Prevalence and predictors of vitamin D insufficiency in women of reproductive age living in northern latitude

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Abstract
Objective: This study assessed the prevalence of vitamin D deficiency (serum 25-hydroxyvitamin D (25OHD) ≤ 50 nmol/l) and insufficiency (serum 25OHD 51–74 nmol/l) during summer and the predictors of serum 25OHD in young women of reproductive age.
Design: Cross-sectional study.
Methods: Between May and September 2006, 153 healthy, ambulatory and essentially Caucasian women, aged 18–41 years, were recruited. Serum 25OHD and parathyroid hormone (PTH) levels were measured, and questionnaires were evaluated.
Results: About 3.9% of women had serum 25OHD ≤ 50 nmol/l with an additional 26.8% in the insufficient range. Most women (56.9%) had their blood sampled in September. Month of blood collection significantly influenced serum 25OHD. Body mass index (BMI) was inversely associated with serum 25OHD, while traveling to a warmer climate during winter/spring and using oral contraceptive pills (OCP) were associated with higher serum 25OHD. Sunscreen was used by 77.8% of women, but only 3.3% reported consuming vitamin D supplements. BMI, serum PTH, travel to a warmer climate, and OCP use were independently and significantly associated with serum 25OHD, after adjustment for the month of sampling, and explained 40% of the variance in serum 25OHD.
Conclusions: In Canada, the prevalence of vitamin D insufficiency is relatively high (30%) during summer in healthy women of reproductive age. Given the expected decrease in serum 25OHD during winter and the low consumption of vitamin D supplements, a high prevalence of vitamin D deficiency and insufficiency is to be anticipated during winter, except maybe for those traveling to a warmer climate.

Introduction
In Canada and throughout the world, vitamin D insufficiency is a major public health issue that has been linked to a range of common chronic diseases including cancers, autoimmune diseases, insulin resistance, and infectious diseases (1–5). In women of reproductive age, it is of particular importance given that maternal vitamin D insufficiency has been associated with gestational diabetes as well as with short- and long-term consequences for the offspring (6–9). Although optimal serum 25-hydroxyvitamin D (25OHD) cut-off values are still debated, a growing body of evidence indicates that the optimal level for multiple health benefits is at least 75 nmol/l (10). Serum 25OHD levels defining vitamin D deficiency and insufficiency are still a matter of controversy; however, recent publications suggest that serum 25OHD levels of 50 nmol/l or less represent vitamin D deficiency, and that levels between 50 and 75 nmol/l define vitamin D insufficiency (11).

The main source of vitamin D is from the conversion of the skin precursor 7-dehydrocholesterol under sunlight exposure. In northern latitudes, the season greatly influences serum 25OHD levels as minimal skin production of vitamin D occurs from October to March (12). During summer, lifestyle factors such as reduced outdoor activities and sunscreen use may prevent from building adequate vitamin D stores (13).

This study aimed to evaluate the prevalence of vitamin D deficiency and insufficiency during summer, and to identify the predictors of serum 25OHD levels in a sample of healthy young women of childbearing age living in northern latitudes.

Subjects and methods
Subjects
Healthy women between the ages of 18 and 41 who attended the outpatient collection center of the Centre
Hospitalier Universitaire de Sherbrooke (CHUS) in Quebec, Canada (latitude 45°N), were consecutively recruited between May and September 2006. A total of 182 women were assessed, but 29 were excluded for the following reasons: pregnancy (n = 1), glucocorticoid use (n = 1), and incomplete questionnaire or laboratory data (n = 27). Women were essentially of Caucasian origin. This study was approved by the ethics committee of the CHUS, and a written informed consent was obtained from all the participants.

Questionnaire and measurements

Participants completed a questionnaire that evaluated medication use, including the use of vitamin D supplements and oral contraceptive pills (OCP), sunlight exposure (h/week spent outdoor with exposition of the face and extremities), sunscreen use (yes/no), and travel to a warmer climate at a latitude below 35°N for at least 1 day during winter/spring (yes/no; number of days). Vitamin D intake was derived from the reported daily consumption of milk (100 IU/cup), egg yolks (20 IU), and fish, including salmon (100 IU/ounce), canned tuna (66 IU/ounce) and mackerel (100 IU/ounce). Body mass index (BMI) was calculated from self-reported body weight and height. Serum 25OHD was measured using the Diasorin RIA (Stillwater, MN, USA) with an average within-batch coefficient of variation (CV) < 10%. Serum intact parathyroid hormone (PTH) was assessed using a second-generation electrochemiluminescence assay (Elecys 2010 platform, Roche Diagnostics) with an average within-batch CV < 5%. Serum calcium, albumin, and inorganic phosphorus were measured using colorimetric methods (Vitros 950 platform, Ortho-Clinical Diagnostics, Raritan, NJ, USA) with CV values of 2.5, 1.5, and 1.8% respectively. All specimens were analyzed in one batch assay.

Statistical analyses

Analyses were performed using SPSS statistical software, version 17 (Chicago, IL, USA). In order to make them normally distributed, BMI and serum 25OHD were log transformed for all the analyses, and vitamin D intake was transformed using the square-root transformation. Data are presented as means ± s.d., or median (interquartile range) for non-normally distributed variables. Simple and multiple linear regression models were used to evaluate the associations between serum 25OHD (dependent variable) and age, BMI, month of blood collection, serum PTH, sunlight exposure, sunscreen use, travel to a warmer climate during winter/spring, dietary vitamin D intake, and use of vitamin D supplements and OCP (independent variables). Only variables with a P value < 0.1 in the simple linear regression model were entered in the multiple linear regression model using a forward method, in order to determine significant independent predictors of 25OHD levels. Differences in mean log serum 25OHD levels by the month of sampling were tested with ANOVA (using post-hoc Tukey’s honestly significant difference (HSD) tests for differences among groups). A P value < 0.05 was considered statistically significant.

Results

Baseline characteristics of the 153 women are presented in Table 1. Most women (56.9%) had their blood collected in September. Sampling was performed in May, June, and July in 3.3, 28.8, and 11.1% of women respectively. Median serum 25OHD was 88.4 nmol/l. The prevalence of vitamin D deficiency was only 3.9%; however, an additional 26.8% fell into the insufficient range. Of note, none of the participants had serum 25OHD levels < 25 nmol/l. While the median time spent outdoor exposing at least the face and extremities was 12 h/week, sunscreen was used by more than 75% of women. Median vitamin D intake from the diet was low (230 IU/day), and only 3.3% reported consuming vitamin D supplements. Most women were on OCP (63.4%).

Age, sunlight exposure, sunscreen use, vitamin D intake, and use of vitamin D supplements were not significantly associated with serum 25OHD (Table 2). Serum PTH and BMI were inversely associated with serum 25OHD, while spending at least 1 day in a warmer climate during winter/spring and using OCP were associated with higher serum 25OHD levels. Women who were using OCP had significantly higher serum 25OHD levels than those who did not (median levels of 94.4 vs 75.0 nmol/l, P < 0.001). The prevalence of vitamin D insufficiency was also less than half that of women on OCP (20.6 vs 48.2%, P < 0.01).

Table 1  Baseline characteristics of the 153 women aged between 18 and 41 years. Data are %, mean ± s.d. or median (25–75th percentiles).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.8 ± 5.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7 (20.5–23.6)</td>
</tr>
<tr>
<td>Overweight (%)</td>
<td>16.3</td>
</tr>
<tr>
<td>Obese (%)</td>
<td>1.3</td>
</tr>
<tr>
<td>Serum 25OHD (nmol/l)</td>
<td>88.4 (70.9–112.1)</td>
</tr>
<tr>
<td>Serum PTH (pmol/l)</td>
<td>2.9 ± 1.1</td>
</tr>
<tr>
<td>Serum calcium (mmol/l)</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Serum phosphorus (mmol/l)</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Sunlight exposure (h/week)</td>
<td>12 (7–25)</td>
</tr>
<tr>
<td>Sunscreen use (%)</td>
<td>77.8</td>
</tr>
<tr>
<td>Holiday in warmer climate (%)</td>
<td>20.9</td>
</tr>
<tr>
<td>Number of days spent in warmer climate</td>
<td>9 (7–19)</td>
</tr>
<tr>
<td>Dietary vitamin D intake (IU/day)</td>
<td>230 (140–348)</td>
</tr>
<tr>
<td>Vitamin D supplements (%)</td>
<td>3.3</td>
</tr>
<tr>
<td>Oral contraceptive use (%)</td>
<td>63.4</td>
</tr>
</tbody>
</table>

Reference ranges for 25OHD, PTH, calcium, and phosphorus are respectively 75–150 nmol/l, 1.6–6.9 pmol/l, 2.10–2.54 mmol/l, and 0.81–1.45 mmol/l.

*Exposure of at least the face and extremities.
*At least 1 day at a latitude below 35°N.
*From food frequency questionnaire.
Month of blood collection significantly influenced serum 25OHD levels ($P<0.001$ by ANOVA); levels were highest in July and lowest in May. Serum 25OHD levels by the month of sampling are depicted in Fig. 1. BMI, serum PTH, travel to a warmer climate, and OCP use were significantly and independently associated with serum 25OHD in multivariable analysis, after adjustment for the month of blood collection ($P=0.03$, $P<0.01$, $P<0.01$, and $P<0.001$ respectively). When put together in the regression model, these factors explained 40% of the variance in serum 25OHD levels (36% if serum PTH is excluded from the model).

### Discussion

This study revealed that during summer in Quebec, Canada, a third of healthy women of reproductive age have serum 25OHD levels $<75$ nmol/l. Given that virtually no skin production of vitamin D occurs during winter and that only 3.3% of women reported consuming vitamin D supplements, a much higher proportion of women with suboptimal serum 25OHD levels is to be expected during that period. A study by Tangpricha et al. (14) revealed that the prevalence of vitamin D deficiency increased from 3 to 30% between summer and winter in young healthy adults aged 18–29 years, and that the risk of vitamin D deficiency was higher in those who did not consume a multivitamin supplement. Very few foods contain vitamin D, and indeed, median dietary vitamin D intake was low with only 58.2% of women reaching the recommended adequate intake (RAI) of 200 IU/day (15). The Institute of Medicine is expected to release in 2010 the revised RAI for vitamin D. Most experts agree that the current RAI is too low, especially during winter in Canada, but also during summer in people who are at high risk of vitamin D deficiency. The Canadian Cancer Society took the first step in 2007 by recommending that all Canadians consume 1000 IU of vitamin D daily during the fall and winter, and all year round for those who are unlikely to produce enough vitamin D during summer (elderly people, people with dark skin, and people who avoid sunlight exposure).

Much work is required to increase public awareness about the importance of reaching, but also maintaining, optimal serum 25OHD levels throughout the year. Vitamin D has long been recognized as an important factor for bone health (16, 17). In recent years, studies associating vitamin D deficiency and insufficiency with an increased risk of adverse outcomes such as insulin resistance, cardiovascular disease, colon and breast cancer, inflammatory diseases, and mortality have cumulated (1–4, 18–20). Of particular relevance to women of reproductive age is the association between vitamin D deficiency and insufficiency and an increased risk of gestational diabetes, preeclampsia, and requirement of a primary C section (9, 21, 22). Low maternal serum 25OHD levels during pregnancy may also result in short-term adverse outcomes for the fetus (e.g. neonatal hypocalcemia and impaired bone development) (8, 23), but also long-term consequences on the offspring (e.g. risk of type 1 diabetes and low bone mass) (6, 7).

BMI, serum PTH, month of blood collection, spending time during winter/spring in a warmer climate, and OCP use were independent predictors of serum 25OHD in this population. BMI has been repeatedly shown to be inversely correlated with serum 25OHD levels (24, 25). Lower serum 25OHD levels with increasing obesity have been attributed to the sequestration of vitamin D in adipose tissue (26). The obesity epidemic that many countries including Canada are facing could potentially greatly impact on the prevalence and prevention of vitamin D deficiency and insufficiency.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Standardized coefficients ($\beta$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$-0.02$</td>
<td>0.83</td>
</tr>
<tr>
<td>Log BMI</td>
<td>$-0.17$</td>
<td>0.04</td>
</tr>
<tr>
<td>Month of blood collection$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>$-0.38$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>June</td>
<td>$-0.37$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>September</td>
<td>$-0.54$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Serum PTH</td>
<td>$-0.28$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Sunlight exposure $\geq 12$ h/week$^b$</td>
<td>$0.06$</td>
<td>0.45</td>
</tr>
<tr>
<td>Sunscreen use</td>
<td>$0.13$</td>
<td>0.09</td>
</tr>
<tr>
<td>Holiday in warmer climate$^c$</td>
<td>$0.20$</td>
<td>0.01</td>
</tr>
<tr>
<td>Square-root of dietary vitamin D intake$^d$</td>
<td>$-0.03$</td>
<td>0.75</td>
</tr>
<tr>
<td>Use of vitamin D supplements</td>
<td>$0.02$</td>
<td>0.78</td>
</tr>
<tr>
<td>Use of oral contraceptive pills</td>
<td>$0.38$</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

*July was the reference category (dummy variables created).

$^a$Exposure of at least the face and extremities.

$^b$Spent at least 1 day at a latitude below 35°N.

$^c$Derived from a food frequency questionnaire.

<table>
<thead>
<tr>
<th>Month of sampling</th>
<th>Serum 25OHD (nmol/l)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>May $(n=5)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June $(n=44)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July $(n=17)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept $(n=87)$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Serum 25-hydroxyvitamin D levels by month of sampling in our population. $P$ values correspond to post-hoc Tukey’s HSD $P$ values after ANOVA tests.

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Interestingly, spending at least a day at a latitude below 35°N during winter/spring was positively associated with serum 25OHD levels. Rucker et al. (25) also showed that travel to lower latitudes (<42°N for ≥1 day) was associated with higher serum 25OHD levels. Whether or not this finding is due to the fact that people traveling to warmer climate increased their area of skin exposed to the sun and/or increased their time spent outdoors in general cannot be determined from our study. Finally, OCP use has been previously reported to increase serum 25OHD by 24 nmol/l during winter and 9 nmol/l during summer (versus women who did not use OCP), after adjustment for age and vitamin D intake (27, 28). The mechanisms by which OCP use affects vitamin D metabolism remain incompletely understood, but are likely attributed to an increase in circulating vitamin D binding protein levels by estrogen (29). If this is the case, the ratio of bound to free serum 25OHD should increase. The clinical implications of reduced free serum 25OHD levels are not known, but OCP use should be taken into account when assessing the vitamin D status of young women.

In contrast to many studies, we found no association between age and serum 25OHD, which could be accounted for the young age of our population. Indeed, a greater than twofold decline in epidermal stores of 7-dehydrocholesterol, the skin precursor of vitamin D3, was demonstrated when elderly people aged 77 and 88 years were compared with individuals aged 8 and 18 years old (30). Contrary to what we anticipated, sunscreen use and sunlight exposure were not associated with serum 25OHD levels. Self-report of sunlight exposure using questionnaires has been shown to poorly correlate with more objective measures, such as dosimetry (31). While the application of sunscreen with a sun protection factor of 8 was shown in a controlled experiment to interfere with the cutaneous synthesis of vitamin D (32), ‘real-life’ trials have failed to document any correlation between sunscreen use and serum 25OHD levels, perhaps due to the inappropriate application of sunscreen (33, 34).

In March 2010, Statistics Canada released a report on the vitamin D status of Canadians (35). In the subgroup of women aged 20–39 years, 63.7% had serum 25OHD levels ≤75 nmol/l, which is much higher than our results (30.7%), and serum levels from April to October were 75.7 nmol/l, which is lower than in our population (88.4 nmol/l). These differences may be explained by predominantly summer sampling of our population that comprises a lower proportion of non-Caucasian women living at a lower latitude (Sherbrooke) than most women sampled in the Statistics Canada report, but also possibly by lower BMIs or increased milk consumption, sunlight exposure, and OCP use in our population. Statistics Canada found that milk consumption more than once a day, sunlight exposure more than 1 h per day, and month of blood collection were positively associated with higher serum 25OHD levels. However, no data are available on other factors that may influence 25OHD levels. Similar contrasting results were found between our study and the study published by Rucker et al. (25) where 188 healthy, ambulatory Caucasians aged 27–89 years living in Calgary, Canada (latitude 51°N), had their serum 25OHD level sampled at four different times of year. Mean serum 25OHD during summer (August or September) was 71.6 nmol/l with 14% of the population having a serum 25OHD level below 50 nmol/l. The lower latitude of Sherbrooke and younger age of our study population may account for the higher serum 25OHD levels found in our study. Differences in lifestyle factors such as sunlight exposure, regular use of tanning beds, sunscreen use, and clothing habits, as well as OCP and vitamin D supplements use, may also account for the discrepancy of results between these studies.

Four other studies focused on the vitamin D status of Canadians (25, 36–38), of which two were conducted in young women in Ontario (latitude 43°N) (36, 37) and one in teenage girls from Québec (38). Vieth et al. (36) recruited 796 women aged 18–35 years sampled once over a year. During summer (from May to October), the percentage of Caucasian women (n = 322) with serum 25OHD levels <40 nmol/l was 71%, with a mean ± s.d. serum 25OHD of 76 ± 28 nmol/l. During that period only, a significant association between multivitamin use and serum 25OHD was found and was attributed to the fact that women who were more physically active and therefore who had greater sunlight exposure were also more likely to consume multivitamins. Mean serum 25OHD levels in this study were much lower than in our study, which could be explained by the different sampling periods. It is also possible that the prevalence of women on OCP was lower in Vieth’s study, but this information was not reported. As opposed to findings by Vieth et al. (36) the consumption of vitamin D supplements did not predict serum 25OHD in our study, perhaps due to the much lower prevalence of women using supplements (3.2 vs 25%). Sunscreen use and sunlight exposure were not assessed in Vieth’s study. However, physical activity, used as a surrogate marker of sunlight exposure, correlated with serum 25OHD levels. Gozdzik et al. (37) reported that the prevalence of vitamin D insufficiency (serum 25OHD 75 nmol/l) during winter among 107 young healthy women comprising mainly Caucasians and Asians was 93%. The prevalence of vitamin D insufficiency in the subgroup of Caucasian women was very high (84.4%). The magnitude of this public health issue also extends to teenage girls. A school-based sample of girls aged 13 and 16 years from Quebec revealed that 99.5 and 93.2% respectively had suboptimal 25OHD levels during winter/spring (38). Those in the lowest category of household income and with a higher BMI had lower serum 25OHD levels. The prevalence of vitamin D insufficiency in this study is much higher than that in
ours, which could be attributed by winter sampling and possibly also the less frequent use of OCP.

Our study has a number of limitations that need to be considered. Although these women were young and healthy, the fact that they were recruited through the outpatient collection center of the hospital could have potentially led to bias. In addition, mainly Caucasian women were included in this study. Therefore, caution must be applied when extrapolating these results to the general population. Another limitation is the lack of serum 25OHD data during winter. While a decrease in serum 25OHD is expected during winter, it would have been interesting to document it especially in this group where the consumption of vitamin D supplements is low. Finally, the tools used to evaluate sunlight exposure and sunscreen use may have lacked the precision necessary to adequately assess the effect of those factors on serum 25OHD as we did not inquire specifically on the time of day spent outdoors and the area of skin exposed to the sun. We also did not ask whether the participants were using a tanning bed on a regular basis. Further studies are needed to clarify these issues.

In conclusion, about a third of women of childbearing age have suboptimal serum 25OHD levels during summer. Given the expected decrease in serum 25OHD during winter and the small proportion of women consuming vitamin D supplements, a much higher prevalence of vitamin D insufficiency is to be anticipated during winter, except maybe for those traveling to a warmer climate. This is of particular importance considering the potential risks associated with vitamin D insufficiency during pregnancy and the long term. Future studies are required to assess 25OHD levels during winter in this population of young women and the specific effect of holidays in lower latitudes. Whether the higher circulating serum 25OHD levels observed in women using OCP reflect vitamin D sufficiency at the cellular level warrants further investigation because this could be an important factor to consider when assessing vitamin D insufficiency in women.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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