The development and evaluation of a food frequency questionnaire used in assessing vitamin D intake in a sample of healthy young Canadian adults of diverse ancestry

Hongyu Wu\textsuperscript{a}, Agnes Gozdzik\textsuperscript{b}, Jodi Lynn Bart\textsuperscript{a}, Dennis Wagner\textsuperscript{c,e}, David E. Cole\textsuperscript{d}, Reinhold Vieth\textsuperscript{c,e}, Esteban J. Parra\textsuperscript{b}, Susan J. Whiting\textsuperscript{a,*}

\textsuperscript{a}College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon SK, Canada S7N 5C9
\textsuperscript{b}Department of Anthropology, University of Toronto at Mississauga, Mississauga, Ontario, Canada L5L 1C6
\textsuperscript{c}Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 3E2
\textsuperscript{d}Departments of Laboratory Medicine and Pathobiology, Medicine, and Paediatrics (Genetics), University of Toronto, Toronto, Ontario, Canada M5G 1L5
\textsuperscript{e}Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5

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Abstract

Little data exist on vitamin D deficiency related with intake, especially for the Canadian population. The purpose of this study was to develop and evaluate a food frequency questionnaire (FFQ) with 37 items for rapid assessment of vitamin D intake in healthy young adults of diverse ancestry. We recruited 107 subjects in Southern Ontario during the late winter of 2007 who completed an FFQ twice (FFQ-1 and FFQ-2, repeated for reproducibility assessment) and a 7-day food diary (for validation). Serum 25-hydroxyvitamin D (25(OH)D), the major biomarker of vitamin D nutritional status, and skin melanin were determined. The FFQ results were highly correlated with 7-day diary results and with serum 25(OH)D concentrations ($r = 0.529, P < .001; r = 0.481, P < .001$, respectively). Modifications to the FFQ, by redefining the large serving size and excluding the fortified orange juice category, improved the validity of the FFQ ($r = 0.602, P < .001; r = 0.520, P < .001$, respectively). The FFQ results were highly correlated ($r = 0.663, P < .001$), but the mean intakes were different ($P < .05$). Using results from a modified version of FFQ-1, we examined dietary intakes in 3 predominant groups: East Asian (n = 27), European (n = 31), and South Asian (n = 32). The European group had higher total vitamin D intake ($P < .05$) and the highest serum 25(OH)D concentrations ($P < .05$), with a trend for dairy products being responsible for this ($P < .10$). Because Canadians are reliant on dietary intakes of vitamin D in the wintertime, especially those with higher skin melanin, our FFQ can monitor and provide information on intake and food group consumption.

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; FFQ, food frequency questionnaire; $\kappa_w$, weighted kappa; LSD, least significant difference (a post-hoc multiple comparison test); PTH, parathyroid hormone; SD, standard deviation.

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* Corresponding author.
E-mail address: susan.whiting@usask.ca (S.J. Whiting).

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1. Introduction

It is now recognized that the function of vitamin D extends far beyond that required for calcium homeostasis. Vitamin D can play a role in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease [1]. Vitamin D is produced in skin through a photolytic process. Vitamin D deficiency is common in situations when cutaneous production and intake are compromised [2]. Many studies have identified a high prevalence of vitamin D insufficiency in healthy adults living in North America [3-7]. Some of these reports also demonstrated the importance of ethnicity, where persons with darker skin color (ie, higher skin melanin levels) had lower vitamin D status [3,6,7]. Vitamin D intakes are not adequate to maintain sufficient vitamin D status in the Canadian population [3,4,8,9], but little is known about vitamin D intakes of subpopulations who are at higher risk of vitamin D deficiency in Canada.

Very little vitamin D is naturally present in foods, except for fatty ocean fish. Fortified foods such as milk, margarines, and orange juice provide vitamin D for Canadians. Because of relatively limited food sources of vitamin D, specific combinations of foods can be listed in a food frequency questionnaire (FFQ) specific for vitamin D. By adding a few other foods high in calcium (such as cheese and yogurt, which are currently not fortifed in Canada), the resulting FFQ may be able to measure both calcium and vitamin D. When compared to other methods for assessing food consumption of individuals, the FFQ has lower respondent burden. The results are easy to collect and process and are taken to represent usual intakes [10,11].

We designed an FFQ for assessing vitamin D (and calcium) intake of a multiethnic group of healthy young Canadian adults. Validation of the FFQ method was undertaken during the winter in order to use the biomarker of vitamin D status, 25-hydroxyvitamin D (25(OH)D) as its concentration would be dependent on dietary intake and body stores after 4 months [1,3,12]. We also used a dietary reference method, 7-day food diary records, in the validation [11]. We have previously published results of dietary intakes from the 7-day diaries and 25(OH)D concentrations in this pilot study [13]. We hypothesized that the validity of an FFQ designed for young adult Canadians of diverse ancestry could be determined in the late winter when sun synthesis of vitamin D would not be a confounder. A secondary objective was to explore the determinates of vitamin D intake in this group [12-14].

2. Methods and materials

2.1. Subject recruitment and data collection

Details of the study have been described elsewhere. Briefly, study recruitment took place during January to March of 2007. Ethical approval was obtained from the University of Toronto Health Sciences Research Ethics Board. Exclusion criteria were age (only participants between the ages of 18 and 30 were recruited for this study), diagnosis of kidney/liver damage or other disorders or diseases that may affect vitamin D metabolism or absorption, use of medications that affect vitamin D metabolism, and recent (< 3 months) exposure to large amounts of UV radiation (tanning salons, visits to tropical locations, and so on). A total of 107 subjects were eligible and agreed to participate, and most of the participants were either students or employees of the university; 105 of them completed both FFQs and 7-day diary. Participants completed a first FFQ (FFQ-1) in the initial visit; they returned their 7-day food diary and completed the second FFQ (FFQ-2) in the second visit (mostly within 2 weeks of the first interview).

2.2. Development and administration of the FFQ

The FFQ administered in this study was based on a previously published questionnaire for vitamin D and calcium intake assessment [15], which had been validated only against 4-day diary records. The FFQ was expanded to include new foods and foods that might be consumed by persons from different ethnicities, such as various fishes, seafood, and soy products. It consisted of 37 items of specific foods or food groups with 9 response options ranging from ‘never or less than 1 per month’ to ‘2+ per day’ for the frequency of consumption. The serving sizes were based on household measures (eg, cups, spoons) and natural units (eg, 1 slice). Respondents were asked to rank serving sizes as medium (described), small (half of medium), or large (twice medium). Additional open-ended questions on the FFQ collected information on nutritional supplement use. Subjects were asked to recall their frequency of consumption of food items over the preceding month.

Vitamin D and calcium contents for each food item in the FFQ were determined using ESHA Food Processor (Version 8.0, ESHA Research, Ore), which included the 1997 Canadian Nutrient File from Health Canada. Fortification amounts for foods recently approved in Canada were updated (orange juice, soy beverage) to present values. Dietary intake was exported to Excel spreadsheet reports to which supplement intake was added. Vitamin D and calcium content of the supplements were obtained from the label declarations.

2.3. 7-Day food diary as a reference method for validation of the FFQ

Detailed instructions were provided to the participants during the initial visit to complete food records on 7 consecutive days. Participants were asked to record, at the time of consumption, all foods and beverages (including snacks) eaten in household measures. A portion size kit consisting of measuring cup and spoons and assorted shapes
was provided. Brand names of products and recipes used were included. Any nutritional supplements consumed were also recorded. Participants returned their 7-day food diaries during the second visit.

Among the 105 participants who provided food diaries, 97% of them had 7 days of records (n = 102); 1% (n = 1) had 6 days; and 2% (n = 2) had 5 days of records due to incomplete recording. The ESHA Food Processor was also used for food records analysis. Appropriate food ingredients and amounts were determined when the records were in recipe format and/or in non-Western dietary patterns. Dietary intake was exported to an Excel spreadsheet. Supplement intake recorded in the diaries was added into the spreadsheet to give total intake.

2.4. Serum 25(OH)D as the biomarker for validation of the FFQ

An aliquot of whole blood was centrifuged, and the serum fraction was removed after clotting and stored at −80°C. The 25(OH)D concentrations were determined using the Diasorin “25-OH Vitamin D TOTAL” competitive chemiluminescence immunoassay on the automated LIAISON analyzer (Stillwater, Minn) [13].

2.5. Statistical analyses

Analyses are based on subjects (N = 105) who had completed all assessment methods (both FFQs, 7-day diary, and biomarker 25(OH)D), and data are presented as means ± standard deviation (SD). Histogram displays were initially examined to determine the shape of the spread required by inferential statistics. Square-root transformations were performed to normalize the distribution of serum 25(OH)D, melanin index, and all intake variables (except energy intake). Paired t tests were used to examine mean vitamin D intakes on the FFQ and the food diary and on the FFQ-1 and FFQ-2. Correlation analyses were used to measure the strength of the relationship between the intakes from the 2 dietary methods and between the 2 methods and biomarker 25(OH)D. Cross-classification and weighted kappa (κw) were used to test the relative agreement between FFQ-1 and 7-day diary and between FFQ-1 and FFQ-2 [11]. Analysis of variance was used to evaluate group difference for serum measurements and intake levels. The least significant difference (a post hoc multiple comparison test) test was used in post hoc analysis for multiple comparisons. The nonparametric tests Kruskal-Wallis and Mann-Whitney were used when transformed data did not satisfy normality. Analyses were performed using SPSS version 15.0 (SPSS Inc, Illinois). A P value < .05 was considered significant.

3. Results

3.1. The characteristics of the study sample

The characteristics of the study sample are shown in Table 1. The subjects, mean age of 20 years, were predominantly of low and normal body weight (44% with body mass index [BMI] <18.5 kg/m2, 44% with BMI ≥18 but <25 kg/m2). Subjects self-identified their ethnicities as European (n = 31), East Asian (n = 27), South Asian (n = 32), African (n = 7), and others (n = 8) who had self-identified more than one ethnicity (n = 7) or indicated as Middle Eastern (n = 1). Sex differences were found for age, BMI, and energy intake (P < .05) but not for melanin index, parathyroid hormone (PTH), 25(OH)D, or vitamin D and calcium intakes (FFQ-1, FFQ-2, or 7-day diary results).

3.2. Validity and reproducibility of the FFQ

When we compared intakes from the 7-day diary and the FFQ-1, there was a highly significant correlation (r = 0.529,
Results using the FFQ-1 were significantly higher than intakes using the FFQ-2 \( (P = .013) \), indicating a change in reporting behavior. Further, \( \kappa w \) suggested somewhat fair agreement. However, the Pearson correlation coefficients between these 2 FFQs were higher than 0.65, indicating good reliability. The correlations and agreement tended to be stronger after using the modified FFQ results.

### 3.3. Vitamin D intake by ethnicity

We focused on 90 participants in 3 groups: East Asian \( (n = 27) \), European \( (n = 31) \), and South Asian \( (n = 32) \), and we applied the FFQ-1M results. Ethnic differences were found in melanin index and serum 25(OH)D concentrations \( (P < .001) \), as well as in vitamin D intake and calcium intake \( (P < .05) \) (Table 4). The European group had higher total vitamin D intake than the other 2 groups \( (P < .05) \). There was no significant ethnic difference among percentages of subjects in each group who had vitamin D intake lower than the adequate intake value of 5μg/d.

Sources of vitamin D intake were categorized from the FFQ as dairy products, nondairy products, and supplements. Intakes of some major vitamin D foods are also presented in Table 4. There was a trend that the European group was relatively higher in dairy products intake \( (P = .089) \) and in cow’s milk consumption \( (P = .074) \). The East Asian group had higher intake of soy milk than the other 2 groups \( (P = .001) \). There was no significant ethnic difference among the percentages of supplement use.

Vitamin D intakes (FFQ-1M) were significantly positively related to serum 25(OH)D concentrations in each of the 3 ethnic groups (East Asian: \( r = 0.498, P < .05 \); European: \( r = 0.493, P < .05 \); South Asian: \( r = 0.421, P < .05 \)), whereas melanin content was not (East Asian: \( r = 0.066, P > .05 \); European: \( r = -0.303, P > .05 \); South Asian: \( r = 0.340, P = .057 \)). Vitamin D intake was also related with serum 25(OH)D when data were combined from all 3 ethnic groups either before or after being controlled for melanin content \( (r = 0.520, P < .001 \text{ vs } r = 0.511, P < .001) \). The BMI was not related with either serum 25(OH)D concentrations \( (r = -0.018, P = .869) \) or with vitamin D intake (FFQ-1M; \( r = -0.087, P = .414 \)).

In Table 4, we also show that there were ethnic differences in mean calcium intake \( (P < .05) \) but no ethnic differences in PTH levels \( (P > .05) \). There was an inverse relationship between serum 25(OH)D concentrations and
PTH levels ($r = -0.273$, $P = .009$) when the 3 ethnic groups were combined. When 25(OH)D concentrations were categorized as $\geq 50$ nmol/L and $<50$ nmol/L, and calcium intakes were categorized as $\geq 1000$ mg/d (adequate intake) and $<1000$ mg/d, PTH levels were significantly lower when vitamin D status was sufficient ($P < .05$) but did not show a difference by calcium intake levels ($P > .05$; Table 5).

### 4. Discussion

We developed and evaluated a cross-cultural semiquantitative FFQ for estimating vitamin D intake in a young adult population. The subjects of our study were recruited from the same population in which a following larger study ($n = 400$) of vitamin D intake and status assessment will be carried out. Thus, we initially piloted the FFQ on a sufficient and representative sample of the population to which the final FFQ will be applied. However, we cannot rule out the possibility that those who participated in this study had a greater interest in and awareness of vitamin D intake than those who would be included in nutrition epidemiological studies using the FFQ alone. Although the subjects were well motivated to complete the food diary for 7 days, it is possible that the food diary was not representative of long-term dietary habits that we expected.

We assessed the validity and reproducibility of the FFQ used in our study. The FFQ was found to be valid. Comparison of means revealed a tendency for higher estimation of intake on the FFQ-1 than the 7-day diary. We

### Table 5

Parathyroid hormone levels by serum 25(OH)D levels or calcium intake ($n = 90$)

<table>
<thead>
<tr>
<th>Serum 25(OH)D</th>
<th>PTH (pmol/L)</th>
<th>Calcium intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;50$ nmol/L ($n = 64$)</td>
<td>3.43 ± 1.07</td>
<td>2.77 ± 0.98</td>
</tr>
<tr>
<td>$\geq 50$ nmol/L ($n = 26$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;1000$ mg/d ($n = 52$)</td>
<td>3.22 ± 1.12</td>
<td>3.26 ± 1.04</td>
</tr>
<tr>
<td>$\geq 1000$ mg/d ($n = 38$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $P < .05$. PTH levels were presented as means ± SD. Calcium intakes were FFQ-1M results.
therefore attempted to eliminate this bias through modification of the FFQ. After modification, the strength of the correlation and the extent of agreement were both enhanced. For reproducibility, the FFQ-1 was highly correlated with the FFQ-2; a significant difference in mean intakes between the 2 periods, however, indicated a bias. The large within-subject variation may be due to the short time frame between the 2 FFQs. Having become aware of the study purpose and having learnt from food diary recording, the subjects might have adjusted their estimation of intake amounts in the FFQ-2.

The Canadian Nutrient File recently added vitamin D values for meat [16]. We investigated whether having these additional contents would be a problem on our 7-day diary and found that the vitamin D from meat intake was only 0.4 ± 0.3 μg/d, just 8.5% of total vitamin D intake. The meat intake level was low in our study sample, and there was no ethnicity difference in meat vitamin D intake. Although updated vitamin D values from meat intake in 7-day diary results did not change intake results, meat-containing foods should be added into the FFQ list for future use.

Our previous report had provided 7-day diary data on ethnic difference in vitamin D intake in healthy young adults [13]. We now show a breakdown of intakes by food groups using modified FFQ results. The dairy group was the greatest food source of vitamin D for each of the 3 groups. This was similar to the vitamin D intake observed in the United States [17]. There was a trend that the European group had higher consumption of dairy products, especially cow’s milk. We believe this is evidence that consumption of dairy products and in particular cow’s milk is higher in European subjects’ diets than in East and South Asian groups. The East Asian group had a significantly higher consumption of fortified soy beverage, but this made little difference to overall mean intake of vitamin D. Thus, we suggest that fortified soy beverage could be an important source of vitamin D for some ethnic groups if consumed more often. We observed that the use of supplements (16%-26%) was low for subjects in all 3 groups.

In our study sample, it was consistently shown in either 7-day diary results [13] or FFQ-1M results that vitamin D intake was associated with serum 25(OH)D levels. The data in our study were collected in late wintertime, when skin production of vitamin D was not possible and previous serum vitamin D stores had been depleted or nearly depleted. This timing allowed for an examination of the importance of dietary intake on vitamin D status in the winter. An inverse relation between measures of BMI and serum 25(OH)D concentrations has been shown in National Health and Nutrition Examination Survey data [18]; however, this relationship was not observed in our study. Our sample subjects had relatively low BMIs, and there were few obesity cases. In other words, the variance of this variable (BMI) might not exist in our study for observing the effect of body fat on 25(OH)D levels that others have reported [19-21].

An inverse relationship between 25(OH)D and PTH was observed in our study. This is consistent with the demonstration that PTH levels are elevated with vitamin D deficiency [22]. The results of our study suggest that for suppressing PTH levels, it is more important to keep sufficient vitamin D status than to raise calcium intake levels. Others have shown calcium intake levels to be important in this regard [23].

Reproducibility in dietary assessment can be difficult to ascertain because in repeated dietary assessments, subjects might show a sequence effect that would result in a change in reported nutrient intakes over time [11]. This effect could be severe if subjects completed the repeated assessment too close in time. Although the 2-week time frame of our study gave us a high response rate of the participants, the subjects likely changed the description of their intake habits in the second FFQ, which was completed soon after the food diary recording. Although the sample size of our study did not limit the overall validation assessment of the FFQ, the relatively small number of subjects in each ethnic group affected the statistical power for vitamin D intake assessment. Another limitation is that most of the subjects in our study were either students or employees of the University of Toronto and, thus, were likely to have different socio-economic status than the general population. Therefore, we cannot generalize our findings to the whole young healthy adults living in Canada.

In summary, the newly developed FFQ was found to be consistently related to the reference method and the biomarker. It is valid and can provide a reasonable estimation of vitamin D intake in healthy young adults of diverse ancestry. The modification of the results decreased the bias, further enhanced the validity, and improved the reproducibility. The modified FFQ is now being used in an ongoing vitamin D study in healthy young adults. As vitamin D and/or calcium enhanced foods enter the marketplace, these can be added to the FFQ to maintain accuracy of the instrument.

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