Original Article

Prevalence and predictors of low serum 25-hydroxyvitamin D levels in rural Canadian children

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Abstract

Objectives: Studies in Canada have reported varying prevalences of low serum 25-hydroxyvitamin D (25(OH)D) levels, but none have been conducted in rural paediatric populations. The purpose of this study was to determine the prevalence and predictors of low vitamin D levels in rural communities.

Methods: We conducted a cross-sectional study of children aged 3 to 15 living in Canadian Hutterite communities. Serum 25(OH)D levels were measured between October 2008 and April 2009 using a chemiluminescence assay. Predictors of vitamin D levels were evaluated using multivariable linear regression. A multilevel model was used to evaluate the impact of individual, household and colony factors on the variation in vitamin D levels.

Results: Serum 25(OH)D levels were available on 743 children/adolescents. The median was 62.0 nmol/L (interquartile range 51.0, 74.0). Levels lower than 50 nmol/L and 75 nmol/L were found in 152 (20.5%) and 565 (76%) children, respectively. Adolescents were at highest risk for levels <75 nmol/L (odds ratio 3.38, 95% confidence interval 2.00, 5.80). Age and latitude were negatively correlated with serum 25(OH)D level. In the multilevel model, most of the variation in levels was associated with individual children.

Conclusion: Low vitamin D levels are a significant problem in rural Hutterite communities in Canada. Adolescents were at greatest risk for low levels and represent an important target group for supplementation. Variation in serum 25(OH)D levels was explained mostly at the individual level. Additional studies are needed to explore factors associated with individuals (e.g., genetics) leading to lower 25(OH)D levels.

Keywords: Canada; Nutrition disorders; Primary care; Serum 25-hydroxyvitamin D; Vitamin D.

Vitamin D is a fat-soluble vitamin important for a variety of cell functions. Its role in bone health and calcium homeostasis is well established and current research has focused on its role in other organ systems. Recent advances have described its association with cardiovascular health (1), autoimmune disease (2) and cancer (3).

With the increasing awareness of its importance, the optimal serum level of 25-hydroxyvitamin D (25(OH)D) has become an area of debate. Current recommendations from the American Academy of Pediatrics (AAP) suggest that serum concentrations of 25(OH)D should be at least 50 nmol/L (4), a

level at which the risk of rickets is low (5). The Canadian Paediatric Society has chosen a higher cut-off of 75 nmol/L (6), based on the levels at which parathyroid hormone production and calcium resorption from the bone are minimized and intestinal calcium absorption is stabilized. There are also several other recommendations from groups such as the Pediatric Endocrine Society (7) and the Institute of Medicine (8). Unfortunately, little is known about the optimal 25(OH)D levels for extra-skeletal health.

Despite available recommendations surrounding serum levels of 25(OH)D and supplementation, evidence suggests that a significant proportion of

children in Canada have low serum 25(OH)D levels, with more than 50% of children being below the Canadian Paediatric Society (CPS) recommendation in most studies (9–14) (Table 1). These studies have been conducted primarily in urban settings and have small sample sizes.

The purpose of the present study was to describe the vitamin D status in Hutterite children in Canada and to identify potential determinants of vitamin D status. Given the homogeneity with respect to diet, ethnicity, clothing and sun exposure in Hutterite colonies, we also wanted to determine whether variation in vitamin D levels was associated with individual, household or community characteristics, in order to evaluate whether environmental factors (community, household) or individual factors (e.g., genetics) contributed more to vitamin D status.

METHODS

Population and study design

The study involved a cross-sectional analysis of data collected from a randomized trial of the effect of influenza vaccination of children on infection rates in Hutterite communities. These communities are rural, self-governing and self-sufficient communal groups of Anabaptists that actively isolate themselves from the influence of the outside world. Children aged 3 to 15 years from 46 colonies were randomly assigned by colony to a vaccination group (influenza versus hepatitis A). Vitamin D levels were collected on these children as part of the study protocol unless blood work was refused or specimen collection was unsuccessful. Details of the randomized controlled trial have previously been described (15).

Covariables of interest were age, sex, presence of underlying conditions, season and latitude. Age was analyzed as both a continuous variable and categorized into less than 5 years, 5 to 9 years and 10 to 15 years. The effect of seasonality was based on the date of the vitamin D level sample and categorized into Winter (November to March) and Other (April to October), to be consistent with previous studies in Canada (11) and based on the National Health and Nutrition Examination Survey (NHANES) (16).

Outcome

The primary outcome was prevalence of vitamin D insufficiency, defined by both AAP (<50 nmol/L) and CPS (<75 nmol/L) guidelines. Vitamin D deficiency was defined as a 25(OH)D level lower than 25 nmol/L. Vitamin D concentration was measured using the serum 25(OH)D level, which is considered to be the most stable circulating vitamin D metabolite and the best marker for individual vitamin D status (17). Sera from the venous blood samples from the start of the randomized controlled trial were frozen until batched analysis was performed according to the manufacturer's instructions using the DiaSorin LIAISON chemiluminescence assay.

Statistical analysis

Descriptive statistics were used to examine the study sample characteristics. Continuous variables were reported using the mean and SD for normally distributed variables and the median and range for non-normally distributed data. Number and percent were reported for dichotomous outcomes. Univariable analyses were conducted using the Spearman correlation coefficient for continuous variables and chi-square or Fisher's exact test for dichotomous variables. Vitamin D levels were analyzed as both a continuous variable and dichotomized based on the AAP (<50 nmol/L) and CPS (<75 nmol/L) recommendations. In the multivariable model, generalized estimating equations were used to adjust for the clustering of data at the colony level. The model was created using a stepwise backwards elimination method, which retained variables with P values <0.10. It was decided a priori to always adjust for age and sex. The continuous dependent variable (serum 25(OH)D level) was log-transformed to correct for positive skewness. Model fit was tested by plotting the standardized residuals

against the standardized predicted values, and assessing the homogeneity of variance.

The covariables from the generalized estimating equation model were then used to develop a mixed effects model to analyze the variation at the colony, household and individual levels. The variances between randomly chosen colonies and randomly chosen households within a colony were reported together with 95% confidence intervals (CIs). All analyses were conducted using SPSS version 20 (SPSS Inc., Chicago, IL).

RESULTS

A total of 1186 children aged 3 to 15 years were included in the study. Vitamin D levels were available on 743 (63%) subjects from 43 colonies and 304 households. Almost all vitamin D levels were sampled from October to December (n=724, 97%). Subject characteristics are presented in Table 2. The mean age was 9.3 years (SD 4.3 years); 52.5% were female. Underlying conditions were present in 2.4% (n=18); the most common condition was asthma (n=11). All participants were of the same ethnicity. The colony latitudes ranged from 49.2°N to 54.8°N with a median of 50.0°N.

The mean serum 25(OH)D level was 64.3 ± 19.7 nmol/L (range 12 to 168). Four participants (0.5%) had serum 25(OH)D levels considered deficient (<25 nmol/L). Serum 25(OH)D levels were lower than 50 nmol/L in 152 children (20.5%) and lower than 75 nmol/L in 565 children (76.0%).

In univariable analyses, age, sex and latitude were associated with serum 25(OH)D levels (Table 2). Age ($r=-0.22$, $P<0.001$) and latitude ($r=-0.26$, $P<0.001$) were negatively correlated with serum 25(OH)D levels (Table 3). For every 1° increase in latitude, the serum 25(OH)D level decreased by approximately 4 nmol/L. Participants aged 10 to 15 years were at higher risk for serum 25(OH)D levels lower than 50 nmol/L (odds ratio [OR] 2.11, 95% CI 1.07, 4.16) and lower than 75 nmol/L (OR 3.38, 95% CI 2.00, 5.80) compared with participants aged 3 to 4. Male sex was associated with higher serum 25(OH)D levels. There was no interaction between age and sex.

In the multivariable analysis accounting for clustering of data, age remained an important predictor of vitamin D level (Table 4). Latitude also remained significant, despite the fact that adjusting for clustering (colony) accounted for some of the latitude effect. Sex was no longer an independent predictor of serum 25(OH)D level or levels lower than 50 nmol/L, but remained an important predictor for levels lower than 75 nmol/L with females at increased risk (adjusted OR 1.69, 95% CI 1.07, 2.65, $P=0.024$). There was no interaction between age and sex.

The multilevel model showed that approximately 19% of the variation in log 25(OH)D levels was associated with households and 9% with colonies (Table 5). Most of the variation (72%) was associated with individuals, even after adjusting for age, sex and latitude.

DISCUSSION

We found that in children and adolescents living in Hutterite communities in Canada, low serum 25(OH)D levels were common, with levels <50 nmol/L in 21% of participants and levels <75 nmol/L in 76% of participants. The majority (97%) of serum 25(OH)D levels measurements were taken in October/November, just after when serum 25(OH)D levels peak in the Northern Hemisphere (18). Therefore, it is likely that even more children have levels below the recommendations later in the winter season.

Our findings are consistent with those reported for children and adolescents in St. John's, Newfoundland and Labrador (10). Although at a lower latitude (47°N), St. John's receives the most rain of all major cities in Canada, which may account for its lower vitamin D levels (19). Other studies of the prevalence of insufficiency in children/adolescents in Canada have shown varying results. Studies from Calgary, Alberta (13) and the Canadian Health Measures

Table 1. Canadian studies of vitamin D levels in children

Author (ref.)	Population	n	Female, n (%)	Location	Latitude	Time period	Mean vitamin D level (nmol/L)	Number (%) deficiency		Variables associated with serum 25-hydroxyvitamin D level	
								<25 nmol/L	<50 nmol/L	<75 nmol/L	
Roth et al. (9)	Age 2–16 years presenting to the emergency department	68	29 (43)	Edmonton, Alberta	52°N	Apr 2003	47.2 (95% CI 44–51)	4 (6)	23 (34)*	NA	Vitamin D intake, age, sex
Newhook et al. (10)	Age 0–14 years presenting to hospital and had blood work	48	21 (44)	St. John's, Newfoundland and Labrador	47°N	Sept 2005 and Mar 2006	60.2	0	17 (35)	37 (77)	Season
Langlois et al. (11)	Survey at 15 sites across Canada; paediatric component: age 6–19	1848	906 (49)	Canadian Health Measures Survey	NA	2007–2009	Age 6–11: 75 (95% CI 70–80); age 12–19: 68.1 (95% CI 64–72)	NA	NA	1076 (58)	NA
Maguire et al. (12)	Healthy children 24–30 months; community paediatric office	91	43 (47)	Toronto, Ontario	43°N	Nov 2007–May 2008	Median: 60 (range 20–126)	1 (1)	29 (32)	75 (82)	Age, cow's milk consumption, BMI
Stoian et al. (13)	Healthy children 2–13 years presenting to hospital for elective surgery	1442	580 (40)	Calgary, Alberta	51°N	Jan–Dec 2006	86.1 ± 35.1 (range 10–323)	29 (2)	NA	568 (39)	Age, ethnicity, season, BMI, dietary intake, time outdoors
Mark et al. (29)	Healthy children age 9, 13 and 16	1753	871 (50)	Quebec	45–48°N	Jan–May 1999	Range 11.4–115.9	114 (6.5) [†]	NA	1712 (98)	Age, BMI (girls)

CI Confidence interval; BMI Body mass index; NA Not available

*Less than 40 nmol/L. [†]Less than 27.5 nmol/L

Survey (CHMS) (11) both found that the prevalence of serum 25(OH)D levels lower than 75 nmol/L was lower (39% and 58%, respectively) than in our study. Possible explanations for the discrepant results include differences in socioeconomic status (20) and lower dietary vitamin D (secondary to availability of fortified food) (9), both shown to be risk factors for lower levels of serum 25(OH)D.

Older children were found to have lower serum 25(OH)D levels, a finding consistent with results from two studies in Calgary (9,13). We found children aged 10 to 15 years to be at the highest risk for insufficiency. Age may be a surrogate for weight, with sequestration of vitamin D in body fat leading to lower

serum 25(OH)D levels (21). Although we were not able to adjust for body mass index in the analysis, it is unlikely that this would have contributed in this population, given its low prevalence of obesity (22) and given that other evidence suggests that body fat percentage does not increase systematically in adolescents (23).

Girls had lower serum 25(OH)D levels compared with boys in univariable analysis, but this effect was not noted after adjustment for age and latitude. The risk associated with sex in other studies has varied. Rates of vitamin D deficiency have been high in studies conducted exclusively in female adolescents (24,25). However, in a study in Edmonton, Alberta (9), males were found to be at higher risk. This was a smaller study with a different population (children presenting to an urban emergency centre), which may account for its discrepant results.

Finally, our study is unique in that we were able to examine the effects of the community, household and individual on variation in 25(OH)D levels. The determinants of vitamin D status are likely multifactorial, including environmental (e.g., exposure to sunlight) and individual (e.g., age, sex, genetics) factors. Recent evidence suggests that genetics contributes to the variability in serum 25(OH)D levels (26), although less than expected based on twin studies (27). We found that most of the variation in serum 25(OH)D levels was explained at the individual level, suggesting that other individual-level predictors may be important. Given the relatively homogeneous distributions of ethnicity, diet and skin colour in this population, it is possible that genetic variants, such as vitamin D receptor polymorphisms, account for some of the differences. In fact, recent genome-wide association studies have shown an association between 25(OH)D level in children and single nucleotide polymorphisms in the both the GC and CYP2R1 genes. These genes code for vitamin D binding protein (GC) and vitamin D 25-hydroxylase (CYP2R1), important proteins in the vitamin D synthesis pathway (28,29).

Our study has two main limitations. First, the study was conducted in Hutterite children and adolescents and the results may not be generalizable to other populations. However, the homogeneity of the population allowed us to look at environmental and individual-level factors contributing to variation in vitamin D levels. Secondly, we did not collect data on body mass index, exposure to sunlight or sources of vitamin D intake.

CONCLUSION

We found that vitamin D insufficiency at levels lower than 50 nmol/L and lower than 75 nmol/L is a significant health problem in rural Hutterite communities in Alberta, Saskatchewan and Manitoba, and these populations should be targeted for supplementation. Older age and living at a higher latitude were

Table 2. Baseline characteristics (n=743)

	Value*
Age in years, mean (SD)	9.3 (3.4)
Female	390 (52.5)
Comorbidities	
≥1 comorbidity†	18 (2.4)
Heart/lung disease	13 (1.7)
Asthma	11 (1.5)
Blood disorder	2 (0.3)
Swallowing/choking disorder	1 (0.1)
ASA use	0
Chronic metabolic condition	1 (0.1)
Kidney/liver disease	1 (0.1)
Cancer/immune deficiency	0
Season	
Winter (Nov–Mar)	372 (50.1%)
Other (Apr–Oct)	371 (49.9%)
Colony latitude, median (IQR)	50.5 (49.2–54.8)
Vitamin D level (nmol/L), median (IQR)	62.0 (51.0, 74.0)
Vitamin D cut-offs	
<25 nmol/L	4 (0.5%)
AAP <50 nmol/L	152 (20.5%)
CPS <75 nmol/L	565 (76.0%)

AAP American Academy of Pediatrics; ASA Aspirin; CPS Canadian Pediatric Society; IQR Interquartile range; SD Standard deviation

*Categorical variables presented as number (%); †Comorbidity: heart/lung disease (including asthma), blood disorder, swallowing/choking disorder, ASA use, chronic metabolic condition, kidney/liver disease, immunodeficiency

Table 3. Predictors of serum 25(OH)D levels; univariable analyses

Variable	Serum 25(OH)D level (nmol/L)		Serum 25(OH)D by threshold*	
	Spearman rank coefficient, P	Beta coefficient, P	OR (95% CI)	
			<50 nmol/L	<75 nmol/L
Age (years)	–0.222, P<0.001	–1.39, P<0.001	1.09 (1.03, 1.15)	1.14 (1.08, 1.20)
3–4		Reference		
5–9		–5.06, P=0.04	1.00 (0.49, 2.04)	1.36 (0.80, 2.29)
10–15		–13.82, P<0.001	2.11 (1.07, 4.16)	3.38 (2.00, 5.80)
Male sex	NA	3.13, P=0.031	0.81 (0.57, 1.2)	0.61 (0.44, 0.86)
Comorbidities	NA	3.85, P=0.413	1.98 (0.73, 5.37)	0.49 (0.19, 1.27)
Latitude	–0.255, P<0.001	–3.72, P<0.001	1.52 (1.33, 1.74)	1.35 (1.15, 1.58)
Season	NA	1.41, P=0.329	1.34 (0.94, 1.92)	0.79 (0.56, 1.11)

CI Confidence interval; NA Not applicable (predictor variable dichotomous); OR Odds ratio

*American Academy of Pediatrics (AAP) cut-off of <50 nmol/L and Canadian Paediatric Society (CPS) cut-off of <75 nmol/L

Table 4. Predictors of serum 25(OH)D level; multivariable analysis

Variable	Beta coefficient *	P value	Vitamin D level, OR (95% CI)	
			<50 nmol/L	<75 nmol/L
Age (years)	-0.022	<0.001	1.12 (1.05, 1.20)	1.16 (1.09, 1.23)
Female	-0.048	0.083	1.28 (0.83, 1.97)	1.69 (1.07, 2.65)
Latitude	-0.064	<0.001	1.60 (1.31, 1.94)	1.43 (1.17, 1.74)

CI Confidence interval; OR Odds ratio

*Outcome variable: log-25(OH)D level

Table 5. Multilevel model

	Beta coefficient*
Fixed component	
Intercept	7.27
Age	-0.023
Sex	0.042
Latitude	-0.058
Random component	
Residual variance	0.0601
Variance (household)	0.0161
Variance (colony)	0.0075
Deviance	198.45

*Outcome variable: log-25(OH)D level

factors associated with lower serum 25(OH)D levels. The majority of variation in serum 25(OH)D levels was at the individual level. Additional studies are needed to explore the potential mechanisms leading to lower serum 25(OH)D levels, especially individual-level factors (e.g., genetics).

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