Association Between Total Duration of Breastfeeding and Iron Deficiency

WHAT’S KNOWN ON THIS SUBJECT: Previous studies have found a relationship between exclusive breastfeeding for ≥6 months and iron deficiency. Little is known about the relationship between total breastfeeding duration, including the period after the introduction of complementary foods, and infant iron status.

WHAT THIS STUDY ADDS: Our results suggest that infants with longer total breastfeeding duration may be at risk for iron deficiency. Our findings highlight a clinically important association warranting additional investigation that may inform future guideline updates regarding assessment of risk for iron deficiency in young infants.

OBJECTIVE: To determine whether there is an association between the total breastfeeding duration and iron stores, iron deficiency, and iron deficiency anemia in healthy urban children.

METHODS: A cross-sectional study of healthy children, aged 1 to 6 years, seen for primary care between December 2008 and July 2011 was conducted through the TARGet Kids! practice-based research network. Univariate and adjusted regression analyses were used to evaluate an association between total breastfeeding duration and serum ferritin, iron deficiency, and iron deficiency anemia.

RESULTS: Included were 1647 healthy children (median age 36 months) with survey, anthropometric, and laboratory data. An association was found between increasing duration of breastfeeding and lower serum ferritin ($P = .0015$). Adjusted logistic regression analysis revealed the odds of iron deficiency increased by 4.8% (95% confidence interval: 2%–8%) for each additional month of breastfeeding. Exploratory analysis suggested an increasing cumulative probability of iron deficiency with longer total breastfeeding duration with an adjusted odds ratio of 1.71 (95% confidence interval: 1.05–2.79) for iron deficiency in children breastfed over versus under 12 months of age. The relationship between total breastfeeding duration and iron deficiency anemia did not meet statistical significance.

CONCLUSIONS: Increased total breastfeeding duration is associated with decreased iron stores, a clinically important association warranting additional investigation. Pediatrics 2013;131:e1530–e1537

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KEY WORDS: breastfeeding duration, serum ferritin, iron deficiency, cross-sectional studies

ABBREVIATIONS: CI—95% confidence interval
CRP—C-reactive protein
OR—odds ratio
WHO—World Health Organization

Dr Maguire conceptualized and designed the study, designed the data collection instruments, analyzed and interpreted the data, drafted the manuscript, critically revised and reviewed the manuscript for important intellectual content, and approved the final manuscript as submitted; Ms Salehi designed the data collection instruments, performed statistical analysis, and approved the final manuscript as submitted; Dr Carsley designed the data collection instruments, analyzed and interpreted the data, performed statistical analysis, drafted the manuscript, and approved the final manuscript as submitted; Dr Birken critically revised and reviewed the manuscript for important intellectual content, and approved the final manuscript as submitted; Ms Carsley designed the data collection instruments, performed statistical analysis, and approved the final manuscript as submitted; Dr Mamdani drafted the manuscript, critically reviewed and reviewed the manuscript for important intellectual content, and approved the final manuscript as submitted; Mr Thorpe and Dr Lebovic analyzed and interpreted the data, performed statistical analysis, and approved the final manuscript as submitted; Dr Khovratovich designed the data collection instruments, coordinated and supervised data collection, approved the final manuscript as submitted; Dr Parkin conceptualized and designed the study, analyzed and interpreted the data, critically revised and reviewed the manuscript for important intellectual content, and approved the final manuscript as submitted; and all authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

(Continued on last page)
Iron is an essential nutritional component for normal neurodevelopment, and yet it is the most common nutrient deficiency among infants and young children worldwide. Data from developed countries suggests a prevalence of iron deficiency of >8% among preschool aged children. Iron deficiency, with or without anemia, has been associated with adverse long-term neurodevelopmental and behavioral outcomes. Rapid growth and increasing nutrient requirements, combined with the consumption of foods with low bioavailable iron, place infants and young children at particular risk for the development of iron deficiency. Risk factors known to be associated with iron deficiency include preterm delivery, low birth weight, chronic illness, low socioeconomic status, certain racial and ethnic backgrounds, early introduction or excessive intake of cow’s milk, prolonged bottle use, and obesity.

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life (defined as feeding with only breast milk without any additional food or water) with introduction of complementary foods at 6 months and continued breastfeeding up to 2 years of age or beyond, based on a systematic review published in 2002. This recommendation has been endorsed by Canada, the United Kingdom, and the United States, among other nations. Although previous research supports a relationship between exclusive breastfeeding for >6 months and iron deficiency, little is known about the relationship between the total breastfeeding duration, including the period after the introduction of complementary foods, and infant iron status.

The primary objective of this study was to determine whether there is an association between the total breastfeeding duration and iron stores in young healthy urban children in a developed country. Secondary objectives included determining the association among total breastfeeding duration, iron deficiency, and iron deficiency anemia.

METHODS

This was a cross-sectional study of healthy urban children seen for routine primary healthcare at a TARGet Kids! participating pediatric or family medicine primary care practice in Toronto, Canada (a high-income, developed country) between December 2008 and June 2011.

Definitions

Infant feeding practices have been classified through a variety of terms. For the purposes of this study, “exclusive breastfeeding” is defined as feeding with only breast milk without any additional foods or liquids. “Nonexclusive breastfeeding” is defined as feeding with breast milk in addition to other fluids or complementary foods. We use the term “total breastfeeding duration” to refer to the duration of breastfeeding of any kind (including exclusive or nonexclusive).

Participants

Parents of healthy children 1 to 6 years of age attending routine primary health care were approached to participate between December 2008 and July 2011. Exclusion criteria for this TARGet Kids! study were any chronic health condition (with the exception of asthma) and laboratory evidence of inflammation (C-reactive protein [CRP] ≥10 mg/L), which could falsely elevate serum ferritin.

Subject Recruitment and Data Collection

TARGet Kids! (The Applied Research Group) is a primary care practice-based research network set in Toronto, Canada, created to examine growth and developmental trajectories of infants and preschool-age children. It was developed as a partnership between researchers at the Pediatric Outcomes Research Team at the Hospital for Sick Children Research Institute, the Applied Health Research Centre at the Li Ka Shing Knowledge Research Institute of St Michael’s Hospital, and primary care physicians in the Section on Community Pediatrics of the Department of Pediatrics and the Department of Family and Community Medicine at the University of Toronto.

Study participants were recruited by research personnel embedded in 7 participating pediatric and family medicine practices. Data were collected prospectively through: a standardized parent completed survey instrument based on the Canadian Community Health Survey; anthropometric measurements including height and weight obtained by trained research assistants; and venous blood sampling collected at the primary care clinic. Medidata RAVE (Medidata Solutions Inc, http://www.mdsol.com) was used as the secure electronic data capture system and data repository for all TARGet Kids! data.

Our main predictor variable, total breastfeeding duration, was determined from the response to the question, “For how long has your child been breastfed?” Maternal recall has been found to be a valid and reliable estimate of breastfeeding duration. Those who had never breastfed were classified as having a total breastfeeding duration of 0 months. Those currently breastfeeding were classified as having a total breastfeeding duration equal to the child’s current age.

Our primary outcome measure was iron stores as measured by serum ferritin (measured using a Roche Modular platform, Roche Diagnostics, http://www.roche.com/diagnostics) performed at the Mount Sinai Services Laboratory (http://www.mountsinaihospitals.com/pages/Home). Iron deficiency was defined as serum ferritin <14 μg/L to be consistent with NHANES 2003–2006, which also used the Roche assay. Iron deficiency anemia was defined as
hemoglobin $\leq 110$ g/L in the presence of iron deficiency. As per current American Academy of Pediatrics' iron screening guidelines, children with elevated CRP ($\geq 10$ mg/L) were excluded from the analysis to minimize the likelihood of falsely elevated serum ferritin from acute illness.

Covariates that we felt might influence the relationship between breastfeeding duration and iron stores included age, gender, birth weight, BMI z score, ethnicity, household income, day-care attendance, age of introduction of solids, age of introduction of cow's milk, and current daily volume of cow's milk intake. Height and weight were measured by using standardized instruments. BMI was calculated as weight in kilograms divided by the height in meters squared. BMI z scores were calculated by using WHO growth standards. Six-digit postal codes were used to obtain the median after-tax neighborhood household income for each participant (using the Statistics Canada Postal Code Conversion File and data from the 2006 Census). Ecological measures of socioeconomic status have been used as proxies for individual-level measures of socioeconomic status.

### Statistical Analysis

Descriptive statistics were performed for the main predictor, outcomes and covariates. Univariate linear regression was used to determine the unadjusted association between the main predictor and serum ferritin levels.

For our primary analysis, multivariable linear regression was performed with adjustment for prespecified, clinically relevant covariates (described above) regardless of traditional statistical significance. Several biologically plausible interactions were considered strategically to achieve a balance between overfitting and interpretation. These included interactions between total breastfeeding duration and each of age, gender, age of introduction of cow's milk, day-care attendance, current daily volume of cow's milk intake, BMI z score, and ethnicity. Interactions were tested for significance using a likelihood ratio test through addition of all biologically plausible interactions to the main effects model. If the joint $P$ value was large (ie, $p > 0.30$), making any interaction unlikely, they were removed from the final model. This approach was used to limit biases and overfitting that can result from standard variable selection approaches. Residual plots of serum ferritin and iron deficiency against total breastfeeding duration were generated to determine the presence of a nonlinear relationship between the main predictor and the primary and secondary outcomes. To explore this nonlinear relationship, a restricted cubic spline regression model was developed.

Secondary analyses included univariate logistic regression to determine the association between our main predictor (total breastfeeding duration) and our secondary outcomes of iron deficiency and iron deficiency anemia. Multivariable logistic regression was then used to adjust for the clinically relevant covariates described earlier. We conducted an exploratory analysis of the odds of iron deficiency in 2 categories of total breastfeeding duration: $\leq 12$ months vs $> 12$ months. All statistical analyses were performed by using the SAS Enterprise Guide 4.3 and R statistical software programs.

Research ethics approval was granted through the Research Ethics Boards of the Hospital for Sick Children and of St Michael's Hospital.

### RESULTS

Eligibility was met and consent obtained for 3696 children (Fig 1). Blood testing was performed in 1699 children. Children with blood testing were slightly older but otherwise appeared similar to children who did not have blood testing (Table 1). Of these, 52 children had CRP $\geq 10$ mg/L and were excluded, leaving 1647 children with survey, anthropometric and laboratory data who were included in the analysis (Table 2). Median age was 36 months (range 12–72 months) and the majority of the children (93%) had received at least some breastfeeding. The median total breastfeeding duration was 10 months (range 0–48 months) with 27% having a total breastfeeding duration of $> 12$ months. The median serum ferritin was 32 $\mu$g/L (range 2–172 $\mu$g/L), the prevalence of iron deficiency and iron deficiency anemia was 9.1% (95% confidence interval [CI]: 7.8%–10.6%) and 1.6% (95% CI: 1.0%–2.3%) respectively. Univariate analysis revealed that serum ferritin decreased by 0.21 $\mu$g/L for every additional month of breastfeeding ($p = 0.006$). This association remained statistically significant after adjusting for clinically relevant covariates (Table 2). For every additional month of breastfeeding, there was a 0.24 $\mu$g/L decrease in serum ferritin in the adjusted model ($p = .001$). Statistically significant covariates associated with decreased serum ferritin included younger age ($p = .04$), higher birth weight ($p = .04$), and higher daily volume of cow's milk consumption ($p < .0001$). Additional exploration identified an association between greater daily cow's milk consumption and both shorter total breastfeeding duration and lower serum ferritin.

A likelihood ratio test between the main effects model and nested model with all hypothesized interaction terms (total breastfeeding duration by age, gender, age of introduction to cow's milk, day-care attendance, current daily volume of cow's milk intake, BMI z score, and ethnicity) yielded a $P$ value sufficiently high to exclude all hypothesized interactions ($p = .4$). Exploration of the effect of child age at follow-up on the relationship between the total duration of...
TABLE 1
Descriptive Characteristics

<table>
<thead>
<tr>
<th>Child Characteristics Without Blood Testing (n = 1953)</th>
<th>With Blood Testing (n = 1699)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>Age, mo</td>
<td>25 (11–72)</td>
</tr>
<tr>
<td>Gender, male</td>
<td>1019 (52)</td>
</tr>
<tr>
<td>Ever breastfed, yes</td>
<td>1737 (93)</td>
</tr>
<tr>
<td>Total breastfeeding duration, mo</td>
<td>11 (0–48)</td>
</tr>
<tr>
<td>0–6 mo</td>
<td>506 (34)</td>
</tr>
<tr>
<td>6–12 mo</td>
<td>585 (39)</td>
</tr>
<tr>
<td>12–24 mo</td>
<td>349 (23)</td>
</tr>
<tr>
<td>&gt;24 mo</td>
<td>56 (4)</td>
</tr>
<tr>
<td>Birth wt, kg</td>
<td>3.18 (0.45–7.00)</td>
</tr>
<tr>
<td>&lt;2.5</td>
<td>177 (11)</td>
</tr>
<tr>
<td>2.5–4.0</td>
<td>1281 (78)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>1781 (11)</td>
</tr>
<tr>
<td>After tax income, Can$</td>
<td>55 818 (14 870–268 530)</td>
</tr>
<tr>
<td>0–14 999</td>
<td>21 (1)</td>
</tr>
<tr>
<td>15 000–29 999</td>
<td>78 (4)</td>
</tr>
<tr>
<td>30 000–49 999</td>
<td>582 (32)</td>
</tr>
<tr>
<td>50 000–79 999</td>
<td>830 (45)</td>
</tr>
<tr>
<td>$80 000</td>
<td>320 (17)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>830 (58)</td>
</tr>
<tr>
<td>Asian (East, Southeast, South)</td>
<td>382 (27)</td>
</tr>
<tr>
<td>African/Caribbean/Latin American</td>
<td>188 (13)</td>
</tr>
<tr>
<td>Other</td>
<td>36 (3)</td>
</tr>
<tr>
<td>z BMI</td>
<td>0.18 (–4.73 to 5.97)</td>
</tr>
<tr>
<td>Underweight (z &lt; –1)</td>
<td>233 (13)</td>
</tr>
<tr>
<td>Normal wt (–1 ≤ z ≤ 1)</td>
<td>1218 (67)</td>
</tr>
<tr>
<td>Overweight (1 &lt; z ≤2)</td>
<td>292 (16)</td>
</tr>
<tr>
<td>Obese (z &gt;2)</td>
<td>85 (5)</td>
</tr>
<tr>
<td>Day-care attendance, yes</td>
<td>826 (45)</td>
</tr>
<tr>
<td>Age of introduction of cow’s milk, mo</td>
<td>12 (0–35)</td>
</tr>
<tr>
<td>Current cow’s milk intake, cups</td>
<td>2.0 (0–5)</td>
</tr>
<tr>
<td>Serum ferritin, µg/L</td>
<td>—</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>—</td>
</tr>
<tr>
<td>Iron deficiency, yes</td>
<td>—</td>
</tr>
<tr>
<td>Iron deficiency anemia, yes</td>
<td>—</td>
</tr>
</tbody>
</table>

Can$, Canadian dollar; —, data not available.

FIGURE 1
Patient recruitment and enrollment flow chart.
breastfeeding and serum ferritin revealed a nonsignificant trend ($P = .11$) toward a stronger association between total breastfeeding duration and serum ferritin in children aged <2 years vs >2 years at follow-up. Our secondary analysis identified a statistically significant association between total breastfeeding duration and the odds of iron deficiency (odds ratio [OR]: 1.026, 95% CI: 1.004–1.050). This association remained statistically significant after adjusting for all covariates (OR: 1.048 for each additional month of breastfeeding, 95% CI: 1.02–1.08). Exploratory analysis using a restricted cubic spline model with 3 knots suggested a slight nonlinear effect with an increasing cumulative probability of iron deficiency with longer total breastfeeding duration (Fig 2). The adjusted OR for iron deficiency for children with a total breastfeeding duration over versus under 12 months of age was 1.71 (95% CI: 1.05–2.79). A trend toward an association between total breastfeeding duration and the odds of iron deficiency anemia was found but did not meet traditional statistical significance (OR: 1.04, 95% CI: 0.98–1.1).

**DISCUSSION**

We have identified an association between increasing total breastfeeding duration and lower iron stores in
children 1 to 6 years of age. The odds of iron deficiency appear to increase by 5% with each additional month of breastfeeding with an increasing cumulative probability of iron deficiency with longer total breastfeeding duration. Children who are breastfed beyond 12 months of age appear to have a 1.7-fold increase in the odds of iron deficiency.

Our study is unique in providing a relatively large sample of children with a wide range of total breastfeeding duration (0–48 months) allowing adjustment for multiple biologically plausible confounders. Furthermore, our study was not limited to exclusive breastfeeding but considered a broader, more inclusive definition of breastfeeding practices. In a survey of Canadian birth mothers in 2006, the proportion who were exclusively breastfeeding at 6 months postpartum was 14.4% (95% CI: 13.5–15.4), whereas that of any breastfeeding at 6 months was 53.9% (95% CI: 52.6–55.2). The US Healthy People 2020 objectives include increasing the proportion of infants who are breastfed exclusively through 6 months from the current 14.1% and increasing the proportion of infants who are breastfed at 1 year from the 22.7%. Therefore, using a definition of total breastfeeding duration, which includes exclusive and non-exclusive breastfeeding, may be highly relevant to current infant feeding practices and current health policy objectives.

Previous studies have examined the relationship between iron status and feeding practices in the first 6 months of life. Exclusive breastfeeding for 6 months compared with exclusive breastfeeding for 0 to 4 months was associated with anemia (hemoglobin <100g/L) at 9 months of age in an observational study of 183 infants in Mexico City (OR: 12.2, 95% CI: 2.4–62.1). A history of late weaning (defined as adding foods and gradually reducing the amount of milk beyond 6 months of age), was associated with iron deficiency anemia in a case-control study of 150 children aged 1 to 2 years in Pakistan (60% of cases and 9% of controls were weaned late, P < .001). An observational study using data from 9930 children who were enrolled in a US Special Supplemental Nutrition Program for Women, Infants, and Children program found that hemoglobin concentrations increased from age 1 to 2 years in those who had been breastfed ≥25 weeks (P < .0001). A cross-sectional survey of 245 children aged 12 to 24 months from US NHANES (1999–2002) found that the odds of low ferritin were less with full breastfeeding for 4 to 5 months compared with full breastfeeding for ≥6 months (OR: 0.19, 95% CI: 0.06–0.57). In a randomized trial, low ferritin was associated with exclusive breastfeeding for 6 months compared with introduction of complementary foods at 4 months in 141 low-income children in Honduras (relative risk: 2.93, 95% CI: 1.13–7.56). In a randomized trial of 77 Canadian infants, low ferritin at 6 months of age was associated with exclusive breastfeeding for 6 months compared with exclusive breastfeeding plus iron supplementation from 1 to 6 months of age (33% vs 7%). The American Academy of Pediatrics Committee on Nutrition (2010) and Section on Breastfeeding (2012) now include recommendations regarding oral iron supplementation in exclusively breastfed infants.

Fewer studies have examined the relationship between breastfeeding practices and iron status beyond the first 6 months and into the second and third years of life. This is relevant, because the WHO recommends that breastfeeding continue up to 2 years of age or beyond. A study of 928 term infants from the Avon Longitudinal Study of Parents and Children examined infant feeding practices in the second 6 months of life and demonstrated that infants at 8 months of age receiving breast milk with or without some cows’ milk but no formula had higher rates of anemia (hemoglobin <110 g/dL) but not low ferritin at 8 and 12 months compared with infants receiving formula at 8 months. Furthermore, infants receiving <6 breastfeeds per day, compared with ≥6 breastfeeds per day, obtained greater energy from solids and greater total iron. The results from our study, which included infants receiving breast milk for up to 48 months, are complementary, suggesting that longer total breastfeeding duration, especially beyond 12 months of age, may be a risk factor for depletion of iron stores.

Limitations of our study include the relatively low prevalence of iron deficiency and iron deficiency anemia (9% and 1.5%, respectively), although these are similar to rates previously reported in Toronto, Canada, and other developed nations such as the United Kingdom and the United States. In addition, total breastfeeding duration was measured by parental self-report, which may be subject to recall error. However, we used the same breastfeeding question used in recent national surveys in Canada and the United States, which have set the standard for breastfeeding measurement. Furthermore, several studies (recently reviewed by Li et al) have found maternal recall to be a valid and reliable estimate of breastfeeding duration. Our analysis for confounders in the association between total breastfeeding duration and serum ferritin levels identified daily volume of cow’s milk intake as a potential confounder. However, when this variable was included in our fully adjusted model, the effect of breastfeeding duration on iron stores remained significant. Finally, although our participation rate without blood testing was similar to several national
surveys on breastfeeding.46,47 the lower participation rate with blood testing reflects the realities of conducting prospective research requiring invasive measures in young healthy children. Our results suggest that infants with longer total breastfeeding duration may be at risk for iron deficiency. To our knowledge, no previous study has documented a relationship between breastfeeding beyond 12 months of age and reduced iron stores. Both the American Academy of Pediatrics5 and the US Preventive Services Task Force recommend assessment of risk for iron deficiency during infancy in infants with prolonged breastfeeding duration and risk for iron deficiency anemia. Although there is currently no risk assessment tool available to guide practitioners, individual risk factors have been identified. Our findings highlight a clinically important association warranting additional investigation, which may inform future guideline updates regarding assessment of risk for iron deficiency in young infants.

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