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

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ABSTRACT

Background and Objectives

Current guidelines for cow's milk consumption in children > age 2 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 vitamin D is fat-soluble, we hypothesized 25-hydroxyvitamin D (25(OH)D) concentration to be positively associated with fat content of milk. The objective was to determine the relationship between fat content of milk consumed and serum 25(OH)D concentration; secondarily, to explore the role volume of milk consumed played in this relationship.

Methods

We completed a cross-sectional study of children 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.

Results

2857 children were included in the analysis. Fat content of milk was positively associated with 25(OH)D ($p=0.03$), and the interaction between volume of milk consumed and milk fat content was statistically significant ($p=0.005$). Children who drank 1% milk needed 2.46 cups (95% CI 2.38-2.54) of milk to have similar 25(OH)D as 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 25(OH)D concentration <50 nmol/L than children who consumed homogenized milk.

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

INTRODUCTION

Vitamin D is a fat soluble steroid that is important for children's growth and development (National Institutes of Health 2011b). Insufficient vitamin D in early childhood is believed to place children at risk for health complications, including rickets (Cooper 1997; Pawley & Bishop 2004). Data from the 2012-2013 Canadian Health Measures Survey identified that 11% of three to five year old children had insufficient serum 25-hydroxyvitamin D levels, which the Institute of Medicine defines as ≤ 50 nmol/L (F. a. N. B. Institute of Medicine 2010; Janz 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 one and two 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 a child's risk of obesity (Gidding et al. 2006; National Institutes of Health 2011a). Vitamin D is a fat soluble hormone and dietary fat is believed to facilitate vitamin D absorption in the GI tract (Borel 2003; Dawson-Hughes et al. 2014). 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 consumption of higher fat content cow's

milk is associated with higher vitamin D stores in children (Dawson-Hughes et al. 2014; Dawson-Hughes et al. 2013).

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

METHODS AND STATISTICS

Subjects and design

This cross-sectional study utilized 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. 2014).

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

Measurements

Clinical data was collected using a standardized, parent completed questionnaires based on the Canadian Community Health Survey ("Canadian Community Health Survey - annual component (CCHS)" 2013) by trained research assistants at each of the participating practices. 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 tested for 25-hydroxyvitamin D in batches on a daily basis.

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

The primary outcome was serum 25-hydroxyvitamin 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 inter-assay 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-hydroxyvitamin D concentration cutoffs of <50 nmol/L and <75 nmol/L, based on recommendations for optimal serum 25-hydroxyvitamin 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 25-hydroxyvitamin D concentration $> 75\text{nmol/L}$ (Godel 2007; Maguire et al. 2014).

Clinically relevant covariates, which might be confounders of the relationship between percent fat content of milk consumed and serum 25-hydroxyvitamin D, were identified through a literature review and pre-specified. These included: children's age, sex (Hagenau et al. 2009; Lagunova et al. 2009; Rockell et al. 2005), skin pigmentation (Maguire et al. 2013), BMI z-score (zBMI), 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 was used to assess skin pigmentation, an acceptable method for skin pigmentation quantification used in dermatological research (Fitzpatrick 1988; Quevedo et al. 1975). Weight was measured with a precision digital scale ($\pm 0.025\%$; SECA); child length using a calibrated length board for children under 2 years, and older children's heights were measured with a stadiometer (SECA, Germany). Growth curves from the World Health Organization were utilized for zBMI calculation (Mei et al. 2002; Pietrobelli et al. 1998; World Health Organization 2006). Child vitamin D supplementation was measured 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 micrograms per daily dose (Health Canada 2007). 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 analysis

Descriptive statistics were performed for our primary exposure, outcome and covariates. Univariate linear regression was used to test the unadjusted association between percent fat content of milk consumed (primary exposure) and child 25-hydroxyvitamin D (primary outcome). Using this model, we chose to compare children who drank homogenized (3.25% fat) milk to 1% milk, as it was expected that few children of this age would consume skim (0.1% fat) milk. After examining residual plots, 25-hydroxyvitamin D was positively skewed and necessitated a log transformation. For our primary analysis, we used multivariable linear regression adjusted for pre-specified biologically plausible sociodemographic, dietary, and environmental factors (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 2010).

For our secondary analysis we explored whether the relationship between volume of milk consumed and 25-hydroxyvitamin D was modified by percent fat content of 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 analysis using multivariable logistic regression to explore the relationship between our primary exposure and 25-hydroxyvitamin D cutoffs 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 was assumed to satisfy the missing at random criteria, so multiple imputation was used on 50 datasets to minimize bias from 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 Board of both 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 analyzed for 2857 participants, who were included in the analysis. Characteristics of children with and without blood sampling appeared clinically similar (see Table 1). The mean age of participants was 2.8 years, and 52.7% were male. Homogenized milk (3.25% fat) was consumed by 48.5% of children, followed by 2% milk consumed by 34.8%, 1% milk consumed by 12.0%, and skim milk consumed by 4.6% of children respectively. On average, children drank 2.1 cups of milk per day. The mean serum 25-hydroxyvitamin D concentration was 87 nmol/L, and 54.1% of children were consuming a daily vitamin D supplement. Children with serum 25-hydroxyvitamin D less than 75 nmol/L constituted 37.5% of the population, and 5.9% of children had serum 25-hydroxyvitamin D less than 50 nmol/L.

Results of the primary analysis are displayed in Table 2. Multivariable linear regression identified that percent fat content of milk was positively associated with serum 25-hydroxyvitamin D ($p=0.03$). Each 1% increase in milk fat content was associated with

a 1.52% (95%CI: 0.83-2.21%) higher 25-hydroxyvitamin D. For example, children who drank homogenized milk had a 4.1 nmol/L (95%CI: 3.57-4.74) higher median 25-hydroxyvitamin D than children who drank the same volume of 1% milk (see Figure 1). Covariates positively associated with serum 25-hydroxyvitamin 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 zBMI ($p=0.01$) and median neighbourhood family income between \$80,000 and \$150,000 ($p=0.03$).

For our secondary analysis (see Table 2), 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 volume of milk consumed and serum 25-hydroxyvitamin 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-hydroxyvitamin D (see Figure 2). Children who drank 1% milk needed to consume 2.46 cups (95% CI 2.38-2.54) of milk to have similar 25-hydroxyvitamin D as children who drank 1 cup of homogenized milk (3.25% fat).

Exploration of 25-hydroxyvitamin D cutoffs of <50 nmol/L revealed that the odds ratio for serum 25-hydroxyvitamin D <50 nmol/L was 1.25 (95% CI 1.14-1.35) 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 serum 25-hydroxyvitamin D concentration <50 nmol/L than children drinking homogenized milk. When we assessed 25-hydroxyvitamin 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 (OR=1.07, 95% CI 0.98-1.16).

DISCUSSION

In this study we have identified a relationship between higher fat content of milk and higher serum 25-hydroxyvitamin D in early childhood. Children who consumed homogenized milk had a 4.1 nmol/L higher median 25-hydroxyvitamin D than children consuming the same volume of 1% milk. Further, milk fat content appeared to modify the relationship between volume of milk consumed and serum 25-hydroxyvitamin D. For children drinking 1 cup of milk, each 1% higher milk fat was associated with a 2.8 nmol/L higher serum 25-hydroxyvitamin 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-hydroxyvitamin 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-hydroxyvitamin D less than 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-hydroxyvitamin D concentration through increased jejunal absorption of vitamin D in the presence of higher dietary fat (Borel 2003; Dawson-Hughes et al. 2014). Results from our primary and secondary analyses were consistent with this hypothesis. A few other studies have examined the effect of dietary fat on large supplemental doses of vitamin D, which yielded inconsistent results on the relationship between dietary fat and serum vitamin D concentration in adults (Dawson-Hughes et al. 2014; Dawson-Hughes et al. 2013; Tangpricha et al. 2003). The relationship between the fat content of cows 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 2011a). 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). While these recommendations were intended to have a positive effect on childhood obesity (Huh et al. 2010; National Institutes of Health 2011a; Scharf et al. 2013), our results suggest that they may have 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 dialog around current guidelines on milk fat recommendations for children.

Strengths of our study include 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.

Limitations of our study include the 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 two 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 one urban 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-hydroxyvitamin D concentration.

We have identified an association between higher milk fat content and higher 25-hydroxyvitamin D in early childhood. Children consuming lower fat milk may be at risk for 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 individual and population levels.

The authors declare that there are no conflicts of interest.

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Shelley Vanderhout and Jonathon Maguire designed the research study, performed statistical analyses, wrote the paper, and had primary responsibility for final content.

Catherine Birken, Patricia Parkin and Deborah O'Connor assisted in refining the study design, and reviewed and revised the manuscript.

Gerald Lebovic and Yang Chen reviewed and revised statistical analysis as well as the manuscript.

All authors have read and approved the final manuscript.

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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, months, mean $\pm$ SD	33.9 $\pm$ 16.3	33.6 $\pm$ 16.8
Sex, males, no. (%)	1506 (53)	1810 (52)
Child 25(OH)D, nmol/L, mean	86.7 $\pm$ 30.0	N/A
Percent fat content of milk		
Skim, no. (%)	131 (5)	120 (3)
1%, no. (%)	344 (12)	311 (9)
2%, no. (%)	996 (35)	1256 (36)
Homo, no. (%)	1386 (49)	1776 (51)
Child zBMI, mean $\pm$ SD	0.2 $\pm$ 1.0	0.2 $\pm$ 1.0
Skin pigmentation, Fitzpatrick scale $\leq$ 3, no. (%)	2247 (79)	2606 (86)
Cow's milk, cups/day, mean $\pm$ SD	2.1 $\pm$ 1.1	2.0 $\pm$ 1.0
Child vitamin D daily supplementation, yes, no. (%)	1547 (54)	1660 (48)
Median Neighbourhood Family Income		
Less than \$30,000, no. (%)	177 (6)	188 (5)
\$30,000-79,999, no. (%)	2111 (74)	2493 (72)
\$80,000-\$149,999, no. (%)	333 (12)	464 (13)
\$150,000 or more, no. (%)	23 (1)	43 (1)
Ethnicity		
Western, no. (%)	1889 (66)	2415 (75)
East/South East Asian, no. (%)	277 (10)	327 (10)
Southwest Asian, no. (%)	207 (7)	206 (6)
African/Caribbean, no. (%)	116 (4)	114 (4)
Mixed Western/Non-Western, no. (%)	132 (5)	137 (4)

TABLE 2
Multivariable Linear Regression

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

[†]The interaction analyzed was between percent fat content of milk and volume of milk consumed.

Figure 1: Adjusted* association between percent fat content of milk consumed and child serum 25-hydroxyvitamin D.

Legend for Figure 1: *Adjusted for age, sex, BMI z-score, season of serum collection, skin pigmentation, daily vitamin D supplementation, non-cow's milk consumption, and median neighbourhood family income. Due to milk volume adjustment, serum 25-hydroxyvitamin D values reflect average intakes of each fat content of milk. Shaded area indicates 95% CI.

Figure 2: Adjusted* association between volume of milk consumed (cups/day) and serum 25-hydroxyvitamin D (nmol/L) with interaction.†

Legend for Figure 2:

---	Skim milk
- . -	1% milk
.....	2% milk
————	Homo milk

*Adjusted for age, sex, BMI 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 percent fat content of milk and volume of milk consumed.