy under-reporters excluded, mean (SD) daily iron
192 intakes increased to 7.5 (2.7) mg, although 19% still had intakes below the EAR and in under-reporters
193 ($n = 167$) alone, mean (SD) intakes were 5.7 (2.7) mg/day and 47% had intakes below the EAR.

194 There were no significant differences in socio-demographic, anthropometric characteristics or
195 macronutrient intakes between iron-fortified product consumers and non-consumers. Iron intakes from
196 non-fortified foods such as meat, breads and grains were also similar between consumers and non-
197 consumers (see Table 2). Formula consumers had a median [IQR] intake of iron-fortified formula of
198 233 [150, 356] ml/day. There were no significant differences in socio-demographic or anthropometric
199 characteristics at two years between formula consumers and non-consumers. Formula consumers had
200 slightly higher daily intakes of energy (1055 [926, 1187] vs. 995 [867, 1144] kcal, $P = 0.023$) consistent
201 with a higher carbohydrate intake (144.6 [119.1, 161.4] vs. 128.6 [106.2, 148.9] g, $P < 0.001$). Protein

202 intakes were lower (37.7 [32.0, 45.8] vs. 40.6 [33.4, 47.5] g, $P = 0.033$) than non-consumers and intakes
203 of fat were the same between the groups. Intakes of foods and overall food groups were also similar,
204 except for a higher intake of unmodified cows' milk in non-consumers of formula (125.0 [68.4, 212.6]
205 vs. 303.0 [175.4, 485.3] ml/day, $P < 0.001$).

206 *Iron status*

207 The distributions of haemoglobin (g/l), serum ferritin ($\mu\text{g/l}$) and MCV (fl) are presented in **Table 3**.
208 Haemoglobin and MCV were measured in 606 children with mean concentrations of 120.5 g/l and 76.0
209 fl respectively; 4.5% ($n = 27$) had haemoglobin concentrations < 110 g/l. Serum ferritin and high
210 sensitivity CRP were measured in 706 children, however 37 children were excluded from the analysis
211 (due to infection, CRP > 5 mg/l), leaving 669 children with valid serum ferritin measurements. In total,
212 555 children had both haemoglobin and a valid serum ferritin measurement. Females had significantly
213 higher median [IQR] MCV (77.0 [75.0, 78.9] vs. 75.6 [73.4, 77.8] fl, $P < 0.05$) than males, with no
214 differences in serum ferritin or haemoglobin concentrations.

215 The prevalence of iron deficiency and iron deficiency anaemia, using multiple cut-off definitions, is
216 presented in **Table 4**. Using WHO definitions, iron deficiency (serum ferritin < 12 $\mu\text{g/l}$) was observed
217 in 4.6% ($n = 31$) of children, while iron deficiency anaemia (iron deficiency + haemoglobin < 110 g/l)
218 was present in five children (1.0%). A further nine children presented with microcytic anaemia
219 (indicated by MCV < 74 fl + haemoglobin < 110 g/l), but had serum ferritin concentrations > 12 $\mu\text{g/l}$.
220 Following an iron series workup on these participants' serum samples, five were diagnosed with iron
221 deficiency anaemia due to abnormal values on > 1 of the haematological indices measured. Therefore,
222 ten (2%) children in total had iron deficiency anaemia.

223 There was a small but significant association between mean daily iron intakes and serum ferritin
224 concentrations (Spearman $r = 0.183$, $P = 0.003$) and MCV ($r = 0.172$, $P = 0.005$). There was no
225 significant association between mean daily iron intakes and haemoglobin concentrations. A clear
226 differentiation in serum ferritin concentrations among different food group consumers is illustrated in
227 **Figure 1(b)**. Iron-fortified product consumers had significantly higher serum ferritin concentrations

228 compared to non-consumers (20.1 [15.7, 27.6] vs. 18.5 [13.6, 21.6] $\mu\text{g/l}$, $P = 0.027$). Formula
229 consumers had higher ferritin concentrations (25.5 [19.6, 33.1] vs. 18.9 [15.1, 26.0] $\mu\text{g/l}$, $P < 0.001$)
230 and MCV (77.0 [74.7, 78.7] vs. 76.0 [73.9, 78.2] fl, $P = 0.012$) than non-consumers.

231 *Cows' milk consumption*

232 From the food diary, unmodified cows' milk (liquid milk only) intakes were stratified at < 400 ml/day
233 and ≥ 400 ml/day. The cut-off of 400 ml/day (two beakers of 150 ml each plus ~ 100 ml serving with
234 breakfast cereal) was chosen based on a preliminary analysis of the current data to identify a threshold
235 that may increase the risk of low iron status as well as the outcomes of previous research (Thane *et al.*,
236 2000, Uijterschout *et al.*, 2014) indicating relatively high intakes in this age group. During the recording
237 period, 91% ($n = 424$) of children consumed cows' milk with a median [IQR] daily intake of 260 [114,
238 452] ml. Among consumers, 31% ($n = 130$) drank ≥ 400 ml/day, with a median [IQR] intake of 528
239 [468, 604] ml/day. Children that consumed < 400 ml/day had a median [IQR] intake of 190 [105, 272]
240 ml/day ($P < 0.001$). Consumers of ≥ 400 ml/day of cows' milk had significantly lower mean daily iron
241 intakes (6.2 vs. 6.9 mg/day, $P = 0.016$), median [IQR] serum ferritin concentrations (18.3 [13.9, 21.8]
242 vs. 20.5 [16.1, 27.6] $\mu\text{g/l}$) and MCV (75.3 [72.5, 77.6] vs. 76.5 [74.3, 78.5] fl) than those that consumed
243 < 400 ml/day (all $P < 0.01$).

244 *Determinants of status*

245 Following adjustment for gender and birth weight, mean daily iron intakes were a significant
246 determinant of serum ferritin concentrations at two years; participants with intakes $< \text{EAR}$ had an
247 increased risk of low ferritin concentrations (OR [95% CI]: 1.94 [1.09, 3.48], $P = 0.025$). Current
248 consumption of iron-fortified formula was protective against low serum ferritin concentrations at two
249 years (0.21 [0.11, 0.41], $P < 0.001$), while the consumption of cows' milk at volumes ≥ 400 ml/day was
250 associated with an increased risk of low serum ferritin concentrations, after adjustment for daily iron
251 intakes (1.95 [1.07, 3.56], $P = 0.03$).

252 In a separate regression, males were three times more likely to present with a MCV < 74 fl (3.05 [2.01,
253 4.63], $P < 0.001$). Iron intakes $< \text{EAR}$ were associated with an increased risk of a MCV < 74 fl (3.03

254 [1.62, 5.65], $P < 0.001$) and iron-fortified formula consumption was associated with a reduced risk of a
255 low MCV (0.43 [0.23, 0.79], $P = 0.007$, adjusted for gender and birth weight). After adjustment for
256 gender and birth weight, cow's milk intakes ≥ 400 ml/day were associated with an increased risk of a
257 low MCV (2.01 [1.09, 3.70], $P = 0.025$), although this was no longer significant after adjustment for
258 daily iron intakes (1.77 [0.95, 3.32], $P = 0.073$). No dietary factors were significant determinants of
259 haemoglobin concentrations < 110 g/l at two years. There were no associations between early feeding
260 methods or any other dietary factors at two years including meat/fish consumption, with any of the iron
261 status indicators assessed in this study. All results remained the same both including and excluding
262 energy under-reporters (from the food diary) from the analysis.

263 **Discussion**

264 This study, the largest study of its kind in Europe to date, has described the iron intakes and status of
265 two year old children in Ireland.

266 Using WHO definitions, 5% of children had iron deficiency and 1% had iron deficiency anaemia; these
267 rates are lower than contemporary data reported in other European countries (Eussen *et al.*, 2015) and
268 in the USA (Brotanek *et al.*, 2007). However, variable diagnostic criteria for infants and young children
269 make it difficult to draw accurate comparisons (Domellof *et al.*, 2002a). Some of the variability may
270 also be explained by differences in the study populations assessed (in demographics and age) and in the
271 analytical methods used to assess haematological indices, especially in the case of immunoassays used
272 to measure ferritin concentrations (World Health Organisation/Centers for Disease Control and
273 Prevention, 2004).

274 Despite a low prevalence of iron deficiency and iron deficiency anaemia using WHO definitions, 21%
275 of participants had serum ferritin concentrations < 15 $\mu\text{g/l}$, a cut-off used by some (Hay *et al.*, 2004,
276 Capozzi *et al.*, 2010) to indicate low iron stores and risk of deficiency. In the clinical setting, serum
277 ferritin cut-offs of 17-18 $\mu\text{g/l}$ are used, along with a combination of haematological indices including
278 haemoglobin, MCV, serum iron and transferrin saturation. This approach was utilised in the current
279 study for nine children that presented with microcytic anaemia, with serum ferritin concentrations > 12

280 $\mu\text{g/l}$; following further investigations, an additional five children were diagnosed with iron deficiency
281 anaemia. Therefore, using both standard definitions and more detailed investigations, ten children in
282 the current study had iron deficiency anaemia. These findings highlight the need for standardisation of
283 the diagnostic criteria in young children, with a need to assess status using multiple haematological
284 indices to ensure no “at risk” children are inadvertently undiagnosed.

285 The prevalence of iron deficiency/depleted stores in the current study is in particular contrast to the last
286 study in Ireland (Freeman *et al.*, 1998), where 50% of two year olds had serum ferritin $< 10 \mu\text{g/l}$,
287 compared to 2% in the present study. Rates of iron deficiency anaemia were also reported as higher, at
288 9.2%. This diversity can be explained in part by differing sample sizes and socio-demographic profiles,
289 with a much larger, more educated sample included in the current study. However, changes in cows’
290 milk consumption patterns may be another explanation.

291 The cows’ milk consumption patterns of young children in Ireland have changed significantly in
292 accordance with national recommendations to wait until a child is one year before cows’ milk is given
293 as a main drink (Food Safety Authority of Ireland, 2011). Intakes of unmodified cows’ milk are now
294 lower; evident as mean intakes of cows’ milk in one-year olds from the NPNS were 302 ml/day (Irish
295 Universities Nutrition Alliance 2012) in comparison to 524 ml/day in one-year olds in the Freeman
296 study (Freeman *et al.*, 1998). Also important is the early introduction of cows’ milk as a drink (at age
297 two months) in the Freeman study, in contrast to the current study where no child was given cows’ milk
298 as a drink before six months. Fifteen percent of participants in the BASELINE cohort consumed cows’
299 milk as their main drink after their first birthday (O’Donovan *et al.*, 2015), while 18% of nine-month
300 olds in the Euro-Growth study of the late 1990s (Freeman *et al.*, 2000) were already habitual cows’
301 milk consumers. In the UK, cows’ milk was consumed as a main beverage in 79% of 12-18 month
302 olds, in line with UK recommendations to move directly to cows’ milk at one year (Lennox *et al.*, 2011).

303 Similarly to other studies (Thane *et al.*, 2000, Gunnarsson *et al.*, 2004), high intakes of cows’ milk were
304 associated with lower serum ferritin concentrations at two years. The exact mechanisms behind the
305 effect of cows’ milk on iron status are still somewhat unclear; however possible explanations are its

306 low iron content (~0.5 mg/l), the presence of components in it that inhibit iron absorption or it may
307 cause occult intestinal blood loss (Ziegler, 2011). It has been suggested that high intakes of cows' milk
308 may displace iron-rich solid food sources from the diet (Thane *et al.*, 2000), however our data do not
309 support this suggestion as the negative influence of high cows' milk intakes remained significant after
310 adjustment for mean daily iron intakes. Due to its influence on iron status, the European Society for
311 Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition recently
312 advised that intakes of cows' milk in young children should not exceed 500 ml/day (Domellof *et al.*,
313 2014). However, our findings, in line with findings in the UK (Thane *et al.*, 2000) and Netherlands
314 (Uijterschout *et al.*, 2014) suggest that intakes in young children should not exceed 400 ml/day. It may
315 be timely to re-examine dietary recommendations in the second year of life.

316 Dietary iron intakes, in particular inadequate intakes, were a significant determinant of iron status in
317 the current study. No association between dietary iron intakes and status was observed in British and
318 Icelandic children previously (Thane *et al.*, 2000, Gunnarsson *et al.*, 2004). A Swedish study displayed
319 age-dependent associations; serum ferritin concentrations were associated with iron intakes at 18
320 months of age but not at 12 months, while haemoglobin was associated with iron intakes before 12
321 months but not after (Lind *et al.*, 2004). These findings suggest that before 12 months of age, dietary
322 iron is directed towards erythropoiesis and after 12 months, it is directed towards iron storage. This
323 dynamic regulation may also provide an explanation for the lack of dietary determinants of haemoglobin
324 concentrations at two years in this study.

325 Iron-fortified product consumption had a positive impact on iron status in this study, with iron-fortified
326 formula consumption an independent determinant of serum ferritin and MCV. Follow-on formula and
327 iron-fortified infant cereals have previously been identified as significant determinants of iron status in
328 infancy (Krebs *et al.*, 2013, Thorisdottir *et al.*, 2013). Our study is one of the first to report on the
329 positive impact of iron-fortified formula specifically in toddlers. Most iron-fortified formula, targeted
330 at 1-3 year olds, are fortified at a level of 1.2 mg iron/100 ml feed, significantly higher than the iron
331 content of unmodified cows' milk (~0.5 mg/l). The European Food Safety Authority have described
332 such formula as one method of increasing intakes of certain micronutrients but concluded that there was

333 “no unique role” for them in the diet of young children in Europe (EFSA Panel on Dietetic Products
334 Nutrition and Allergies, 2013). Amongst consumers, formula contributed to increased iron intakes and
335 reduced inadequate intakes, similar to previous observations (Ghisolfi *et al.*, 2013, Walton and Flynn,
336 2013). While energy intakes were higher (~60 kcal/day, attributable probably to the increased
337 carbohydrate contribution of ~16 g/day from the formula), we did not observe major differences in
338 dietary intakes or any differences in growth patterns of participants in the current study. As iron-
339 fortified products were the main dietary sources of iron in our study, the positive impact of these
340 products on iron status points to a potentially important role of fortified products in the diets of young
341 children. Further investigations are required to establish whether improving iron status in the second
342 year of life (over and above prevention of iron deficiency) is linked to favourable growth and
343 development outcomes in early and middle childhood.

344 Similar to a study in Norwegian children (Hay *et al.*, 2004), males had significantly lower MCV
345 compared to females. These gender differences have been observed in infants previously (Domellof *et*
346 *al.*, 2002b, Thorsdottir *et al.*, 2003), although other studies have observed no differences in early
347 childhood (Sherriff *et al.*, 1999, Gunnarsson *et al.*, 2004). Explanations for these differences are
348 limited, although suggestions include genetic or hormonal factors, altered growth rates, smaller iron
349 stores at birth in males or even increased intestinal losses in males (Domellof *et al.*, 2002b).

350 Mean daily iron intakes in the current study were similar to intakes observed in other European national
351 surveys (Ocké *et al.*, 2008, Kytälä *et al.*, 2010, Bates *et al.*, 2014), however slightly lower than those
352 reported by the NPNS of Ireland (6.8 vs. 7.6 mg/day) (Irish Universities Nutrition Alliance, 2012). The
353 differing age profiles of these two studies may contribute to this variation, as all children in the current
354 study were aged 23-25 months while the age of those in the NPNS ranged from 24-35 months. Only
355 7% of intakes were attributed to haem iron sources; intakes from meat/meat products were low, a
356 possible explanation as to the lack of association observed between meat consumption and iron status
357 in this study. This diet of predominately non-haem iron sources appears to be typical of young children
358 in other countries also (Fox *et al.*, 2006, Domellof *et al.*, 2014).

359 The main strengths of the Cork BASELINE Birth Cohort Study are its prospective, longitudinal design,
360 with its multidisciplinary approach to research. The use of validated, detailed assessments and standard
361 operating procedures are other strengths. Dietary data were collected prospectively, although all forms
362 of self-reported dietary assessment are subject to error (Livingstone *et al.*, 2004). The generalizability
363 of our results to the rest of Ireland may be somewhat limited, due to the region-based recruitment and
364 high proportion of educated women in the study, although other socio-demographic characteristics of
365 the cohort compare well with national reports (O'Donovan *et al.*, 2014).

366 We have presented the prevalence of iron deficiency and iron deficiency anaemia in young children in
367 Ireland, using a variety of definitions. Compliance with a clear, simple national recommendation to
368 limit cows' milk intakes in young children appears to have made a major contribution to the improved
369 iron status observed in this study. Voluntary fortification of foods popular amongst young children
370 appears to increase iron status but further studies are required to assess the public health significance of
371 this in a high-resource setting with a low prevalence of iron deficiency. At risk subgroups of young
372 children should continue to be targeted with policies to improve iron status.

373 **Key Messages**

- 374 • Standardisation of the diagnostic criteria for iron deficiency and iron deficiency anaemia in
375 infants and young children is required.
- 376 • Compliance with national recommendations to limit cows' milk intakes in young children
377 appears to have made a major contribution to improved iron status.
- 378 • Voluntary fortification of foods popular amongst young children with iron also appears to have
379 contributed to improved iron status.

380

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517 **Table 1** Maternal and child characteristics of participants of the Cork BASELINE Birth Cohort Study
 518 with a completed food diary and/or blood sample at two years ($n = 911$)

	Median [IQR] or %
Maternal	
Age at delivery (years)	32.0 [29.0, 34.0]
Caucasian	99
Born in Ireland	85
Attended third level education	86
Relationship status, single	5
Iron supplement user (in pregnancy)	73
Child	
Gender, male	53
Birth weight (kg)	3.5 [3.2, 3.8]
Gestational age (weeks)	40.3 [39.3, 41.0]
<i>Infant Feeding</i>	
Any breastfeeding (hospital discharge)	72
Age first given solids (weeks)	20.0 [17.0, 22.0]
<i>24-month assessment</i>	
Age (years)	2.1 [2.1, 2.2]
Weight (kg)	12.9 [12.0, 13.9]
Height (m)	0.88 [0.86, 0.90]
BMI (kg/m ²)	16.7 [15.9, 17.6]
Iron supplement user ^a	2
Iron-fortified formula consumer ^a	19

519 IQR: interquartile range; BMI: body mass index.

520 ^a Prevalence data collected from participant questionnaires at 24-month assessment.

Table 2 Mean percentage and actual (mg/day) contribution of food groups to total iron intakes ($n = 468$)

Food Groups	Total Population		Iron-fortified Product Consumers		Iron-fortified Product Non-Consumers		Formula Consumers		Formula Non-Consumers	
	$n = 468$		$n = 422$		$n = 46$		$n = 96$		$n = 372$	
	%	mg/day	%	mg/day	%	mg/day	%	mg/day	%	mg/day
Breakfast Cereals	31	2.10	32	2.25	17	0.81	17	1.63	36	2.23
Breads and Grains	14	0.93	13	0.94	18	0.89	10	0.97	15	0.92
Iron-fortified Formula	11	0.76	12	0.85	—	—	40	3.72	—	—
Meat and Meat Products	10	0.68	9	0.66	16	0.79	6	0.57	11	0.70
Biscuits and Cakes	7	0.47	7	0.48	6	0.33	6	0.58	7	0.44
Vegetables	5	0.37	5	0.37	9	0.43	4	0.36	6	0.38
Fruit	6	0.40	6	0.40	7	0.35	4	0.38	6	0.40
Milk, Yoghurts, Cheeses	4	0.25	4	0.25	5	0.25	2	0.23	4	0.26
Pulse incl. Baked Beans	4	0.30	4	0.29	8	0.40	3	0.22	5	0.32
Nutritional Supplements	1	0.06	1	0.04	5	0.26	1	0.05	1	0.06
Other ^a	7	0.48	7	0.47	9	0.51	7	0.60	9	0.48
Mean (SD) Daily Intake (mg/day) ^b	6.8 (2.6)		7.0 (2.5)		5.0 (2.2)		9.3 (2.8)		6.2 (2.1)	
% Below the EAR ^c	30		26		65		4		36	

SD: standard deviation; EAR: Estimated Average Requirement.

^a Other includes eggs and egg dishes, fish and fish dishes, beverages, confectionary, savoury snacks, soups and sauces.

^b Mean (SD) daily intake calculated using the population proportion (Krebs-Smith *et al.*, 1989) and mean proportion methods. Results were same for both methods.

^c EAR = 5.3 mg/day (UK Department of Health 1991, SACN 2010).

Table 3 Distribution of haematological indices in two year olds from the Cork BASELINE Birth Cohort Study

	<i>n</i>	Mean	SD	Median	10th centile	25th centile	75th centile	90th centile
Haemoglobin (g/l)	606	120.5	7.1	120.0	112.0	116.0	125.0	129.0
Serum ferritin ($\mu\text{g/l}$)	669	24.6	16.7	19.8	13.4	15.5	27.3	39.4
MCV (fl)	606	76.0	3.8	76.2	72.1	74.1	78.3	79.8

SD: Standard Deviation; MCV: Mean Corpuscular Volume.

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Table 4 Prevalence of iron deficiency and iron deficiency anaemia in two year olds from the Cork BASELINE Birth Cohort Study, according to international definitions

Definition	% (n)^a	Studies (country) using definition
<i>Iron Deficiency</i>		
SF <10 µg/l	1.8 (12)	NHANES (USA), NDNS (UK), Zhou <i>et al.</i> 2012 (Australia)
SF <12 µg/l	4.6 (31)	World Health Organisation 2004, SACN 2010 (UK)
SF <15 µg/l	20.8 (139)	Hay <i>et al.</i> 2004 (Norway), Copozzi <i>et al.</i> 2010 (Italy)
SF <12 µg/l + MCV <74 fl	2.6 (15)	Michaelsen <i>et al.</i> 1995 (Denmark), Gunnarsson <i>et al.</i> 2004 (Iceland)
<i>Iron Deficiency Anaemia</i>		
Hb <110 g/l + SF <10 µg/l	0.2 (1)	NHANES (USA), NDNS (UK),
Hb <110 g/l + SF <12 µg/l	1.0 (5)	World Health Organisation 2004, SACN 2010 (UK)
Hb <110 g/l + SF <15 µg/l	1.3 (7)	Hay <i>et al.</i> 2004 (Norway)
Hb <105 g/l + SF <10 µg/l	0.2 (1)	Zhou <i>et al.</i> 2012 (Australia)
Hb <105 g/l + SF <12 µg/l + MCV <74 fl	0.4 (2)	Michaelsen <i>et al.</i> 1995 (Denmark), Gunnarsson <i>et al.</i> 2004 (Iceland)

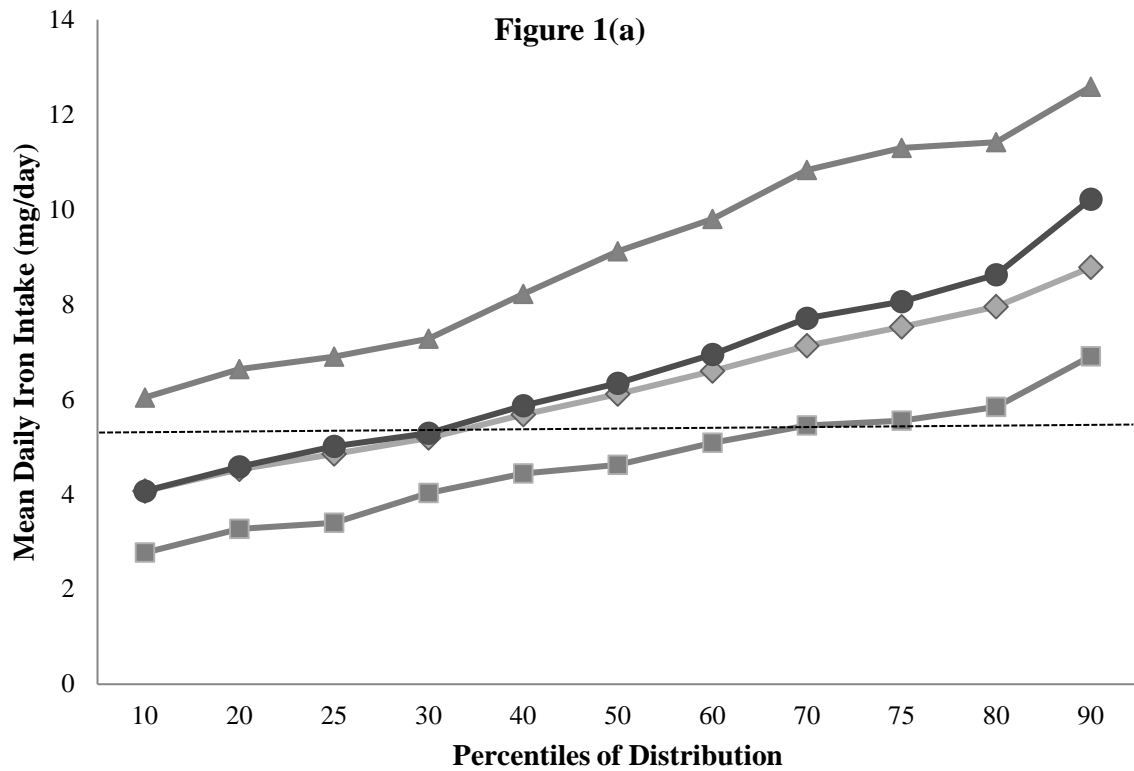
SF: serum ferritin; MCV: mean corpuscular volume; Hb: haemoglobin; NHANES: National Health and Nutrition Examination Survey; NDNS: National Diet and Nutrition Survey; SACN: Scientific Advisory Committee on Nutrition.

^a Prevalence (% and *n*) in the current study, according to each definition.

Figure 1

(a) Distributions of mean daily iron intakes (mg/day) among food group consumers.

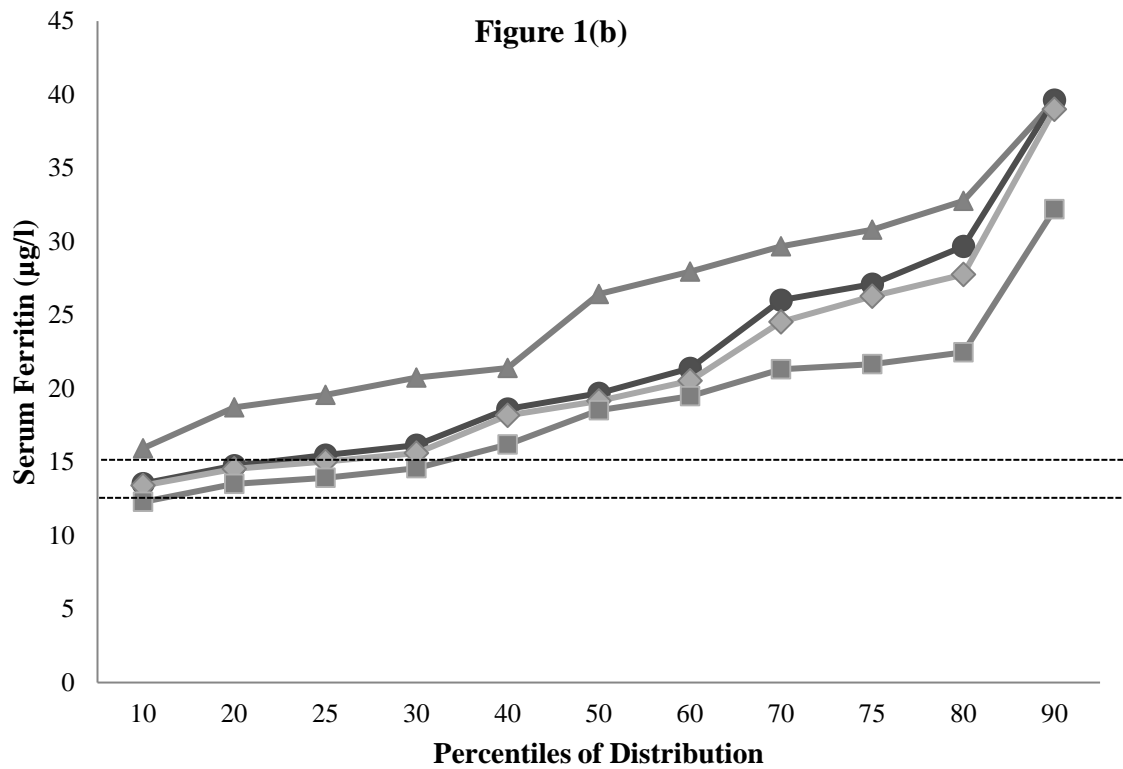
Total population (●, $n = 461$), iron-fortified breakfast cereal only (excluding all formula) consumers (◆, $n = 322$), formula consumers (▲, $n = 95$) and base diet (excluding all iron-fortified products) consumers (■, $n = 44$). Iron supplement users ($n = 7$) excluded. Dashed line indicates UK estimated average requirement (5.3 mg/day).



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(b) Distributions of serum ferritin concentrations ($\mu\text{g/l}$) among food group consumers.

Total population (\bullet , $n = 257$), iron-fortified breakfast cereal only (excluding all formula) consumers (\blacklozenge , $n = 173$), formula consumers (\blacktriangle , $n = 51$) and base diet (excluding all iron-fortified products) consumers (\blacksquare , $n = 33$). Iron supplement users ($n = 6$) excluded. Serum ferritin cut-offs of 12 and 15 $\mu\text{g/l}$ are indicated on the chart by the dashed lines.



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