NRC Research Press

ARTICLE

Higher milk fat content is associated with higher 25-hydroxyvitamin D concentration in early childhood

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Abstract: Current guidelines for cow's milk consumption in children older than age 2 years suggest 1% or 2% milk to reduce the risk of obesity. Given that milk is the main dietary source of vitamin D for North American children and that vitamin D is fat soluble, we hypothesized 25-hydroxyvitamin D (25(OH)D) concentration to be positively associated with the fat content of milk. The objective was to determine the relationship between the fat content of milk consumed and the serum 25(OH)D concentration; our secondary objective was to explore the role that the volume of milk consumed played in this relationship. We completed a cross-sectional study of children aged 12–72 months in the TARGetKids! research network. Multivariable linear regression was used to test the association between milk fat content and child 25(OH)D, adjusted for clinically relevant covariates. The interaction between volume of milk and fat content was examined. Two thousand eight hundred fifty-seven children were included in the analysis. The fat content of milk was positively associated with 25(OH)D ($p = 0.03$), and the interaction between the volume of milk consumed and the milk fat content was statistically significant ($p = 0.005$). Children who drank 1% milk needed 2.46 cups (95% confidence interval (CI) 2.38–2.54) of milk to have a 25(OH)D concentration similar to that of children who drank 1 cup of homogenized milk (3.25% fat). Children who consumed 1% milk had 2.05 (95% CI 1.73–2.42) times higher odds of having a 25(OH)D concentration <50 nmol/L compared with children who consumed homogenized milk. In conclusion, recommendations for children to drink lower-fat milk (1% or 2%) may compromise serum 25(OH)D levels and may require study to ensure optimal childhood health.

Key words: vitamin D, pediatrics, cow's milk, fat, epidemiology, nutrition.

Résumé : Les directives actuelles au sujet de la consommation de lait de vache chez les enfants >2 ans suggèrent du lait 1 % ou 2 % afin de diminuer le risque d'obésité. Étant donné que le lait est la principale source alimentaire de vitamine D pour les enfants de l'Amérique du Nord et que la vitamine D est liposoluble, nous posons l'hypothèse selon laquelle la concentration de 25-hydroxyvitamine D (25(OH)D) est positivement associée au contenu en gras dans le lait. Cette étude a pour objectif de déterminer la relation entre le contenu en gras du lait consommé et la concentration sérique de 25(OH)D et, en deuxième lieu, d'examiner l'effet du volume de lait consommé sur cette relation. Nous avons effectué une étude transversale chez des enfants âgés de 12 à 72 mois inscrits dans le réseau de recherche *TARGetKids!* On effectue une analyse de régression linéaire multiple pour vérifier l'association entre le contenu en gras du lait et 25(OH)D chez l'enfant en prenant en compte les covariables pertinentes sur le plan clinique. On examine aussi l'interaction entre le volume de lait et le contenu en gras. L'analyse inclut 2857 enfants. Le contenu en gras du lait est positivement associé à 25(OH)D ($p = 0{,}03$) et l'interaction entre le volume de lait consommé et le contenu en gras du lait est statistiquement significative ($p = 0{,}005$). Les enfants consommant du lait 1 % doivent boire 2,46 tasses (intervalle de confiance (IC) 95 % : 2,38-2,54) de lait pour avoir une concentration de 25(OH)D similaire à celle des enfants qui consomment 1 tasse de lait homogénéisé (3,25 % de gras). Les enfants qui consomment du lait 1 % ont 2,05 plus de chances (IC 95 % : 1,73-2,42) de présenter une concentration de 25(OH)D < 50 nmol/L que les enfants qui consomment du lait homogénéisé. En conclusion, les recommandations selon lesquelles les enfants devraient boire du lait moins gras (1 % ou 2 %) pourraient compromettre le niveau sérique de 25(OH)D et devraient entraîner des études pour s'assurer de la santé optimale de l'enfant. [Traduit par la Rédaction]

Mots-clés : vitamine D, pédiatrie, lait de vache, gras, épidémiologie, nutrition.

Received 2 December 2015. Accepted 11 January 2016.

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Introduction

Vitamin D is a fat-soluble steroid that is important for children's growth and development (National Institutes of Health 2011*b*). Insufficient vitamin D in early childhood is believed to place children at risk of health complications, including rickets (Cooper et al. 1997; Pawley and Bishop 2004). Data from the 2012–2013 Canadian Health Measures Survey concluded that 11% of 3- to 5-year-old children had insufficient serum 25-hydroxyvitamin D (25(OH)D) levels, which the Institute of Medicine defines as ≤50 nmol/L (Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2010; Janz and Pearson 2013).

Cow's milk has been identified as the main dietary source of vitamin D for children (Garriguet 2008; Health Canada 2012; Maguire et al. 2013). To prevent vitamin D insufficiency, vitamin D fortification of cow's milk in the United States and Canada is standardized at approximately 100 IU per 250 mL of milk (Faulkner et al. 2000).

Current professional guidelines from the National Institutes of Health and the American Academy of Pediatrics recommend that children between the ages of 1 and 2 years consume 2 cups per day of homogenized milk (3.25% milk fat), and for older children, low-fat (2% or 1%) milk is recommended, to reduce dietary fat and lower the child's risk of obesity (Gidding et al. 2006; National Institutes of Health 2011*a*). Vitamin D is a fat-soluble hormone, and dietary fat is believed to facilitate vitamin D absorption in the gastrointestinal tract (Borel 2003; Dawson-Hughes et al. 2015). Fat stimulates bile secretion, which breaks down lipid globules and allows lipolytic enzymes to work on a greater surface area, thus enhancing fat and fat-soluble vitamin absorption into the bloodstream (Bowen 2001). It has yet to be explored whether the consumption of cow's milk with a higher fat content is associated with higher vitamin D stores in children (Dawson-Hughes et al. 2013, 2015).

In this study, we hypothesized that children who consume milk with a higher fat content would have higher serum 25(OH)D relative to children who consume milk with a lower fat content. Our primary objective was to evaluate the relationship between the fat content of cow's milk consumed and serum 25(OH)D in early childhood. Our secondary objective was to determine if the known relationship between the volume of milk consumed and 25(OH)D was modified by the percent fat content of the milk.

Materials and methods

Subjects and design

This cross-sectional study used data from TARGetKids! (The Applied Research Group for Kids). TARGetKids! is a practice-based research network and collaboration between researchers in the Faculty of Medicine at the University of Toronto and primary care practitioners in the university's Department of Paediatrics and Department of Family and Community Medicine (Carsley et al. 2015).

Healthy children were recruited between September 2008 and August 2014 during routine primary health-care visits at 9 primary health-care practices located in Toronto, Ontario, Canada (latitude 43.4°N). Exclusion criteria were conditions affecting growth (e.g., failure to thrive), severe developmental delay, or other chronic health conditions (with the exception of asthma).

Measurements

Clinical data were collected by trained research assistants at each of the participating practices using standardized, parent-completed questionnaires based on the Canadian Community Health Survey (Canadian Community Health Survey - annual component (CCHS) 2013). Research assistants took physical measurements of each child, and trained phlebotomists collected venous blood samples. Serum samples were sent on ice to Mount Sinai Services Laboratory (mountsinaiservices.com) in Toronto, Canada, and were tested for 25(OH)D in batches on a daily basis.

The primary exposure was percent fat content of milk consumed. This was measured by the following request: "Please specify your child's diet for the past 3 days: skim, 1%, 2%, or homogenized milk".

The primary outcome was serum 25(OH)D concentration as a continuous variable, which was measured using a 2-step competitive chemiluminescence assay (LIAISON 25 OH Vitamin D TOTAL, DiaSorin) (Carter et al. 2004). This assay had an intra-assay imprecision of 7.2% at a concentration of 213 nmol/L and an interassay imprecision of 4.9% at 32 nmol/L, 8.9% at 77 nmol/L, and 17.4% at 213 nmol/L, all of which are within acceptable limits for biochemical measurements (Maunsell et al. 2005; Singh et al. 2006). Vitamin D testing was monitored using the UK DEQUAS external quality assessment scheme (Carter et al. 2004). Our secondary outcomes were serum 25(OH)D concentration cut-offs of <50 nmol/L and <75 nmol/L, chosen on the basis of recommendations for optimal serum 25(OH)D concentration from the Institute of Medicine and the Canadian Paediatric Society, respectively (Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2010; Godel 2007). Parathyroid hormone production and calcium reabsorption from bone have been shown to be minimized in most children with a 25(OH)D concentration >75 nmol/L (Godel 2007; Maguire et al. 2013).

Clinically relevant covariates, which might be confounders of the relationship between percent fat content of milk consumed and serum 25(OH)D, were identified through a literature review and were prespecified. These included children's age, sex (Hagenau et al. 2009; Lagunova et al. 2009; Rockell et al. 2005), skin pigmentation (Maguire et al. 2013), body mass index *z* score (*z*BMI), daily vitamin D supplementation, daily volume of milk consumed, date of blood collection, non–cow's milk consumption, and median neighbourhood family income (Statistics Canada 2014). The Fitzpatrick scale, an acceptable method for skin pigmentation quantification used in dermatological research (Fitzpatrick 1988; Quevedo et al. 1975), was used to assess skin pigmentation. Weight was measured with a precision digital scale (±0.025%) (seca, Germany); child length was measured using a calibrated length board for children under 2 years of age, and older children's heights were measured with a stadiometer (seca). Growth curves from the World Health Organization were used for *z*BMI calculation (Mei et al. 2002; Pietrobelli et al. 1998; World Health Organization 2006). Child vitamin D supplementation was defined as currently taking a multivitamin and (or) vitamin D supplement daily. All vitamin D-containing supplements in Canada marketed for children contain 400 IU or 10 μg per daily dose (Health Canada 2007). The volume of milk consumed per day was measured by the question, "How many cups of milk does your child drink in a typical day?". Postal codes were used to generate after-tax median family income using the Statistics Canada Postal Code Conversion File, which was based on the 2011 Canadian census (Map & Data Library 2013).

Statistical analyses

Descriptive analyses were performed for our primary exposure, outcome, and covariates. Univariate linear regression was used to test the unadjusted association between the percent fat content of milk consumed (primary exposure) and child 25(OH)D (primary outcome). Using this model, we chose to compare children who drank homogenized (3.25% fat) milk with those who drank 1% milk, because it was expected that few children of this age would consume skim (0.1% fat) milk. After examining residual plots, 25(OH)D was positively skewed and necessitated a log transformation. For our primary analysis, we used multivariable linear regression adjusted for prespecified biologically plausible sociodemographic, dietary, and environmental factors (as outlined above). All covariates were included in the final model regardless of statistical significance, to prevent biased regression coefficients and falsely inflated R^2 values (Little 2002). To adjust for season, a sinusoidal function was applied to the date of blood sample collection (Barnett and Dobson 2010).

For our secondary analysis, we explored whether the relationship between the volume of milk consumed and 25(OH)D was modified by the percent fat content of the milk. We accomplished this by adding an interaction term between percent fat content of milk and volume of milk consumed to our primary model. This interaction was tested at a significance level of $\alpha = 0.05$. We also repeated the primary and secondary analyses using multivariable logistic regression to explore the relationship between our primary exposure and 25(OH)D cut-offs of 50 and 75 nmol/L, as recommended by the Institute of Medicine and the Canadian Paediatric Society, respectively (Godel 2007; Institute of Medicine 2015).

To assess multicollinearity, the variance inflation factor (VIF) was used (O'Brien 2007). All covariates had VIF values <3. Missing data were assumed to satisfy the missing-at-random criteria, so multiple imputation was used on 50 data sets to minimize bias from the missing data (Little 2002). No variable contained more than 11% missing information. Data analysis was performed using R version 3.1.1 (R Core Team 2014).

The research ethics boards of The Hospital for Sick Children and St. Michael's Hospital approved this study, and consent was obtained from all parents of participating children.

Results

Of the 6320 children who consented to participate in the TARGetKids! cohort, blood samples were obtained and from analyzed for 2857 participants, who were included in the analysis. The characteristics of the children with and without blood sampling appeared to be clinically similar (Table 1**)**. The mean age of the participants was 2.8 years, and 52.7% were male. Homogenized milk (3.25% fat) was consumed by 48.5% of the children, followed by 2% milk consumed by 34.8%, 1% milk consumed by 12.0%, and skim milk consumed by 4.6%. On average, the children drank 2.1 cups of milk per day. The mean serum 25(OH)D concentration was 87 nmol/L, and 54.1% of the children were consuming a daily vitamin D supplement. Children with a serum 25(OH)D level <75 nmol/L constituted 37.5% of the population, and 5.9% of children had a serum 25(OH)D level <50 nmol/L.

Results of the primary analysis are displayed in Table 2. Multivariable linear regression identified that the percent fat content of milk was positively associated with serum 25(OH)D ($p = 0.03$). Each 1% increase in milk fat content was associated with a 1.52% (95% confidence interval (CI) 0.83%–2.21%) higher 25(OH)D. For example, children who drank homogenized milk had a 4.1 nmol/L (95% CI 3.57–4.74) higher median 25(OH)D than that of children who drank the same volume of 1% milk (Fig. 1). Covariates positively associated with serum 25(OH)D included vitamin D supplementation ($p < 0.0001$), light skin pigmentation ($p < 0.0001$), higher volume of cow's milk consumption ($p < 0.0001$), lower *z*BMI ($p = 0.01$), and median neighbourhood family income between \$80 000 and \$150 000 ($p = 0.03$).

Our secondary analysis (Table 2) showed that the interaction between daily volume of milk consumption and percent fat content of the milk consumed was also statistically significant ($p = 0.005$). This suggested that milk fat content was an effect modifier of the relationship between the volume of milk consumed and serum 25(OH)D. For children who consumed 1 cup of milk per day, those drinking milk with 1% higher fat had a 2.79 nmol/L (95% CI 1.22–4.39) higher median serum 25(OH)D (Fig. 2). Children who drank 1% milk needed to consume 2.46 cups (95% CI 2.38–2.54) of milk to have a 25(OH)D similar to that of children who drank 1 cup of homogenized milk (3.25% fat).

Exploration of 25(OH)D cut-offs of <50 nmol/L revealed that the odds ratio for serum 25(OH)D <50 nmol/L was 1.25 (95% CI 0.14–1.35) per 1% lower fat content of milk consumed. For example, children drinking 1% milk had 2.05 (95% CI 1.73–2.42) times higher odds of having a serum 25(OH)D concentration <50 nmol/L compared with children drinking homogenized milk. When we assessed 25(OH)D at <75 nmol/L, we did not identify an association between percent fat content of milk consumed and serum 25(OH)D concentration <75 nmol/L (odds ratio 1.07; 95% CI 0.98–1.16).

Table 1. Characteristics of children who participated in the study and nonparticipants.

Child characteristics	Children with blood sample, *n* = 2857	Children without blood sample, *n* = 3463
Age, mo	33.9±16.3	33.6±16.8
Sex, male	1506 (53)	1810 (52)
25(OH)D, nmol/L	86.7±30.0	na
Percent fat content of milk		
Skim	131 (5)	120 (3)
1% fat	344 (12)	311 (9)
2% fat	996 (35)	1256 (36)
Homogenized	1386 (49)	1776 (51)
*z*BMI	0.2±1.0	0.2±1.0
Skin pigmentation, Fitzpatrick scale ≤3	2247 (79)	2606 (86)
Cow's milk (cups/d)	2.1±1.1	2.0±1.0
Vitamin D daily supplementation (yes)	1547 (54)	1660 (48)
Median neighbourhood family income		
<\$30 000	177 (6)	188 (5)
\$30 000–\$79 999	2111 (74)	2493 (72)
\$80 000–\$149 999	333 (12)	464 (13)
≥\$150 000	23 (1)	43 (1)
Ethnicity		
Western	1889 (66)	2415 (75)
East/Southeast Asian	277 (10)	327 (10)
Southwest Asian	207 (7)	206 (6)
African/Caribbean	116 (4)	114 (4)
Mixed Western/non-Western	132 (5)	137 (4)

Note: Data are presented as means ± SD or *n* (%). 25(OH)D, 25-hydroxyvitamin D; na, not applicable; *z*BMI, body mass index *z* score.

Discussion

In this study, we identified a relationship between higher fat content of milk and higher serum 25(OH)D in early childhood. Children who consumed homogenized milk had a 4.1 nmol/L higher median 25(OH)D than that of children consuming the same volume of 1% milk. Furthermore, milk fat content appeared to modify the relationship between the volume of milk consumed and serum 25(OH)D. For children drinking 1 cup of milk, each 1% higher milk fat was associated with a 2.8 nmol/L higher serum 25(OH)D, which is similar to the effect of an additional cup of milk (Maguire et al. 2013). Children who drank 1% milk needed to consume more than double the volume of milk to have the same 25(OH)D as children who drank homogenized milk. We also found that children who drank 1% milk had a 2-fold increased odds of serum 25(OH)D being <50 nmol/L, relative to children drinking homogenized milk.

Given that vitamin D is a fat-soluble vitamin, we hypothesized that higher milk fat may be associated with higher serum 25(OH)D concentration through increased jejunal absorption of vitamin D in the presence of higher dietary fat (Borel 2003; Dawson-Hughes et al. 2015). The results of our primary and secondary analyses were consistent with this hypothesis. Several other studies have examined the effect of dietary fat on large supplemental doses of vitamin D, but they yielded inconsistent results regarding the relationship between dietary fat and serum vitamin D concentration in adults (Dawson-Hughes et al. 2013, 2015; Tangpricha et al. 2003). To the best of our knowledge, the relationship between the

Table 2. Multivariable linear regression.

	Without interaction*			With interaction*		
Child characteristics	Change in 25(OH)D, % (95% CI)	Change in median 25(OH)D, nmol/L (95% CI)	*p*	Change in 25(OH)D, % (95% CI)	Change in median 25(OH)D, nmol/L (95% CI)	*p*
Percent fat content of milk	1.52 (0.83 to 2.21)	1.26 (0.16 to 2.33)	0.0266	3.41 (1.49 to 5.36)	2.79 (1.22 to 4.39)	0.0004
Age (mo)	−0.01 (−0.10 to 0.10)	−0.01 (−0.08 to 0.08)	0.8558	−0.01 (0 to 0)	−0.01 (0 to 0)	0.8907
Sex (male)	−1.77 (−4.11 to 0.60)	−1.45 (−3.37 to 0.49)	0.1465	−1.71 (−3.92 to 1.01)	−1.40 (−3.22 to 0.82)	0.1661
*z*BMI	−1.57 (−2.76 to −0.40)	−1.29 (−2.26 to −0.33)	0.0115	−1.55 (−2.42 to −0.40)	−1.27 (−2.96 to −0.33)	0.0123
Season (sine month)	0.57 (−1.90 to 2.33)	0.47 (−0.90 to 1.91)	0.5030	0.59 (−1.00 to 2.02)	0.49 (−0.82 to 1.66)	0.4772
Skin pigmentation (Fitzpatrick scale ≤3)	9.48 (5.87 to 13.20)	7.78 (4.81 to 10.83)	<0.0001	9.43 (6.18 to 12.75)	7.73 (5.07 to 10.45)	<0.0001
Volume of milk consumed (cups/d)	3.61 (2.33 to 4.81)	2.96 (1.91 to 3.95)	<0.0001	8.37 (5.13 to 11.63)	6.87 (4.20 to 9.53)	<0.0001
Vitamin D daily supplementation (yes)	11.26 (8.55 to 14.0)	9.23 (7.01 to 11.48)	<0.0001	11.20 (8.33 to 13.88)	9.19 (6.83 to 11.38)	<0.0001
Non-cow's milk consumption (yes)	−3.07 (−7.23 to 1.21)	−2.52 (−5.93 to 0.99)	0.1600	−2.74 (−6.76 to 2.02)	−2.25 (−5.54 to 1.66)	0.2128
Median neighbourhood family income						
<$30 000	−4.57 (−9.34 to 0.50)	−3.75 (−7.65 to 0.41)	0.0744	−4.72 (−9.52 to 0.30)	−3.87 (−7.80 to 0.25)	0.0638
$30 000–79 999	Reference value					
$80 000–149 999	4.41 (0.5 to 8.55)	3.62 (0.41 to 7.01)	0.0275	4.49 (1.00 to 8.33)	3.68 (0.82 to 6.83)	0.0239
≥$150 000	−2.71 (−15.21 to 11.63)	−2.22 (−5.93 to 0.99)	0.6957	−3.39 (−15.63 to 10.52)	−2.78 (−12.82 to 8.62)	0.6124
Interaction: type of milk consumption × volume of milk consumed	na			−1.80 (−2.96 to −1.00)	−1.48 (−2.42 to 0.82)	0.0050

Note: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; na, not applicable; *z*BMI, body mass index *z* score.
*The interaction analyzed was between the percent fat content of the milk and the volume of milk consumed.

Fig. 1. Association between percent fat content of milk consumed and child serum 25-hydroxyvitamin D, adjusted for age, sex, body mass index *z* score, season of serum collection, skin pigmentation, daily vitamin D supplementation, non-cow's milk consumption, and median neighbourhood family income. Because of milk volume adjustment, serum 25-hydroxyvitamin D values reflect average intakes of each fat content of milk. Shaded area indicates 95% confidence interval.

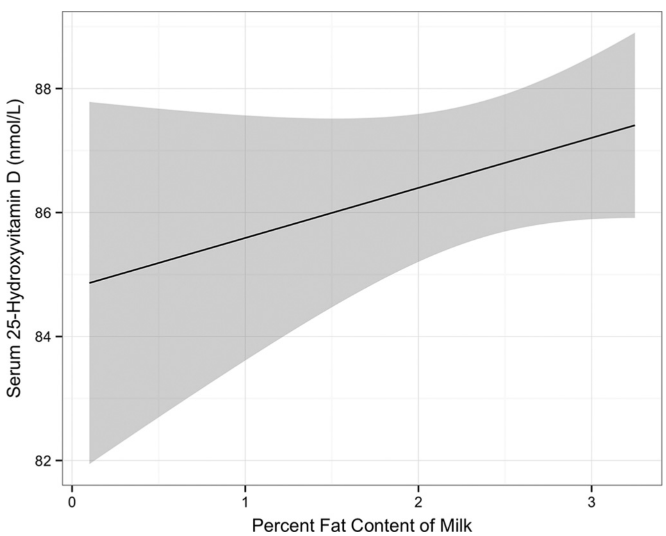
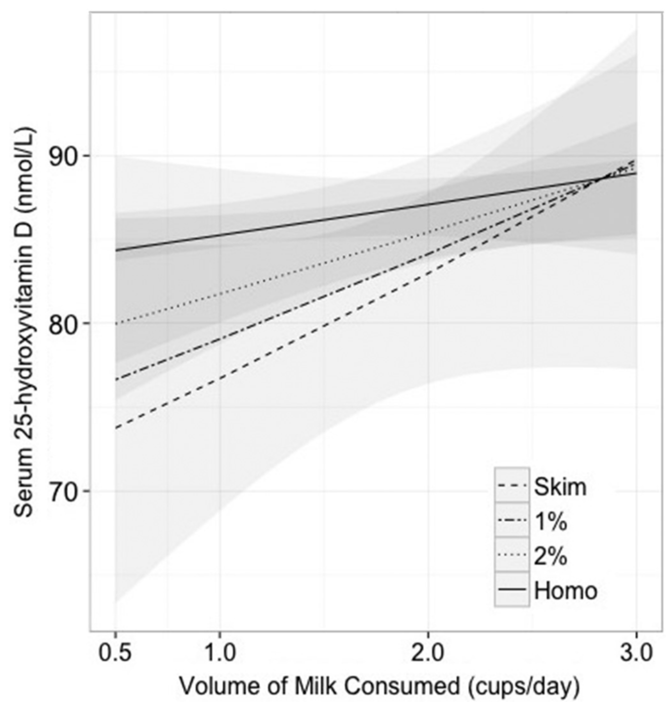


Fig. 2. Association between volume of milk consumed (cups/d) and serum 25-hydroxyvitamin D (nmol/L), adjusted for age, sex, body mass index *z* score, season of serum collection, skin pigmentation, daily vitamin D supplementation, non-cow's milk consumption, and median neighbourhood family income. The interaction analyzed was between the percent fat content of the milk and the volume of milk consumed.



fat content of cow's milk and children's vitamin D stores has not been studied previously.

The National Institutes of Health and the American Academy of Pediatrics recommend that children between 1 and 2 years of age consume homogenized milk to ensure ideal growth and development (National Institutes of Health 2011*a*). Older children are advised to drink reduced-fat (1% or 2%) milk to maintain a lower-fat diet, specifically limiting cholesterol and saturated fat (Gidding et al. 2006). Although these recommendations were intended to have a positive effect on childhood obesity (Huh et al. 2010; National Institutes of Health 2011*a*; Scharf et al. 2013), our results suggest that they may have had the unintentional effect of reducing children's vitamin D stores. Children consuming lower-fat milk may benefit from vitamin D supplementation, particularly those with other risk factors for vitamin D deficiency (American Academy of Pediatrics 2014). We hope the results of this study will create dialogue around the current guidelines on milk fat recommendations for children.

One of the strengths of our study is that we included data from a large, healthy cohort from an urban North American primary care research network. Our sample size, combined with clinically rich data, allowed us to have sufficient power to account for numerous biologically plausible potential confounders.

The limitations of our study include its cross-sectional design; thus, causation cannot be concluded from our results. Parent-reported questionnaire data may have been affected by recall bias. The majority of children consumed moderate amounts (about 2 cups per day) of higher-fat milk (2% or 3.25% fat), with fewer children drinking low-fat (skim or 1%) milk, or very high or low milk volumes, which may have limited our statistical power at the extremes. Our population was from 1 North American urban setting and may not be representative of other urban populations of children. Overall, 54% of participants consumed a daily vitamin D-containing supplement, which may have resulted in a relatively high mean serum 25(OH)D concentration.

We identified an association between higher milk fat content and higher 25(OH)D in early childhood. Children consuming lower-fat milk may be at risk of vitamin D deficiency and may benefit from drinking a higher volume of milk or consuming a daily vitamin D supplement. These findings may be informative for future guidelines on healthy milk consumption for children and may be clinically important at both the individual and the population level.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors thank all of the participating families and practitioners, and the pediatric and family medicine practices that are currently involved in the TARGetKids! research network. They also thank the TARGetKids! Collaboration Steering Committee (Tony Barozzino, Brian Chisamore, Mark Feldman and Moshe Ipp); the research team (Matthew D'Ascanio, Diviya Elango, Nadia Kabir, Kanthi Kavikondala, Tarandeep Malhi, Laurie Thompson and Mandy Tran); Magda Melo and Patricia Nguyen at the Applied Health Research Centre; and Azar Azad at the Mount Sinai Services Central Laboratory. Shelley M. Vanderhout and Jonathon L. Maguire designed the research study, performed the statistical analyses, wrote the paper, and had primary responsibility for the final content.

Author contributions: Catherine S. Birken, Patricia C. Parkin, and Deborah L. O'Connor assisted in refining the study design and reviewed and revised the manuscript. Gerald Lebovic and Yang Chen reviewed and revised the statistical analyses and the manuscript. All authors read and approved the final version.

Funding: Funding of the TARGet Kids! research network was provided by the Canadian Institutes of Health Research (CIHR) Institute of Human Development Child and Youth Health (grant number MOP-106532), the CIHR Institute of Nutrition, Metabolism and Diabetes, and the St. Michael's Hospital Foundation. The Paediatric Outcomes Research Team is supported by a grant from The Hospital for Sick Children Foundation. The funding agencies had no role in the design and conduct of the study; the collection, management, analysis, and interpretation of the data; or the preparation, review, and approval of the manuscript.

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