Prevalence and predictors of low vitamin D concentrations in urban Canadian toddlers

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OBJECTIVES: To determine the prevalence of low vitamin D concentrations in a cohort of healthy two-year-old children living in a large Canadian city, and to explore whether body mass index (BMI) and cow's milk intake are associated with low vitamin D concentrations.
METHODS: A cross-sectional study was performed on healthy two-year-old children attending a well-child visit in Toronto, Ontario (latitude 43.4°N). Dietary exposures were measured by questionnaire. The primary outcome was the prevalence of low vitamin D concentrations (25-hydroxyvitamin D concentration of lower than 50 nmol/L or lower than 75 nmol/L).
RESULTS: A total of 91 healthy children 24 to 30 months of age were recruited between November 2007 and May 2008. The prevalence of low vitamin D concentrations (lower than 50 nmol/L) was 32% (29 of 92, 95% CI 22% to 42%) and the prevalence of vitamin D concentrations of lower than 75 nmol/L was 82% (75 of 91, 95% CI 73% to 89%). Using multivariable logistic regression, the odds of vitamin D concentrations being lower than 50 nmol/L decreased by 0.44 (95% CI 0.2 to 0.96) for each additional cup of cow's milk intake per day and increased by 1.2 to 2.6 per unit BMI depending on BMI level (P=0.07).
CONCLUSIONS: A total of 30% to 80% of toddlers in the present study's urban Canadian setting demonstrated low vitamin D concentrations – the highest prevalence of low vitamin D in toddlers outside of Alaska. Modifiable factors associated with low vitamin D were lower cow's milk intake and higher BMI. The vitamin D status of toddlers in urban Canada may require specific attention.

Key Words: *Obesity; Primary care; Toddlers; Vitamin D*

La prévalence et les prédicteurs de faibles concentrations de vitamine D chez les tout-petits des régions urbaines canadiennes

OBJECTIFS : Déterminer la prévalence de faibles concentrations de vitamine D au sein d'une cohorte d'enfants en santé de deux ans qui vivent dans une grande ville canadienne et explorer si l'indice de masse corporelle (IMC) et la consommation de lait de vache s'associent à de faibles concentrations de vitamine D.
MÉTHODOLOGIE : Les chercheurs ont mené une étude transversale auprès d'enfants en santé de deux ans de Toronto, en Ontario (latitude 43,4 °N) qui se présentaient à leur visite d'enfant en santé. Ils ont mesuré leur exposition alimentaire par questionnaire. L'issue primaire était la prévalence de faibles concentrations de vitamine D (concentration de 25-hydroxyvitamine D inférieure à 50 nmol/L ou à 75 nmol/L).
RÉSULTATS : Au total, les chercheurs ont recruté 91 enfants en santé de 24 à 30 mois entre novembre 2007 et mai 2008. La prévalence de faibles concentrations de vitamine D (inférieure à 50 nmol/L) était de 32 % (29 sur 92, 95 % IC 22 % à 42 %) et la prévalence de concentrations de vitamine D inférieures à 75 nmol/L était de 82 % (75 sur 91, 95 % IC 73 % à 89 %). Selon la régression logistique multivariable, le risque que les concentrations de vitamine D soient inférieures à 50 nmol/L diminuait de 0,44 (95 % IC 0,2 à 0,96) par tasse supplémentaire de lait de vache consommée par jour et augmentait de 1,2 à 2,6 par unité d'IMC selon le taux d'IMC (P=0,07).
CONCLUSIONS : Au total, de 30 % à 80 % des tout-petits de la présente étude en milieu urbain canadien présentaient de faibles concentrations de vitamine D, soit la plus forte prévalence de faibles concentrations de vitamine D chez des tout-petits à l'extérieur de l'Alaska. Les facteurs modifiables associés au faible taux de vitamine D étaient une faible consommation de lait de vache et un IMC plus élevé. Il faudrait peut-être s'intéresser tout particulièrement au taux de vitamine D des tout-petits des régions urbaines du Canada.

The role of vitamin D in skeletal development and maintenance of normal serum calcium levels is well established (1). The risk of rickets is seemingly very low when serum 25-hydroxyvitamin D concentrations are greater than 50 nmol/L in children (2). Largely based on this finding, the American Academy of Pediatrics (AAP) (3) recommends that 25-hydroxyvitamin D concentrations in children be greater than 50 nmol/L, whereas the Canadian Paediatric Society (CPS) (4) has recommended that 25-hydroxyvitamin D concentrations should be greater than 75 nmol/L based on extrapolation from adult data.

There is emerging evidence, from both single-centre studies (5-8) and national surveys (9-11), that children of all ages in North America have vitamin D concentrations that are significantly lower than either the AAP or CPS recommendations (Table 1). However, less is known specifically about the

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TABLE 1
North American studies of low vitamin D including children older than one year of age

First author (reference)	Location	Year(s)	Latitude	Age	Season(s)	n	Vitamin D, nmol/L	
							<50, %	<75, %
Kumar (9)	Nationally representative US sample (NHANES)	2001–2004	NA	1–21 years	Not available	6275	9*	70
Mansbach (10)	Nationally representative US sample (NHANES)	2001–2006	NA	1–5 years	Winter for lower latitudes; summer for higher latitudes	1799	14	63
Gordon (7)	Boston, Massachusetts, US	2005–2007	42°N	8–24 months	All seasons	133	12	40
Gordon (6)	Boston, Massachusetts, US	2001–2003	42°N	11–18 years	All seasons	307	42	NA
Gessner (5)	Alaska, US	2001–2002	58–61°N	6–23 months	All seasons	133	11*	31†
Roth (22)	Edmonton, Alberta	2003	52°N	2–8 years	Spring	35	17*	NA
Newhook (8)	St John's, Newfoundland and Labrador	2005–2006	47°N	0–14 years	Autumn/spring	48	35	77
Langlois (11)	Nationally representative Canadian (CHMS)	2007–2009	NA	6–11 years	All seasons	453	4	51
Maguire (current study)	Toronto, Ontario	2007–2008	43°N	24–30 months	Winter/spring	91	32	82

**Lower than 37.5 nmol/L; †Lower than 62.5 nmol/L. CHMS Canadian Health Measures Survey; NA Not available; NHANES National Health and Nutrition Examination Survey; US United States*

vitamin D status of Canadian urban toddlers during the winter. They may be at particular risk of having low vitamin D concentrations when they transition from vitamin D-supplemented breastfeeding or vitamin D-fortified infant formula to a diet containing vitamin D-fortified cow's milk and solid foods (2).

The primary objective of the study was to determine the prevalence of low vitamin D concentrations in a cohort of healthy two-year-old children who were seen for routine primary care and lived in a large Canadian city during the winter and spring. A secondary objective was to explore associations between low vitamin D concentrations and modifiable factors such as body mass index (BMI) and cow's milk intake.

METHODS

Subjects and design

A cross-sectional study was performed with concurrent measurement of dietary and lifestyle exposures, as well as 25-hydroxyvitamin D concentration by blood test.

Healthy children between 24 and 30 months of age were recruited from a three-physician (SJ, MP, CT) community-based paediatric group practice located in downtown Toronto, Ontario (latitude 43.4°N), during their well-child visits between November 2007 and May 2008. The practice is hospital and university affiliated, and provides primary care for approximately 10,000 children. Exclusion criteria were a birth weight of lighter than 2 kg, and acute or chronic illness (except for asthma).

Outcome variables

The primary outcome was prevalence of low vitamin D concentrations. Two estimates were calculated based on the recommendations of the AAP (25-hydroxyvitamin D concentration of lower than 50 nmol/L) (3) and the CPS (25-hydroxyvitamin D concentration lower than 75 nmol/L) (4). Blood was obtained by venipuncture at the time of the well-child visit, and samples were transported daily on ice to the biochemistry laboratory at The Hospital for Sick Children (Toronto). Serum samples were analyzed for 25-hydoxyvitamin D using liquid chromatography-tandem mass spectrometry, which has been shown to compare favourably with radioimmunoassay methods for children older than one year of age (12). One instrument (4000 Q TRAP LC/MS/MS system, Applied Biosystems, USA) was used for all samples and was regularly calibrated according to the Vitamin D External Quality Assessment Scheme, which is an internationally recognized protocol (13). Intra-assay imprecision was 3.2% and interassay imprecision was 6.8% at a 25-hydroxyvitamin D concentration of 135 nmol/L.

Secondary outcomes included whether BMI and cow's milk intake were associated with low vitamin D concentrations. Data were collected using a standardized data collection form completed by parents. Nutritional survey questions were adapted from the Canadian Community Health Survey (14). Cow's milk intake was queried in cups per day (1 cup = 250 mL). BMI was calculated as the weight in kilograms divided by height in metres squared (15,16). Weight was measured using a precision digital scale (±0.025%; SECA, Germany) and standing height was measured using a stadiometer (SECA).

The following covariates were also collected: age, sex, birth weight, ethnicity, maternal age and education level, duration of breastfeeding, vitamin D supplementation when breastfed, bottle use, daily juice intake, daily multivitamin use, daily snacking (consumption of potato chips or sweets or more than two cups of soda daily), weekly time outdoors, season of serum sampling (winter [November through March] versus spring [April through May]) and skin pigmentation. Skin pigmentation was recorded by a research assistant using the Fitzpatrick scale, which is a skin pigmentation classification system that is widely used in dermatological research (17-19).

Statistical analysis

Using the convenience sample, the prevalence of low vitamin D concentration (based on the AAP and CPS definitions) and its associated 95% CI was estimated. Data were analyzed using SAS 9.0 (SAS Institute Inc, USA) for Windows (Microsoft Corporation, USA). Fisher's exact test and Student's *t* test were used to test for associations between low vitamin D concentrations and categorical and continuous variables, respectively. Logistic regression was used to examine the relationship among milk intake, BMI and low vitamin D concentrations. For the purpose of the present analysis, the most conservative cut-off for vitamin D (25-hydroxyvitamin D concentration of lower than 50 nmol/L) was used as the binary outcome.

The study was approved by the Research Ethics Board of The Hospital for Sick Children, and parents of all participating children consented to participation in the study.

TABLE 2
Subject characteristics

		Vitamin D <50 nmol/L (n=29)					Vitamin D ≥50 nmol/L (n=62)				
	n*	Lower quartile	Median	Upper quartile	Mean ± SD	n (%)	Lower quartile	Median	Upper quartile	Mean ± SD	n (%)
Age of child, months	91	24.10	24.16	24.48	24.30±0.55		24.06	24.40	25.75	25.18±1.67	
Sex	91										
Female						14 (48)					29 (47)
Male						15 (52)					33 (53)
Season of visit	86										
Spring						6 (22)					24 (41)
Winter						21 (78)					35 (59)
Birth weight, kg	90	3.18	3.36	3.60	3.31±0.42		3.04	3.23	3.47	3.32±0.42	
Maternal age, years	91	33.0	37.0	38.0	35.1±4.6		32.0	35.0	38.0	34.3±4.4	
Maternal education	91										
Public school						0 (0)					0 (0)
High school						4 (14)					8 (13)
University or college						25 (86)					54 (87)
Daily cow's milk intake (250 mL cups)	79	0.98	1.50	2.00	1.40±0.67		1.25	1.75	2.06	1.73±0.64	
Daily juice intake (250 mL cups)	72	0.25	0.75	1.00	0.70±0.45		0.31	0.50	0.75	0.65±0.54	
Bottle use	85										
No						22 (81)					41 (71)
Yes						5 (19)					17 (29)
Daily snacking	85										
No						13 (48)					28 (48)
Yes						14 (52)					30 (52)
Weight, kg	85	11.8	13.0	13.6	12.6±1.2		11.5	12.1	1.3.4	12.5±1.4	
Body mass index, kg/m^2	85	15.4	16.6	17.4	16.4±1.4		14.8	15.6	16.5	15.7±1.3	
Feeding at 9 months	88										
Breast only						7 (24)					18 (31)
Bottle only						16 (55)					29 (49)
Breast and bottle						6 (21)					12 (20)
Ever used vitamin D drops	80										
No						7 (28)					14 (25)
Yes						18 (72)					41 (75)
Multivitamin use	85										
No						21 (78)					37 (64)
Yes						6 (22)					21 (36)
Outdoor time, h/week	85										
0–4						16 (59)					29 (50)
>4						11 (41)					29 (50)
Always uses sunscreen	85										
No						10 (37)					27 (47)
Yes						17 (63)					31 (53)
Skin pigmentation	85										
1–3						23 (85)					48 (83)
4–6						4 (15)					10 (17)

*The lower quartile, median and upper quartile are for continuous variables. *Represents the number of nonmissing values*

RESULTS

Study population

A total of 136 children were approached to participate during their routine two-year well-child visit between November 2007 and May 2008. Of these, 10 children (7%) did not meet the inclusion criteria because of acute illness, 26 parents (19%) did not consent and nine children (7%) had an insufficient quantity of blood drawn. Therefore, 91 children (67%) had anthropometric data, survey data and bloodwork completed, and were included in the final analysis. The population was mainly Caucasian (70%) with highly educated mothers (87% had university or college education) and a high frequency of breastfeeding at nine months of age (47%). Subject characteristics are presented in Table 2.

Prevalence of low vitamin D concentration

The median vitamin D concentration was 60 nmol/L, ranging from 20 nmol/L to 126 nmol/L. Low vitamin D concentration (lower than 50 nmol/L) was found in 29 of 91 children (32%, 95% CI 22% to 42%). One child had a vitamin D concentration of lower than 25 nmol/L (1%, 95% CI 0% to 6%) and 75 children had a vitamin D concentration of lower than 75 nmol/L (82%, 95% CI 73% to 89%).

Variables associated with low vitamin D concentration

Comparison of the demographic characteristics of patients with 25-hydroxyvitamin D concentrations of lower than 50 nmol/L versus 50 nmol/L or greater are found in Table 2. Children with

TABLE 3
Logistic regression model for low vitamin D concentration (lower than 50 nmol/L)

Characteristic	OR* (95% CI)	P
Cow's milk intake (per 250 mL cup daily)	0.44 (0.2–0.96)	0.04
Body mass index 14–15 kg/m^2	2.6 (0.8–8.3)	0.07†
Body mass index 17–18 kg/m^2	1.2 (0.6–2.6)	

**OR represents the increase in odds per unit predictor; †P value includes two degrees of freedom (body mass index 14 kg/m^2 to 15 kg/m^2, and 17 kg/m^2 to 18 kg/m^2)*

low vitamin D concentrations were slightly younger (median 24.2 months versus 24.4 months, P=0.01), more likely to consume a smaller daily volume of cow's milk (median 1.5 cups versus 1.75 cups, P=0.049), and had a higher BMI (median BMI 16.6 kg/m^2 versus 15.6 kg/m^2, P=0.05). There was a trend toward low vitamin D concentrations being more common during the winter than during the spring (78% versus 22%), but this was not statistically significant (P=0.15).

Multivariable analysis

Results of the logistic regression analysis are shown in Table 3. Lower cow's milk intake and higher BMI were independently associated with low vitamin D concentrations (total model P=0.035). There was no interaction between milk intake and BMI (likelihood ratio test P=0.53). Exploratory analysis suggested that the relationship between milk intake and low vitamin D was linear. Nonlinearity was demonstrated in the relationship between BMI and a low vitamin D concentration. To describe the nonlinearity, ORs for low vitamin D concentration per unit of change in BMI are presented separately for a BMI of 14 kg/m^2 to 15 kg/m^2 (below the 50th percentile for this age group representing normal weight) and 17 kg/m^2 to 18 kg/m^2 (above the 85th percentile for this age group representing overweight) in Table 3.

DISCUSSION

The results of the present cross-sectional study show that 32% of a cohort of healthy two-year-old urban children in Toronto had 25-hydroxyvitamin D concentrations below those recommended by the AAP (lower than 50 nmol/L) and 82% had concentrations below those recommended by the CPS (lower than 75 nmol/L) during the winter and spring. With regard to modifiable risk factors associated with low vitamin D concentrations, our findings suggest that lower cow's milk intake and higher BMI are associated with low vitamin D concentrations in this population of toddlers (25-hydroxyvitamin D lower than 50 nmol/L).

The present study is unique because it focused on an urban population of healthy (primarily Caucasian) children in a narrow age range, seen for routine primary care during winter in Canada.

The prevalence of low vitamin D concentrations found in the present cohort of 24-month-old toddlers is higher than that reported for infants and toddlers younger than 24 months of age in Boston (USA) (7), and similar to infants and toddlers in Alaska (5) and children in St John's (Newfoundland and Labrador), which is one of the cloudiest of Canadian cities (8) (Table 1). This difference does not appear to be a function of latitude because Toronto is only 1.5 degrees north of Boston but at least 14 degrees south of Alaska. The difference may be a function of season because we chose to measure vitamin D during the winter and spring; however, we did not find a significant difference in vitamin D concentrations between winter and spring (November through March versus April through May). Furthermore, others (7) have reported a paradoxical relationship between 25-hydroxyvitamin D concentration and season in infants and toddlers, with lower concentrations found in April through September than October through March. Population differences may explain this; however, children in the present study had lighter skin pigmentation and higher socioeconomic status than children from Boston – two factors that are thought to be associated with higher vitamin D concentrations (7,20).

We suggest that age is partly responsible for the higher prevalence of low vitamin D concentrations in the present study's cohort. While Canadian guidelines support supplementation of breastfed infants with 400 IU of vitamin D per day during the first year of life (4,21), the discontinuation of vitamin D supplementation when transitioning to low vitamin D-containing solid foods may have a negative impact on vitamin D status, resulting in a rebound of low vitamin D concentration in the second and third years of life.

Our finding that cow's milk intake was a significant moderator of vitamin D concentration is consistent with this hypothesis because vitamin D-fortified cow's milk (100 IU of vitamin D per 250 mL cup in Ontario) is likely the major dietary source of vitamin D for young children (22). Others have reported a positive association between volume of cow's milk intake and vitamin D levels in toddlers. Gordon et al (7) found a 7.75 nmol/L increase in 25-hydroxyvitamin D level per additional cup of cow's milk ingested by toddlers 12 to 24 months of age, which is similar to our finding of a 6.9 nmol/L increase per cup. Kumar et al (9), using National Health and Nutrition Examination Survey data, reported that children who drank milk less than once per week had an OR for low vitamin D concentration (25-hydroxyvitamin D of lower than 37.5 nmol/L) of 2.9.

The inverse association between BMI and vitamin D that we report is consistent with that reported for adults, adolescents and toddlers (6,7,9,23-26). It has been proposed that increased adiposity decreases vitamin D bioavailability because of deposition of this fat-soluble vitamin in fatty tissues (25).

The present study has several limitations. First, cross-sectional designs can identify associations but cannot determine causality. Second, this population is not generalizable to all urban Canadian children because it is primarily a population of children with lightly pigmented skin who were born to highly educated mothers, seen in a single practice and lived in Toronto. Third, our small convenience sample resulted in relatively wide 95% CIs around the prevalence point estimates. To narrow the 95% CIs to plus or minus 5% would have required a sample size of approximately 250 children.

CONCLUSION

The results of the present study suggest that two-year-old children living in an urban Canadian city are at particular risk for wintertime vitamin D concentrations below those recommended by the AAP and the CPS. It also suggests that young children rely on fortified cow's milk to maintain their vitamin D status. Cow's milk intake is important to the vitamin D status of toddlers. However, it is unclear whether increasing cow's milk intake raises vitamin D concentrations sufficiently to meet suggested concentrations. Universal vitamin D supplementation for children beyond breastfeeding and through childhood may be required, as has recently been recommended by the AAP (3).

AUTHOR CONTRIBUTIONS: The authors completed all aspects of the study (design, conduct, data collection, management, analysis, interpretation, manuscript preparation and

approval) independent of the funding organization. **Study concept and design:** Drs Maguire and Parkin. **Acquisition of data:** Drs Maguire, Parkin, Jacobson, Peer and Taylor. **Analysis and interpretation of data:** Drs Maguire and Parkin, and Mr Thorpe. **Drafting of the manuscript:** Dr Maguire. **Critical revision of the manuscript for important intellectual content:** Drs Parkin, Macarthur and O'Connor. **Statistical analysis:** Drs Maguire and Parkin, and Mr Thorpe. **Obtained funding:** Drs Maguire and Parkin. **Administrative, technical and material support:** Dr Parkin. **Study supervision:** Drs Maguire and Parkin.

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