Serum 25-Hydroxyvitamin D Concentrations Fluctuate Seasonally in Young Adults of Diverse Ancestry Living in Toronto 1,2

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Abstract

Previous research indicates that circulating vitamin D levels are low in many otherwise healthy adults and that there is considerable seasonal variation in 25-hydroxyvitamin D [25(OH)D] concentrations at high latitudes. We examined seasonal variation in 25(OH)D levels in a sample of young adults of diverse ancestry living in the Greater Toronto Area. Three hundred and fifty-one (351) healthy young adults completed both a fall and winter visit during this study. The study was conducted over 2 y (y 1: fall 2007 to winter 2008 and y 2: fall 2008 to winter 2009). At both visits, each participant’s serum 25(OH)D concentration was measured. Information was also obtained on skin pigmentation (measured via reflectometer), vitamin D intake, and extent of sun exposure. Overall, the serum 25(OH)D concentration was 54.4 ± 1.3 nmol/L in the fall and 38.4 ± 1.1 nmol/L in the winter. Concentrations differed among ancestral groups at both visits (P < 0.001), with South Asians and East Asians having substantially lower concentrations than Europeans. Skin pigmentation (r² = 0.14; P < 0.001), supplemental vitamin D intake (r² = 0.09; P < 0.001), sun exposure (r² = 0.04; P < 0.001), and study year (r² = 0.02; P = 0.017) were predictors of fall 25(OH)D concentrations. During the wintertime, serum 25(OH)D concentrations were associated with concentrations taken in the fall (r² = 0.45; P < 0.001), supplemental (r² = 0.15; P < 0.001) and dietary vitamin D intake (r² = 0.06; P < 0.001), and with study year (r² = 0.02; P = 0.009). Our study confirms that serum 25(OH)D concentrations undergo strong seasonal variation at high latitudes and are influenced by vitamin D intake, skin pigmentation, and sun exposure. J. Nutr. 140: 2213–2220, 2010.

Introduction

Previous research has shown that vitamin D concentrations are low in the general population, particularly at high latitudes, where marked seasonal fluctuations have also been observed (1–7). Such findings are important, because low vitamin D levels have been found to be associated with many chronic diseases, including osteoporosis, cancer, diabetes, cardiovascular disease, rheumatoid arthritis, multiple sclerosis, and microbial infections (8–16). Additionally, it has been reported that at higher latitudes, individuals of non-European ancestry are more likely to have vitamin D insufficiency than those of European ancestry (4,5,17–19).

The circulating concentration of serum 25-hydroxyvitamin D [25(OH)D] 20 is the primary indicator of vitamin D status, because it measures the vitamin D from all available sources (cutaneous synthesis, diet, or supplements) (20). Vitamin D experts have recommended that concentrations of serum 25(OH)D in adults should be in excess of 75 nmol/L for multiple health outcomes, including bone health (21,22). Accordingly, recent reports refer to serum 25(OH)D levels > 75 nmol/L as optimal, between 75 and 50 nmol/L as insufficient, and <50 nmol/L as deficient (23). In our study, we report the proportion of the participants in our sample under 3 widely used thresholds, 25, 20 nmol/L, and 50 nmol/L.

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5 Abbreviations used: 25(OH)D, 25-hydroxyvitamin D; 25(OH)D3, 25-hydroxycholecalciferol; 25(OH)D2, 25-hydroxyergocalciferol; CHMS, Canadian Health Measures Survey; GTA, Greater Toronto Area; LC-MS/MS, liquid chromatography tandem MS; PTH, parathyroid hormone; UVR, UV radiation.
Several studies have reported that many Canadians have suboptimal 25(OH)D levels, particularly during the winter (1–3,24–27). However, estimates of vitamin D insufficiency among Canadians may actually be higher than previously reported, because past studies have focused almost exclusively on individuals of European ancestry (2,3) and individuals with darker skin have a higher risk of vitamin D insufficiency. Furthermore, few studies have collected information on all of the major factors influencing vitamin D levels, such as seasonality, skin pigmentation, vitamin D intake, BMI, and sun exposure.

In a previous study, we reported that wintertime vitamin D levels in East Asian and South Asian adults living in the Greater Toronto Area (GTA) were much lower than in their European counterparts (4). However, this previous study did not evaluate seasonal variation in vitamin D levels and had a relatively small sample size. In the present paper, we expanded our previous work by: 1) examining 25(OH)D concentrations and vitamin D intake in a larger sample of young adults of diverse ancestry living in the GTA; 2) assessing seasonal trends in vitamin D status stratified by ancestry; and 3) evaluating the impact of skin pigmentation (measured quantitatively by skin reflectometry), vitamin D intake (measured via a FFQ), UVB exposure, sex, and BMI on seasonal vitamin D levels. The present study addressed the following questions: Are there significant differences in vitamin D levels among ancestral groups? What is the extent of the drop in vitamin D concentrations from the early fall, when cutaneous vitamin synthesis is possible, to the winter, when there is not enough UVB to synthesize vitamin D in the skin? Does the magnitude of the decrease differ across ancestral groups? Which are the most important factors influencing vitamin D levels in early fall and winter? Answering these questions is important for devising strategies to ensure optimal vitamin D levels in the diverse Canadian population.

Participants and Methods

Study population and recruitment. Participants were recruited at the University of Toronto Mississauga (Ontario, Canada) campus (43° N) during the fall of both 2007 and 2008. Our study was advertised to the University of Toronto Mississauga campus (43° N) during the fall of both 2007 and 2008. Most of the participants were either students or employees of the university.

Eligibility for the study was assessed using a questionnaire that was completed prior to study enrollment. Subjects had to be between 18 and 35 y old. The exclusion criteria were: age, diagnosis of kidney or liver disease or other active or chronic diseases potentially affecting vitamin D metabolism or absorption (such as inflammatory bowel disease, cystic fibrosis) (28), or use of medications that affect vitamin D metabolism (including corticosteroids and anticonvulsants) (15,29). Consumption of vitamin D supplements was not a criterion for exclusion, because we were interested in evaluating supplement use and its effect on serum 25(OH)D.

In this initial questionnaire, potential participants also provided information about ancestry (personal, parental, and grandparental places of birth, ethnicity, language, and current residence). Based on this information, a stratified sampling strategy was used to ensure adequate representation of participants of diverse ancestry. The recruitment target was 100 participants per ancestral group. This study was approved by the University of Toronto Health Sciences Research Ethics Board and all participants provided written informed consent.

Data collection. Participants met with the researchers twice during the study. The first visit was arranged for the early fall (beginning of September to the middle of October). The second visit was scheduled for the winter (beginning of January until the end of February) (30). The study was conducted over 2 calendar years (y 1: fall 2007 and winter 2008; y 2: fall 2008 and winter 2009).

During the first visit, participants completed a personal questionnaire that assessed ancestry (as described above) and also included general questions about health (with particular emphasis on history of vitamin D-related disorders) and diet (e.g. if the participant was vegetarian or lactose intolerant). Study staff measured weight and height, and BMI (kg/m²) was calculated. Participants were asked to complete a FFQ and a UV radiation (UVR) exposure questionnaire and provide a blood sample. During the second visit, participants repeated the protocol (except the personal questionnaire) and were reimbursed for their participation. Participants reporting that they had traveled to a sunny destination between the 2 visits (n = 6) or had used a tanning salon (n = 12) continued to participate, but their winter values were excluded from our analyses. Additionally, 3 participants in y 1 and 6 participants in y 2 of the study attended the fall visit but did not attend the winter visit. Overall, a total of 351 participants attended the first visit and 322 met the criteria for having their second visit data included in the study.

For data analysis, participants were grouped into broad geographic regions: East Asia, Europe, Middle East, Africa, and South Asia. For example, individuals who stated that their ancestors were from China, Japan, and Korea were grouped as East Asian, and those who reported ancestors from India and Pakistan were grouped as South Asian. Individuals who reported being of multiple ancestries were placed into a subgroup designated as “Other.”

Sun exposure. Extent of sun exposure was calculated from a UVR exposure questionnaire. The questionnaire asked the participants to estimate the amount of time spent outdoors during the previous 3 mo. Questions included “During the summer, how much time on average per day do you spend in the sun between 9 a.m. to 5 p.m.?” and the options provided included <5 min, 5–30 min, <1 h, 1–2 h, and >2 h.

Measuring pigmentation using reflectometry. Constitutive skin pigmentation (pigmentation in unexposed areas of the skin) was measured in the inner upper arm using a narrow-band reflectometer (Dermaspectrometer, Cortex Technology) at both visits (31). A detailed description of the method can be found elsewhere (4).

Biochemical analyses. An aliquot of whole blood was centrifuged and the serum fraction was removed after clotting and stored at −80°C. Serum parathyroid hormone (PTH), calcium, and phosphate were measured on the automated Modular Analytics Serum Work Area (Roche).

Vitamin D concentrations were measured using liquid chromatography tandem MS (LC-MS/MS). Serum/plasma spiked with an internal standard was extracted with 1 mL of methyl tert-butyl ether. The methyl tert-butyl ether phase was evaporated and redissolved in 1 mL of 80% methanol and extracted with 1 mL of heptane. The methanol phase was completely evaporated and the residue dissolved in 100 μL of 50% methanol and analyzed by LC-MS/MS. Chromatographic separation was achieved using linear gradient HPLC (Agilent 1200) on a 1.8-μm column (Agilent XDB-C8 Eclipse) starting at 63% methanol to 100% methanol during 4 min. MS analysis was performed using atmospheric pressure chemical ionization on an API5000 LC-MS/MS (Applied Biosystems/Sciex). The ion transitions monitored were 401.4 → 383.4 (25-hydroxycholecalciferol), 417.4 → 399.4 (24,25-dihydroxycholecalciferol), 413.4 → 395.4 (25-hydroxyergocalciferol), and 407.5 → 389.4 (Δ7,25-hydroxyergocalciferol). Analyst software (version 1.4.2) mediated data acquisition, peak-area integration, and comparison against the standard curve to calculate the concentration of unknowns. The standard curve was derived from calibrators analyzed within the same analytical run.

The LC-MS/MS method used in this study has not been previously published and was extensively validated. The between-day CV ranged from 3 to 6.9% for 25-hydroxycholecalciferol [25(OH)D3] (depending on the mean serum concentration of the samples) and from 3.1 to 10.4% for 25-hydroxyergocalciferol [25(OH)D2]. The within-day CV was 5.1% for 25(OH)D3 and 7.2% for 25(OH)D2. The r² values for the comparison of the LC-MS/MS estimates and the certified concentration
values of the National Institute of Standards and Technology Standard Reference Material 972 were >99.9% for both 25(OH)D3 and 25(OH)D2. Similarly, the $r^2$ value for the comparison of the concentrations of the Vitamin D External Quality Assessment Scheme samples for the LC-MS/MS method used in this study and the mean of the Vitamin D External Quality Assessment Scheme LC-MS results was 97.1%. Finally, the $r^2$ values for the comparison of estimates obtained with this LC-MS/MS method and estimates using the Diasorin RIA method ($n = 50$) and the DiaSorin Liaison method ($n = 45$) were 89.8 and 90.6%, respectively. A subset of samples ($n = 10$) was assayed separately and the CV was 2.3% for total serum 25(OH)D [arithmetic sum of 25(OH)D3 and 25(OH)D2]. Elsewhere, the term 25(OH)D refers to the summed total of the 2 vitamers.

**Nutritional analyses.** Daily intake of vitamin D from dietary and supplemental sources was estimated using a FFQ. We previously validated this FFQ for assessment of vitamin D and calcium intake in young adults of diverse ancestry (32). In the validation study, the FFQ results were correlated with 7-d food diary results ($r = 0.602; P < 0.001$) and the vitamin D intakes estimated from the FFQ were associated with serum 25(OH)D concentrations ($r = 0.520; P < 0.001$). Participants were provided with portion size aids and recorded their food, beverage, and supplement intakes. The FFQ were analyzed with the computer program Food Processor (version 8.0 and its revisions, ESHA Research, using the Canadian Nutrient File 2007b values from Health Canada); Canadian foods were always chosen where Canadian fortification was different from elsewhere (e.g. margarine and breakfast cereals).

**Statistical analyses.** ANOVA was used to determine whether there were significant differences in serum 25(OH)D and vitamin D intake among the 3 ancestral groups. Post hoc analyses were conducted with Bonferroni’s method. Repeated-measures ANOVA was used to assess the seasonal variation of serum 25(OH)D concentrations and vitamin D intake.

Multiple linear regression was used to examine how well the measured predictors (sex, BMI, vitamin D intake, and skin pigmentation) were able to explain variation in serum 25(OH)D levels in the fall and winter. In the fall visit, serum 25(OH)D was the dependent variable and the predictors were BMI, sex, skin pigmentation, sun exposure, reported dietary vitamin D intake, reported supplemental vitamin D intake, and study year. For the winter visit, BMI, sex, skin pigmentation, reported dietary vitamin D intake, reported supplemental vitamin D intake, and calendar year were inserted as possible predictive variables of vitamin D status in the first model of serum 25(OH)D. To assess the effect of fall vitamin D levels on wintertime levels, we introduced fall serum 25(OH)D as another independent variable in a second regression model for the wintertime sample. Each analysis controlled for sex and study year, because significant sex and study year differences were found for 25(OH)D and vitamin D intake (1-way ANOVA; data not shown). Outliers and influential points were assessed using normality tests (Shapiro-Wilk and Anderson-Darling) and Cook’s distance, respectively. No outliers were identified in the analyses of 25(OH)D and vitamin D intake.

Models for dependent variables 25(OH)D and total vitamin D intake displayed nonconstant error variance. This was corrected by applying natural log transformation to the responses. Results are presented for the log-transformed data. All statistical tests were performed with SPSS (version 15.0) and Minitab (version 15.1.1).

Power analysis was conducted using the software G*Power (version 3) (33). At a significance level of $\alpha = 0.05$, this study had >97% power for detecting a medium effect (Cohen’s $f = 0.25$) for the ANOVA models, >98% power to detect a medium within-subject effect ($f = 0.25$) in the repeated-measures ANOVA models, and >99% power to detect a medium effect ($f^2 = 0.15$) in the fall and winter regression models.

**Results**

**Sample characteristics**

A total of 351 participants (225 females, 126 males) were analyzed in the fall and 322 (202 females, 120 males) in the winter. The response rate was 92%, due to study drop-out and exclusion of individuals who had visited tanning salons and/or traveled to sunny locales in the winter. The majority of the participants were of African, East Asian, European, Middle Eastern, or South Asian ancestry. Because of the small sample size of the African ($n = 12$), Middle Eastern ($n = 16$), and “Other” ($n = 13$) groups, they were not included in subsequent analyses.

**Serum 25(OH)D concentrations stratified by ancestry**

Mean 25(OH)D in the fall visit for the full sample was 54.4 ± 1.3 nmol/L. The highest 25(OH)D concentrations were observed in the European sample and the lowest in the South Asian sample, whereas the East Asian sample had intermediate value. All 3 groups differed from each other (ANOVA; $P < 0.001$) (Table 1). During the winter, the serum 25(OH)D concentrations were substantially lower, with a mean value of 38.4 ± 1.1 nmol/L. Again, the European sample had the highest 25(OH)D concentrations, followed by the East Asian and South Asian samples, respectively. Whereas the European sample differed from the other 2 groups (ANOVA; $P < 0.001$), the East Asian and South Asian groups did not significantly differ in the wintertime.

Serum 25(OH)D concentrations were categorized using thresholds of 25, 50, and 75 nmol/L (Table 2). We observed that a higher proportion of non-Europeans had serum 25(OH)D concentrations < 50 nmol/L and 25 nmol/L in both visits. However, the proportion of individuals with 25(OH)D < 25 or 50 nmol/L increased substantially in all groups in the winter visit. Cohen’s $\kappa$ was calculated to determine the proportion of agreement between the categories at both visits. This was likely due to the strong seasonal drop in 25(OH)D concentrations, which is reported below.

**Seasonal variation in 25(OH)D**

We observed a mean decrease in paired serum 25(OH)D levels of 16 nmol/L (from 54.4 to 39.4 nmol/L) from fall to winter (Fig. 1). Participants of European ancestry experienced the largest absolute decline in mean serum 25(OH)D concentrations (24.5 nmol/L), followed by East Asians (14.9 nmol/L) and South Asians (8.1 nmol/L). The absolute decrease in 25(OH)D was correlated to baseline 25(OH)D concentrations; i.e. individuals with higher baseline 25(OH)D concentrations showed the greatest absolute drop in 25(OH)D levels, both in the total sample and in each of the subgroups (Fig. 2). When considering the relative decline in 25(OH)D, measured as the ratio of winter:fall 25(OH)D, the winter 25(OH)D means were 69, 68, and 78% of the baseline fall values for the East Asian, European, and South Asian samples, respectively.

Repeated-measures ANOVA revealed seasonal differences (within-subject effects) for 25(OH)D concentrations ($P < 0.001$). Season had a significant within-subject interaction with year for 25(OH)D concentrations ($P = 0.010$).

In the repeated-measures ANOVA analysis, ancestry had a between-subject effect on 25(OH)D ($P < 0.001$), which is in agreement with the ANOVA results given above.

**Vitamin D intake**

Fall vitamin D intake was 7.60 ± 0.40 µg/d (304 IU/d) for the total sample, with no significant differences observed among the 3 groups. During the winter, vitamin D intake was 7.24 ± 0.44 µg/d (290 IU/d) and again no significant differences were observed among groups (Table 1). Total vitamin D intake was...
comprised of dietary intake and intake from vitamin D supplements. Significant differences in dietary intake were observed among the 3 groups in the fall (P = 0.030), with South Asians having the highest intake and East Asians having the lowest. Fall vitamin D supplement intake did not differ. During the winter visit, neither dietary intake nor supplement intake differed among the 3 samples (Table 1).

We also examined the proportion of vitamin D intake coming from the diet compared with supplements at fall vs. winter visits. In both seasons, the majority of vitamin D intake came from dietary sources (Table 1). However, the proportion of supplemental vitamin D intake was higher in the winter (39%) than in the fall (31%). This was primarily due to an absolute decrease in dietary vitamin D intake and a slight increase in vitamin D supplements during the winter. The overall increase in supplemental vitamin D intake was traced to a substantial rise in winter supplement intake in a few participants, because the fraction of all individuals taking vitamin D supplements was similar in the fall and winter (22% in the fall vs. 23% in winter). Furthermore, some differences were observed in the pattern of supplement use between ancestry groups: the percentage of East Asians who took vitamin D supplements dropped from 20% in the fall to 14% in winter, whereas among Europeans there was an increase from 27 to 29% and in the South Asian group there was an even more pronounced increase from 21% in the fall to 27% in winter.

Repeated-measures ANOVA results showed a trend toward seasonal differences in total vitamin D intake (P = 0.060). This was primarily because of lower dietary vitamin D intake during the winter (P = 0.032). Supplemental vitamin D intake did not show similar seasonal patterns (P = 0.73).

### TABLE 1 Characteristics of the total sample and by ancestral subgroup

<table>
<thead>
<tr>
<th>Visit and variables</th>
<th>Total sample</th>
<th>East Asian</th>
<th>European</th>
<th>South Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>351</td>
<td>104</td>
<td>110</td>
<td>95</td>
</tr>
<tr>
<td>Age, y</td>
<td>21.1 ± 0.16</td>
<td>20.4 ± 0.23</td>
<td>22.1 ± 0.38</td>
<td>20.7 ± 0.19</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.2 ± 0.23</td>
<td>22.3 ± 0.36</td>
<td>23.6 ± 0.47</td>
<td>23.3 ± 0.38</td>
</tr>
<tr>
<td>Skin pigmentation, melanin index</td>
<td>34.6 ± 0.39</td>
<td>32.4 ± 0.27a</td>
<td>30.4 ± 0.28a</td>
<td>39.2 ± 0.62a</td>
</tr>
<tr>
<td>Serum 25(OH)D, nmol/L</td>
<td>54.4 ± 1.32</td>
<td>48.2 ± 1.50b</td>
<td>76.9 ± 2.32b</td>
<td>37.5 ± 1.55b</td>
</tr>
<tr>
<td>Serum calcium, mmol/L</td>
<td>2.42 ± 0.01</td>
<td>2.41 ± 0.01</td>
<td>2.43 ± 0.02</td>
<td>2.41 ± 0.02</td>
</tr>
<tr>
<td>Serum phosphate, mmol/L</td>
<td>1.20 ± 0.01</td>
<td>1.22 ± 0.02</td>
<td>1.19 ± 0.02</td>
<td>1.21 ± 0.02</td>
</tr>
<tr>
<td>Serum PTH, pmol/L</td>
<td>3.66 ± 0.08</td>
<td>3.33 ± 0.11</td>
<td>3.43 ± 0.13</td>
<td>4.26 ± 0.15</td>
</tr>
<tr>
<td>Total vitamin D Intake, μg/d</td>
<td>7.60 ± 0.40</td>
<td>6.31 ± 0.55</td>
<td>8.39 ± 0.80</td>
<td>7.77 ± 0.65</td>
</tr>
<tr>
<td>Diet</td>
<td>5.21 ± 0.19</td>
<td>4.74 ± 0.34b</td>
<td>5.21 ± 0.32b</td>
<td>5.64 ± 0.34a</td>
</tr>
<tr>
<td>Supplements</td>
<td>2.38 ± 0.34</td>
<td>1.57 ± 0.39</td>
<td>3.18 ± 0.73</td>
<td>2.14 ± 0.50</td>
</tr>
<tr>
<td><strong>Winter</strong></td>
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<td></td>
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<tr>
<td>n</td>
<td>321</td>
<td>92</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>Age, y</td>
<td>21.4 ± 0.17</td>
<td>20.7 ± 0.24</td>
<td>22.6 ± 0.41</td>
<td>21.0 ± 0.20</td>
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<td>BMI, kg/m²</td>
<td>23.5 ± 0.24</td>
<td>22.6 ± 0.41</td>
<td>23.8 ± 0.52</td>
<td>23.6 ± 0.41</td>
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<tr>
<td>Skin pigmentation, melanin index</td>
<td>33.1 ± 0.41b</td>
<td>30.9 ± 0.31b</td>
<td>28.1 ± 0.27b</td>
<td>37.9 ± 0.57a</td>
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<tr>
<td>Serum 25(OH)D, nmol/L</td>
<td>38.4 ± 1.06b</td>
<td>33.3 ± 1.60b</td>
<td>52.4 ± 2.00b</td>
<td>28.4 ± 1.53b</td>
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<td>Serum calcium, mmol/L</td>
<td>2.40 ± 0.01b</td>
<td>2.39 ± 0.01ab</td>
<td>2.38 ± 0.01b</td>
<td>2.42 ± 0.01a</td>
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<tr>
<td>Serum phosphate, mmol/L</td>
<td>1.21 ± 0.01b</td>
<td>1.26 ± 0.02</td>
<td>1.17 ± 0.02</td>
<td>1.22 ± 0.02</td>
</tr>
<tr>
<td>Serum PTH, pmol/L</td>
<td>4.23 ± 0.11</td>
<td>3.96 ± 0.17</td>
<td>4.14 ± 0.20</td>
<td>4.66 ± 0.21</td>
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<tr>
<td>Total vitamin D Intake, μg/d</td>
<td>7.24 ± 0.44</td>
<td>5.63 ± 0.51</td>
<td>7.48 ± 0.76</td>
<td>7.59 ± 0.76</td>
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<tr>
<td>Diet</td>
<td>4.39 ± 0.16</td>
<td>4.18 ± 0.29</td>
<td>4.29 ± 0.23</td>
<td>4.59 ± 0.31</td>
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<tr>
<td>Supplements</td>
<td>2.85 ± 0.41</td>
<td>1.45 ± 0.41</td>
<td>3.20 ± 0.72</td>
<td>3.01 ± 0.86</td>
</tr>
</tbody>
</table>

1 Values are means ± SE. Means in a row with superscripts without a common letter differ, P < 0.05. *Different from fall, P < 0.05.

Factors affecting vitamin D status

**Fall.** Approximately 23% of the variation in serum 25(OH)D concentrations was explained by the linear combination of the variables tested (r² = 0.23; P < 0.001) (Table 3). Skin pigmentation (P < 0.001), sun exposure (P < 0.001), vitamin D supplements (P < 0.001), and study year (P = 0.010) were significant predictors of 25(OH)D levels. Controlling for all the other variables in the model, reported supplemental vitamin D intake showed a positive correlation with serum 25(OH)D and it alone explained ~9% of the variance in 25(OH)D concentrations, whereas skin pigmentation explained ~14%, reported sun exposure 4%, and study year 2% (Table 3).

**Winter.** In winter, 27% of the variation in serum 25(OH)D was explained by the variables included in the regression (r² = 0.27; Table 2).

### TABLE 2 Proportion of individuals at different serum vitamin D concentration thresholds for both the fall and winter visits, stratified by ancestry

<table>
<thead>
<tr>
<th>Visit</th>
<th>nmol/L</th>
<th>East Asian</th>
<th>European</th>
<th>South Asian</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
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<td><strong>Fall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>2 (2)</td>
<td>0 (0)</td>
<td>17 (18)</td>
<td>23 (8)</td>
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</tr>
<tr>
<td>≥25 &lt;50</td>
<td>63 (61)</td>
<td>12 (11)</td>
<td>62 (65)</td>
<td>153 (44)</td>
<td></td>
</tr>
<tr>
<td>≥50 &lt;75</td>
<td>30 (29)</td>
<td>48 (44)</td>
<td>13 (14)</td>
<td>109 (31)</td>
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<tr>
<td>≥75</td>
<td>9 (8)</td>
<td>50 (45)</td>
<td>3 (3)</td>
<td>66 (19)</td>
<td></td>
</tr>
<tr>
<td><strong>Winter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>29 (33)</td>
<td>6 (7)</td>
<td>46 (50)</td>
<td>94 (29)</td>
<td></td>
</tr>
<tr>
<td>≥25 &lt;50</td>
<td>52 (57)</td>
<td>40 (41)</td>
<td>40 (43)</td>
<td>146 (46)</td>
<td></td>
</tr>
<tr>
<td>≥50 &lt;75</td>
<td>9 (10)</td>
<td>42 (43)</td>
<td>5 (5)</td>
<td>68 (21)</td>
<td></td>
</tr>
<tr>
<td>≥75</td>
<td>2 (2)</td>
<td>9 (9)</td>
<td>2 (2)</td>
<td>13 (4)</td>
<td></td>
</tr>
</tbody>
</table>
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Our results are consistent with previous studies reporting seasonal changes in serum 25(OH)D in Canadians (1–3,36). Liu et al. (36) examined 25(OH)D concentrations in adults living in long-term residences in Toronto, finding that 38% had 25(OH)D < 40 nmol/L in the fall compared with 60% in the spring (36). Vieth et al. (3) observed seasonal differences in their sample of young white women from Toronto; in that study, mean summer 25(OH)D was 76 nmol/L, with a decrease to 58 nmol/L during the winter. Rucker et al. (2) also observed seasonal fluctuations in serum 25(OH)D in western Canadians, with a drop in 25(OH)D from 71.6 nmol/L in the summer to 57.3 nmol/L in winter. Sloka et al. (1) studied pregnant women in Newfoundland and Labrador and found that mean 25(OH)D concentrations were 52.1 nmol/L in winter (January to March) compared with 68.6 nmol/L in summer (July to September) (1). Vieth (37) recently proposed that the surprising increased risk of prostate and pancreatic cancers reported in people with higher serum 25(OH)D at higher latitudes is attributable to a greater seasonal amplitude of serum 25(OH)D with higher latitude. The present results are consistent with that perspective, because the greatest seasonal variability in both relative and absolute fluctuations in 25(OH)D occurred in the participants with the highest summertime levels.

The recent Canadian Health Measures Survey (CHMS) examined 25(OH)D levels in a representative sample of Canadians aged 6–79 y from 2007 to 2009 (38). For those aged 20–39 y, there was a small but significant seasonal effect on 25(OH) D concentrations, from a mean of 69 nmol/L measured during the months with UVB (April to May) to a mean of 60 nmol/L in the months lacking UVB (November to March) (38). It is important to note that the CHMS survey did not measure seasonal changes in 25(OH)D in the same individuals. The CHMS also examined ethnicity and reported that the mean 25(OH)D concentration among white Canadians aged 20–39 y was 70 nmol/L compared with 48 nmol/L for Canadians of other ancestry (a group that included anyone who did not report their ethnicity as “White” due to small sample size of other ethnicities) (38). The CHMS survey reported that the age group with the lowest 25(OH)D concentrations were adults aged 20–39 y, indicating that young adults are at greatest risk for vitamin D inadequacy.

We also explored in detail the main factors influencing serum 25(OH)D levels during the fall and the winter. Fall levels of 25(OH)D were primarily influenced by skin pigmentation and supplement vitamin D intake. Our data indicate that for the fall, 1 unit increase in melanin index is associated, on average, with a
Studies of other European and high-latitude populations also found dietary vitamin D intake to be a robust predictor of 25(OH)D levels (43–45). Although direct vitamin D intake measurements were not conducted in the NHANES surveys in the US, milk consumption was found to be a significant predictor of 25(OH)D (46). In the present study, vitamin D intake was a significant predictor of 25(OH)D during both fall (supplemental vitamin D intake only) and winter (both supplemental and dietary vitamin D intake) visits, further indicating the key role that vitamin D intake plays in maintaining circulating 25(OH)D concentrations at high latitudes.

In our sample, mean vitamin D intake met or exceeded the recommended Adequate Intake of 5 μg/d (200 IU/d) for this age group (47) in the total sample and each ancestral group at both visits. However, we observed low 25(OH)D concentrations in many individuals, particularly during the winter. Of the 183 individuals who reported an intake in excess of 5 μg/d in the fall, 78% had serum 25(OH)D < 75 nmol/L, 45% had concentrations < 50 nmol/L, and 4% had concentrations < 25 nmol/L. At the winter visit, 92, 62, and 10% of the 146 individuals who reported vitamin D intakes >5 μg/d had serum 25(OH)D concentrations < 75, 50, and 25 nmol/L, respectively. In agreement with previous studies, our findings show that current Health Canada recommendations are insufficient for maintenance of optimal serum 25(OH)D, particularly in winter (3,4,48). The Institute of Medicine is currently conducting a review of the Dietary Reference Intakes for vitamin D, funded jointly by the U.S. and Canadian governments, with a final report expected later this year (49).

In 2007, the Canadian Cancer Society announced new vitamin D guidelines, recommending that “adults living in Canada should consider taking vitamin D supplementation of
Intakes in excess of 25 μg/d during the fall and winter and that “adults at higher risk of having lower vitamin D levels should consider taking vitamin D supplementation of 1000 μg/d all year round. This includes people who are older, with dark skin, who don’t go outside often, and who wear clothing that covers most of the skin” (50). Our study lends support to the recommendations of the Canadian Cancer Society, which take into account risk factors for vitamin D insufficiency (seasonality, UVR exposure, and skin pigmentation). Our results indicate that higher vitamin D intakes are necessary to maintain adequate 25(OH)D concentrations year-round in young adults, particularly among those of non-European ancestry. It is important to note that mean total vitamin D intake in South Asians was higher than in East Asians during both seasons and higher than Europeans during the winter. However, South Asians had the lowest mean 25(OH)D concentrations during both seasons (37.5 ± 1.5 nmol/L in the fall and 29.4 ± 1.5 nmol/L in the winter), most probably due to the fact that participants of South Asian ancestry had, on average, darker skin pigmentation (Table 1), which negatively influences endogenous vitamin D synthesis during the summer months. Given the very low 25(OH)D concentrations in some young adults in this study, it is possible that intakes in excess of 25 μg/d (1000 μg/d) may be needed to raise and maintain their 25(OH)D above 75 nmol/L year-round. Further work is required to elucidate the vitamin D intake needed to achieve and maintain optimal vitamin D levels in individuals of different ancestry.

Interestingly, we found study year to be a modest but significant predictor of 25(OH)D concentrations. This association can be explained in part by differences in incident solar radiation in the Toronto area over the 2007 and 2008 summers. Based on preliminary data from the UVR monitoring station at Toronto (Station 65), the average daily spectral irradiation (kJ m⁻² nm⁻¹) was higher for almost all measured wavelengths in 2007 compared with 2008 for the months of March to October (300, 305, 305, 310, 315, and 325 nm; the amount measured at 295 nm was higher in 2008 than 2007). The sum of the average daily spectral irradiation between the months of March and October for the UVB spectrum (290–320 nm) was higher in 2007 than in 2008 [11.7 vs. 11.1 (kJ m⁻² nm⁻¹)] (51). Furthermore, during the summer of 2008, there was far more precipitation and far fewer sunny days. Precipitation between the months of March and September of 2007 was only 245 mm, whereas during the same time in 2008, the precipitation was 410 mm (52).

This study has a number of limitations. The sample mostly consisted of young adults that were recruited at a university setting and may not reflect the general population of young people either in the Toronto area or elsewhere in Canada. However, although this study featured only 3 well-represented ancestral groups, it is more representative of the population diversity in the GTA than previous studies. The 2006 Canadian census found that visible minorities represent 43% of the population of metropolitan Toronto, the 2 largest groups being those of South Asian and Chinese ancestry (53). More than one-half (54%) of all the South Asians resident in Canada live in the Toronto area, where they represent nearly one-third (32%) of all visible minorities and comprise 14% of Toronto’s population (53). In 2006, individuals of Chinese ancestry comprised almost a quarter (22%) of all visible minorities and 10% of the total population of the city of Toronto. Nevertheless, our sample encompassed only a subset of the population diversity found in Canada and more particularly in Canadian metropolitan areas (53). Although in our study we considered many of the factors known to affect vitamin D levels (vitamin D intake, skin pigmentation, seasonality, UVR exposure, BMI), we did not include some predictors that have been associated with vitamin D levels in previous studies (e.g., smoking).

In conclusion, we report that low levels of serum 25(OH)D, the main indicator of vitamin D status, are widespread in healthy young adults of diverse ancestry living in the GTA, particularly in individuals of non-European ancestry. The main predictors of vitamin D status are vitamin D intake (particularly from supplements) and skin pigmentation. Additionally, there is a substantial decline in vitamin D levels from the fall to the winter and this drop is proportional to baseline vitamin D levels.

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**Literature Cited**


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