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Low Vitamin D Status in a Representative Sample of Youth From Québec, Canada

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BACKGROUND: Adequate vitamin D status is important for bone growth and mineralization and has been implicated in the regulation of autoimmunity, metabolic function, and cancer prevention. There are no reports of population-based studies on the vitamin D status of Canadian youth, a population with mandatory fortification of foods.

METHODS: We measured plasma 25-hydroxyvitamin D [25(OH)D], the best indicator of vitamin D status, in a school-based cross-sectional sample of representative French Canadian youth (n = 1753) ages 9, 13, and 16 years living in Québec (latitude: 45°–48°N). Blood samples were collected from January to May 1999. We defined 25(OH)D deficiency as ≤27.5 nmol/L, hypovitaminosis as ≤37.5 nmol/L, and optimal as >75.0 nmol/L.

RESULTS: More than 93% of youth in each age and sex group had suboptimal 25(OH)D concentrations. The prevalence of 25(OH)D deficiency increased with age in both sexes ($P < 0.0001$). It was 2%, 3%, and 13% in 9-, 13-, and 16-year-old boys and 2%, 8%, and 10% in 9-, 13-, and 16-year-old girls. Girls with higher body mass index and girls from households with lower income had lower 25(OH)D concentrations. These effects were not observed in boys.

CONCLUSIONS: Inadequate vitamin D status is a potentially serious public health problem among children and adolescents in Québec. Youth living at high latitudes in countries with and without mandatory fortification of vitamin D are likely at heightened risk of 25(OH)D deficiency. These results call for renewed efforts to ensure adequate vitamin D intake among growing children and adolescents.

Vitamin D facilitates the intestinal absorption of calcium and phosphorus and plays an important role in bone mineralization *(1)*. It follows that maintaining optimal 25-hydroxyvitamin D [25(OH)D][7] concentrations is particularly important during the growth period in children and adolescents, when much of adult bone mass is established *(2)*. Vitamin D is believed to exert physiological effects beyond the skeletal system *(3)*. Indeed, the identification of vitamin D–specific nuclear receptors, in a number of tissues, indicates that this hormone plays a role in several physiological processes including cancer prevention *(4)*, immune regulation *(5)*, and glucose homeostasis *(6)*.

Ultraviolet activation of 7-dehydrocholesterol in the epidermis is the predominant source of cholecalciferol or vitamin D_3 *(7)*. This secosteroid is transported to the liver where it is hydroxylated to yield 25(OH)D. When dietary calcium intake is low, the parathyroid glands respond to minute decreases in ionized serum calcium by releasing parathyroid hormone (PTH). PTH, in turn, regulates the final hydroxylation of vitamin D in the renal mitochondria, yielding the biologically active hormone, 1-α,25-dihydroxycalciferol *(8)*. Active vitamin D has 3 target tissues relevant to calcium metabolism. First, active vitamin D initiates the breakdown of bone tissue, releasing calcium into the serum; second, it increases the absorption of dietary calcium in the gut; finally, active vitamin D increases the reabsorption of calcium in the distal tubule of the kidney *(3)*.

At high latitudes, cutaneous vitamin D synthesis is season dependent, and from approximately October to March supplements or fortified foods are the only widely available sources *(9)*. The 1997 Institute of Medicine guidelines for dietary intake of vitamin D were meant to prevent the seasonal increase in PTH associated with lower vitamin D status, assuming little or no cutaneous production of vitamin D *(10)*. In-

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[7] Nonstandard abbreviations: 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; BMI, body mass index.

creased plasma concentrations of PTH are a marker of bone remodeling. Consistently increased concentrations of PTH are associated with an increase in risk for osteoporosis in later life *(11)*.

Several studies have documented vitamin D deficiency and hypovitaminosis from populations inhabiting a wide range of latitudes, including male adolescents in Paris *(12)*, white adolescent girls in Maine *(13)*, inner-city adolescent girls in Manchester, UK *(14)*, and school-aged youth from Lebanon *(15)*. Despite this, there are very few countries with fortification of vitamin D in staple foods. Mandatory fortification does exist in the US, Canada, and Finland, where staple foods, such as fluid milk, are fortified and have been shown to increase vitamin D intake consistent with dietary intake guidelines proposed by the Institute of Medicine *(16)*. Since fortification, however, an expert consensus among vitamin D researchers has stated that current levels of food fortification are inadequate to support optimal vitamin D status in adults *(17)*. In youth, little is known regarding the effectiveness of fortification in mitigating vitamin D deficiency or supporting optimal vitamin D status. Further, there are no reports of large population-based studies on the vitamin D status of youth at high latitudes in a country with mandatory fortification of staple foods with vitamin D.

Because 25(OH)D is widely recognized as the best indicator of vitamin D status *(7)*, we assessed the vitamin D status of a representative sample of French Canadian children and adolescents in Québec (45°–48°N) by measuring 25(OH)D concentration in stored plasma samples collected from January to May, the nadir for skin vitamin D synthesis. We also tested the influence of age, sex, adiposity, and sociodemographic characteristics on 25(OH)D concentrations.

Materials and Methods

STUDY POPULATION

The study population comprised children and adolescents who participated in the Québec Child and Adolescent Health and Social Survey (QCAHS), a school-based survey conducted between January and May 1999. Details on the survey design and methods have been reported *(18)*. Briefly, the QCAHS used a cluster sampling design to draw 3 independent, provincially representative samples of youth ages 9, 13, and 16 years. Questionnaire and anthropometric data were available for 83% (1267 of 1520), 79% (1186 of 1498), and 81% (1212 of 1495) of eligible 9-, 13-, and 16 years-olds, respectively. Our current analysis was restricted to French Canadians, who made up 80% (1019 of 1267), 79% (931 of 1186), and 78% (942 of 1212) of the 9-, 13-, and 16-year-olds, respectively. Sixty-three percent (638 of 1019), 69% (640 of 931), and 75% (709 of 942) of 9-, 13-, and 16-year-olds provided a fasting blood sample. Of 1987 blood specimens available for analysis, 234 (12%) were excluded because parents refused consent for analyses other than glucose and lipids, because the samples had thawed on arrival at the laboratory, or because they were of insufficient quantity for the 25(OH)D assay. There were no differences in sex, body mass index (BMI) *z* score, or parental income among youth for whom blood samples were studied vs those not studied. The Ethics Review Board of CHU Sainte-Justine approved this study. Written informed assent and consent were obtained from participants and their legal guardians, respectively.

VARIABLES

Height was measured to the nearest 0.1 cm at maximal inspiration using a measuring tape and a triangular level. Weight was measured in light indoor clothing with shoes removed. BMI was calculated by dividing weight by the square of height (kg/m^2). We categorized youth as overweight if their BMI was ≥85th and <95th or obese at ≥95th percentile values for their sex and age according to the 2000 US CDC growth charts *(19)*. We categorized household income as superior, upper middle, lower middle, or lowest based on total income and number of persons living in the household *(20)*; household income was coded as missing if parents did not respond to the income question. We used the location of each school as a proxy for the location of the participants' residences, and schools were classified as rural or urban based on the census classification used by Statistics Canada.

After an overnight fast, venous blood was collected between 0800 and 1000 in 1 g/L EDTA collection tubes and placed on ice. Samples were centrifuged on site within 45 min of collection, transported on dry ice, and stored at −80 °C. In 2007, we used RIA for the quantitative determination of plasma 25(OH)D (Immunodiagnostic Systems Ltd.). Our laboratory participates in the International Vitamin D External Quality Assessment Scheme on vitamin D metabolites and meets the performance target set by the Advisory Panel for Data Analysis. The interassay CV was 5.9% at 30.6 nmol/L and 6.0% at 109.4 nmol/L. To assess the stability of 25(OH)D over time, the measurement of 25(OH)D was repeated 26 months apart for 39 randomly chosen plasma samples; the concordance correlation coefficient *(21)*, which evaluates the degree to which pairs of observations fall on the 45° line through the origin, was 0.87.

DEFINING CUTOFFS FOR VITAMIN D

We used 3 cutoffs to describe vitamin D status: deficiency, hypovitaminosis, and optimal. In the absence

of rickets or osteomalacia, there are no outcome-based criteria to define vitamin D deficiency, though it has been suggested that pediatric vitamin D deficiency can be defined as plasma 25(OH)D <25–30 nmol/L *(22)*. The Institute of Medicine defines vitamin D deficiency as 25(OH)D ≤27.5 nmol/L *(23)*. For hypovitaminosis D, we used a 25(OH)D cutoff of ≤37.5 nmol/L, a value reported in the literature *(24)*. The value for optimal 25(OH)D concentration was >75 nmol/L, a value thought to be consistent with both improved bone health *(25)* and other health outcomes *(26)* in adults.

STATISTICAL ANALYSIS

We computed age- and sex-specific *z* scores for BMI using a SAS program developed by the CDC based on the 2000 US CDC growth charts *(27)*. To take the complex study design into account, sampling weights and clustering effects by school were estimated and incorporated into computations of prevalence, percentile values, and 95% CIs. We used the nonparametric method developed by Hutson *(28)* to estimate percentiles and their CIs. Because of low observed frequencies, we calculated exact binomial 95% CIs *(29)* for the prevalence of the 3 concentrations of 25(OH)D. We tested differences in the prevalence between sexes and across ages using a likelihood ratio test in a generalized linear logistic model to take the clustering effect within school into account. We tested the association between mean 25(OH)D, sex, and age in univariate regression analysis. We used hierarchical maximum likelihood regression to estimate regression coefficients for univariate and multivariate associations. Explanatory variables were treated as fixed effects, and clustering between individuals in the same school was treated as a random effect. We stratified analyses by sex because of a significant age × sex interaction ($P < 0.0001$) in a model including sex, age, BMI *z* score, month of blood draw, parental income, and location of residence. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Inc.).

Results

Selected characteristics of participants are shown in Table 1. The majority of blood samples (78%) were drawn between January and March. Plasma 25(OH)D concentrations ranged from 11.4 to 115.9 nmol/L. Mean age- and sex-specific plasma 25(OH)D concentrations and selected percentile values are presented in Table 2. With the exception of 16-year-old girls, the value of the 95th percentile in each age and sex group was below the optimal concentration of 75 nmol/L. We observed no significant differences in mean 25(OH)D between sexes ($P = 0.9$). In both sexes combined, 13- and 16-year-olds had significantly lower mean 25(OH)D than 9-year-olds ($P < 0.0001$).

Table 1. Selected characteristics of study participants.

Characteristic	Boys, %	Girls, %
n	882	871
Age, years		
9	30.9	30.1
13	31.7	29.3
16	37.4	40.6
Household income		
Superior	14.2	11.6
Upper middle	31.3	34.9
Lower middle	27.4	27.8
Lowest	12.6	12.4
Missing	14.5	13.3
Area of residence		
Urban	54.4	54.9
Rural	45.6	45.1
Month of blood draw		
January–March	78.1	78.2
April–May	21.9	21.8
BMI category[a]		
Normal weight	77.5	78.5
Overweight	12.8	13.3
Obese	9.7	8.52

[a] Normal weight is defined as ≤85th percentile, overweight is the 85th to 95th percentiles, and obese is ≥95th percentile of the 2000 US-CDC growth charts *(19)*.

More than 10% of 16-year-olds were 25(OH)D deficient (Table 3). The prevalence of deficiency increased significantly with age in both sexes. As many as 38% of 16-year-old boys and 30% of 16-year-old girls had 25(OH)D concentrations consistent with hypovitaminosis. The prevalence of hypovitaminosis increased significantly with age in both sexes. 25(OH)D concentrations were not optimal in the vast majority of children and adolescents.

Girls in the lowest category of household income had significantly lower plasma 25(OH)D than those in the superior income category after adjustment for age, month of blood draw, area of residence, and BMI *z* score (Table 4). Male and female participants with missing income had the lowest concentrations of 25(OH)D. In contrast to boys, BMI *z* score was significantly associated with 25(OH)D concentrations in girls: a 1 SD increase in BMI was associated with a 1.7 nmol/L decrease in 25(OH)D. There was no associa-

Table 2. Selected percentile values and mean plasma 25(OH)D (nmol/L) by sex and age.[a]

	Boys			Girls		
	9 years	13 years	16 years	9 years	13 years	16 years
n	284	293	305	275	249	347
Percentile						
5th	35.3 (30.9–37.8)	30.1 (27.3–31.5)	23.5 (20.0–24.9)	30.3 (29.0–34.8)	25.3 (22.4–27.7)	24.6 (22.4–25.9)
25th	44.7 (42.9–45.9)	37.2 (35.0–38.3)	33.0 (30.2–35.2)	42.6 (41.2–43.4)	34.9 (33.2–36.2)	34.4 (32.8–37.8)
50th	51.1 (49.7–52.7)	44.0 (42.2–45.4)	41.1 (39.7–43.6)	48.6 (46.7–50.2)	40.2 (38.8–42.6)	44.7 (42.4–47.0)
75th	57.2 (56.5–58.7)	50.2 (49.2–51.1)	51.6 (49.3–53.2)	55.2 (53.7–57.0)	47.1 (45.7–49.4)	56.7 (52.9–60.0)
95th	70.3 (67.3–74.2)	60.9 (55.9–65.3)	66.6 (62.3–70.8)	65.8 (62.5–68.9)	57.9 (55.3–63.9)	78.8 (73.1–89.7)
Mean	51.5 (50.2–52.7)	43.9 (42.8–45.1)	42.7 (41.2–44.3)	48.6 (47.3–49.9)	41.3 (40.0–42.6)	47.3 (45.4–49.1)

[a] Data are percentile (95% CI) and mean (95% CI).

tion in either sex between plasma 25(OH)D and month of blood draw or residence in rural or urban setting.

Discussion

Our study, the first to examine vitamin D status during winter and spring months in a representative, population-based sample of French Canadian youth in the province of Québec, revealed a relatively high prevalence of 25(OH)D deficiency, in particular among 16-year-old boys and girls. We did not detect any differences in 25(OH)D concentrations between samples collected in January–March and April–May, suggesting that the period of deficiency or hypovitaminosis extended beyond the winter months. Because Canada, the US, and Finland are the only countries with mandatory fortification of vitamin D in staple foods, our results likely represent a best-case scenario for vitamin D status of white youth at high latitudes.

Our results concur with those of others to suggest that vitamin D deficiency and hypovitaminosis D are widespread among children and adolescents at high latitudes. For example, Das et al. *(14)* reported a mean serum 25(OH)D concentration of 37.3 nmol/L at the end of May in white girls from Manchester, UK, though the sample size was small. A population-based sample of adolescent girls from Denmark, Finland, Ireland, and Poland taken during February and March

Table 3. Percent of participants according to 3 cutoff values of plasma 25(OH)D by age and sex.[a]

Cutoff value	9 years	13 years	16 years	P^b
Deficient: ≤27.5 nmol/L				
Boys	1.5 (0.3–4.3)	3.3 (1.3–6.7)	12.6 (9.0–17.3)	<0.0001
Girls	1.5 (0.2–4.5)	7.9 (4.4–12.7)	10.1 (6.7–14.4)	<0.0001
P^c	NS[d]	0.036	NS	
Hypovitaminosis: ≤37.5 nmol/L				
Boys	7.8 (4.6–12.2)	25.5 (19.9–31.7)	37.8 (31.9–43.9)	<0.0001
Girls	13.0 (8.6–18.6)	34.5 (27.8–41.8)	29.9 (24.4–35.8)	<0.0001
P^c	NS	0.020	0.016	
Suboptimal: ≤75 nmol/L				
Boys	97.3 (94.2–99.1)	99.7 (97.8–100)	98.8 (96.7–99.8)	NS
Girls	98.7 (95.8–99.8)	99.5 (96.9–100)	93.2 (89.4–95.9)	0.0003
P^c	NS	NS	0.002	

[a] Data are % (95% CI).
[b] Differences across ages.
[c] Differences between sexes.
[d] NS, not significant ($P \geq 0.05$).

Table 4. Associations between 25(OH)D concentration (nmol/L) and selected sociodemographic and anthropometric variables.

	Boys (n = 878)			Girls (n = 867)		
Explanatory variable	β[a,b]	SE	*P*	β[a,b]	SE	*P*
Age, years						
9	Referent	—	—	Referent	—	—
13	–6.9	1.0	<0.0001	–6.7	1.3	<0.0001
16	–8.6	1.1	<0.0001	–1.2	1.2	NS
Month of blood draw						
April–May	Referent	—	—	Referent	—	—
January–March	0.13	1.0	NS	–1.5	1.2	NS
Household income						
Superior	Referent	—	—	Referent	—	—
Upper middle	–1.4	1.3	NS	–1.2	1.6	NS
Lower middle	–2.1	1.3	NS	–1.9	1.6	NS
Lowest	–2.4	1.6	NS	–4.8	1.9	0.010
Missing	–3.5	1.5	0.022	–6.8	1.9	0.0003
Area of residence						
Urban	Referent	—	—	Referent	—	—
Rural	–0.30	0.88	NS	–0.65	1.1	NS
BMI *z* score (1 SD)	–0.58	0.38	NS	–1.7	0.42	0.0001

[a] β, regression coefficient; *P*, probability value for the regression coefficient; NS, not significant ($P \geq 0.05$); BMI, body mass index.
[b] Difference, in nmol/L, of plasma 25(OH)D per unit increment of the explanatory variable.

reported a median serum 25(OH)D of 29.4 nmol/L *(30)*. Similar findings were reported among youth in Philadelphia *(31)* and New Zealand *(32)*. In Canadian children, rickets is an ongoing concern. Ward et al. *(33)* reported 104 cases of vitamin D–deficient rickets between July 2002 and June 2004 (incidence rate 2.9/100 000). Affected children had a mean age of 1.4 years, and many were dark skinned or residing in Northern Canada. Both skin color and living in the north have been associated with decreased cutaneous synthesis of vitamin D *(3)*.

In youth living in Québec, a high prevalence of vitamin D deficiency and hypovitaminosis associated with lower-than-recommended dietary calcium intake as revealed in a 1999 province-wide survey *(18)* may prevent the attainment of optimal bone mass, which may in turn affect the development of osteoporosis in later life. A 3-year prospective study of 171 peripubertal Finnish youth reported that baseline vitamin D deficiency [defined as 25(OH)D ≤20 nmol/L] in a context of high dietary calcium intake (1575 mg/day) was associated with a decrease in bone mineral content of the lumbar spine 3 years later in older girls *(34)*. Furthermore, recent findings from observational and randomized trials suggest that optimal 25(OH)D and adequate dietary calcium intake are important for the prevention of cancer *(4)*, type 1 diabetes *(35)*, type 2 diabetes *(36)*, and other health outcomes *(3)*.

Similar to a study of New Zealand youth *(32)*, our analyses showed an inverse association between 25(OH)D concentrations and body weight in girls. The sequestration of vitamin D into adipose tissue is thought to explain this association *(37)*. Our finding that girls of low socioeconomic position had lower concentrations of 25(OH)D was not consistent with a report from Philadelphia in which caregiver education and annual income were used as measures of socioeconomic position *(31)*. Youth whose parents did not respond to the question on income had the lowest concentrations of 25(OH)D. Nonresponse is generally thought to be most common among individuals in highest and lowest socioeconomic position *(38)*. Further research is needed to explore the association between 25(OH)D concentrations and socioeconomic position.

Because we restricted the sample to French Canadians, the findings may not be generalizable to other groups in which there may be a higher risk of deficiency due to darker skin color *(3)*. There is no widely agreed consensus on threshold to define vitamin D deficiency

in pediatric populations. We have therefore elected to use multiple cut points and present selected percentile values to facilitate comparisons with other studies. Our blood specimens were collected in 1999; however, it is unlikely that 25(OH)D status has changed substantially since 1999. Our plasma samples have been stored since 1999, raising concerns over the stability of 25(OH)D—loss of analyte during storage would increase prevalence of abnormality. It has been shown, however, that 25(OH)D is stable under repeated freeze/thaw cycles *(39)*. Moreover, we have estimated a concordance coefficient of 0.87 in 39 random samples in which 25(OH)D was measured twice 26 months apart. Underestimation of vitamin D_2 is a limitation with Immunodiagnostic Systems RIA because, according to the manufacturer, the assay detects only 75% of this metabolite. Fluid milks in Canada, however, are fortified with vitamin D3 *(40)* and the major vitamin suppliers to Canada use vitamin D_3 in supplements. Finally, we did not collect information on the sources of vitamin D, namely dietary or supplement intake and sun exposure.

In conclusion, 25(OH)D deficiency and hypovitaminosis were highly prevalent in children and adolescents living in Québec during winter/spring 1999, a population for whom staple foods were fortified with vitamin D. No recent representative surveys of vitamin D status in youth are available in Québec and none are available elsewhere in Canada. This lack calls for urgent monitoring of indicators of vitamin D status in Canada and elsewhere. In that respect, Vieth et al. *(17)*, in their editorial, have recently called for international agencies to reassess the dietary recommendations for vitamin D to ensure optimal concentrations of 25(OH)D. The findings of our study strongly support such a plea.

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