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Validity and Reproducibility of a Short Food Frequency Questionnaire in Assessing Calcium and Vitamin D Intake in Canadian Preschoolers

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Abstract

Background: The use of inaccurate dietary assessment methods jeopardizes the understanding of the relationship between diet and health. Vitamin D and calcium are important nutrients that support growing bone. To our knowledge, there is no validated Food Frequency Questionnaire (FFQ) suitable for capturing usual intakes of vitamin D and calcium in preschool children. Therefore, the aim of this study was to evaluate the reproducibility and validity of a brief semi-quantitative FFQ designed to assess usual vitamin D and calcium intakes of Canadian preschoolers attending day care using both dietary and biochemical comparators.

Methods: The FFQ was validated on a subsample ($n = 53$) of a study assessing vitamin D intake and status of preschoolers in Montreal ($n = 508$). Dietary data was collected by observation at day care and dietary recall by caregivers for the 24-h assessment and a 30-day FFQ. Dietary assessment was repeated within 30 days. Plasma 25-hydroxyvitamin D (25(OH)D) concentration in capillary blood samples was measured using a chemiluminescence assay (Liaison, Diasorin Inc.). Differences between groups were tested using paired t-tests or Mann–Whitney tests. Pearson or Spearman rank correlations were tested depending on the distribution of the data.

Results: There were no significant differences in median calcium and vitamin D intakes between the two FFQs and no difference between the two 24-h recalls. Calcium (home, day care, total dietary) and vitamin D (home and total) intakes from FFQ 2 were significantly higher than those of 24-h recalls (1 and 2). A positive correlation was observed between the energy adjusted total vitamin D intake from the 24-h recalls 1 and 2 and 25(OH)D concentration ($r = 0.30$, $p = 0.03$). Using FFQ 2, a positive correlation with 25(OH)D was observed with total vitamin D intake ($r = 0.48$, $p < 0.01$). Cross-classification between FFQ 2 and 24-h dietary recall 1 and 2, showed good agreement for dietary vitamin D (92.4%) and calcium (94.3%) as well as good agreement between 25(OH)D and FFQ 2 (86.7%) and between 24-h dietary recalls 1 and 2 and 25(OH)D concentration (81.1%).

Conclusion: This semi-quantitative FFQ could be a promising tool to assess usual dietary and supplemental calcium and vitamin D intakes of Canadian preschoolers attending day care.

Keywords: *Vitamin D; Calcium; Preschool children; Food frequency questionnaire; Validity and reproducibility*

Abbreviations: %DV: percentage Daily Value; 24-h recall: 24 hour recalls; 25(OH)D: 25-Hydroxyvitamin D; CCHS: Canadian Community Health Survey; CHMS: Canadian Health Measures Survey; CNF: Canadian Nutrient File; CV: Coefficient of Variation; FFQ: Food Frequency Questionnaires; LoA: Limit of Agreements; SD: Standard Deviation

Background

Dietary assessment methods are used to identify foods and nutrients associated with health and disease [1]. These methods include 24 hour recalls (24-h recall), food records and food frequency questionnaires (FFQ) [2] and the use of inaccurate methods can jeopardize this relationship [1]. Even though valid, 24-h recalls and food records should be performed on several days to capture usual intakes, which would increase participant burden [3]. On the other hand, a FFQ is more practical in epidemiological research, as one FFQ could capture dietary intake over extended periods of time [4].

Vitamin D is naturally available in foods including fatty fish and eggs. Fortified sources are also available on the Canadian market; including milk, margarines, and orange juice. Development of a FFQ including these vitamin D sources and foods high in calcium (such as cheese and yogurt) would be very useful in studies designed to examine the determinants of vitamin D status [5].

According to the most recent report on vitamin D status of Canadians, children 3-5 years had the highest rate (89%) of sufficient levels of vitamin D (25-hydroxyvitamin D (25(OH)D) ≥ 50 nmol/L) which is recommended for bone health [6]. The Canadian Health Measures Survey (CHMS) included a one year FFQ to capture usual intakes of foods by individual people [7] whereas the Canadian Community Health Survey (CCHS) relied on 24-h recall methodology to examine intakes of groups of Canadians from infancy and beyond [8]. In order to explain vitamin D status in such young children, often more than one caregiver needs to be surveyed regarding intakes of foods that contain natural, fortified or supplemental vitamin D; this was problematic in the CCHS data [9]. To our knowledge, there is no validated FFQ suitable for capturing usual intakes of foods that contain vitamin D and calcium in children of preschool age. Moreover, since a high proportion of preschool age children have more than one caregiver, the FFQ should be evaluated for accuracy against gold standard methodology such as food observation. Previous work suggests that mixed methodology is a valid approach among this age group [10,11] as the estimated mean intake from observation studies was similar to actual weighed intakes [10].

Therefore, the aim of this study was to evaluate the reproducibility and validity of a brief semi-quantitative FFQ designed to assess usual vitamin D and calcium intakes of Canadian preschoolers attending day care using both dietary and biochemical comparators.

Methods

Participants

This validation study was part of a larger study assessing vitamin D intake and status of Canadian preschool age children in Montreal, Quebec, Canada (n = 508). The FFQ was validated on a representative sample (n = 53, 10%) of the population and across the 1-y study.

In brief, preschoolers (2 through 5 years) were recruited between May 2010 and June 2011 from a random sample of licensed day cares (n = 77) in the Montreal area (73°W, 45°N). Day cares were selected to represent 10% of all licensed day care centers from the Ministry of Family and seniors (Ministère de la Famille et des Ainés) in 91% of the regions in Montreal, Canada. Inclusion criteria was healthy term born children and between the ages of 2 and 6 years. Exclusion criteria included diseases known or associated with disturbances of bone, known or suspected serious chronic illness of childhood, use of medications known to affect bone and/or mineral ion metabolism in the past 3 months, history of prior treatment for biochemically confirmed vitamin D deficiency and severe anemia. A more detailed description of the population characteristics and survey methodology is available elsewhere [12].

Ethics

This study was approved by the McGill University Faculty of Medicine Institutional Review Board and all caregivers provided bilingual (French and English) written informed consent prior to inclusion in the study.

Dietary data

Dietary data was collected by observation at day care and dietary recall by caregivers for the 24-h assessment and FFQ designed to capture foods and supplements containing calcium and vitamin D in preschoolers. Data regarding food and beverages consumed at the day care was done by a registered dietitian, who was trained to observe and record all food consumed during day care hours (07h00 to 16h00). In order to complete a 24-h dietary intake, a registered dietitian contacted the child's primary care giver by telephone and used a multi-pass recall method to record foods eaten outside of day care during the same 24-h period.

The semi-quantitative FFQ was completed by a registered dietitian who examined the day care's menu and interviewed both the child's educator and the primary caregiver. The FFQ was used to reflect the previous month's intake of vitamin D and calcium from 13 commonly consumed sources (including milk, margarine, eggs, fish, vitamin D supplements and multivitamin) that were divided into several subgroups. One month was selected based on this, representing just over 1 half-life of circulating 25(OH)D [13], and since recall bias could be problematic for longer durations [14]. The Canadian Nutrient File (CNF) and percentage daily value (%DV) on nutrition labels were used to determine the vitamin D content of foods. Food labels were used to assess the vitamin D content variability between brand names and food products. Dietary intake observations, 24-h recall data and FFQ questionnaires were repeated within 30 days in a sub-sample to allow for nutrient adjustment and reliability of the FFQ. Data was analyzed using Nutritionist Pro™ (Axxya Systems LLC, Stafford, TX, US) and the CNF version 2010b.

Laboratory analysis

A 1 mL capillary blood sample (lithium heparin) was collected by a registered nurse using finger lance at day care. All blood samples were obtained between 08h00 to 12h00. Samples were stored on ice for transportation to McGill University where all samples were centrifuged at 3000g and 4°C for 20 min and plasma aliquots were stored at -80°C for subsequent analysis. Plasma 25(OH)D concentration was measured using a chemiluminescence assay (Liason, Diasorin, Mississauga, Canada). The 25(OH)D inter-assay and intra-assay coefficient of variation (CV) were 7.3 and 5.1% for the low 25(OH)D control (39.8 nmol/L) and 7.1 and 2.8% for the high 25(OH)D control (130.3 nmol/L). The accuracy using the mid-range of the manufacturer's specifications was 95.6%.

Statistical analysis

All statistical analysis was conducted using SAS (v9.2, Cary, USA). Normal continuous variables were expressed as mean±standard deviation (SD) and non- normal continuous variables were expressed as median (interquartile range). Categorical variables were expressed as proportions (%). Statistical significance was set at $p<0.05$. All entered data were screened for accuracy and outliers (>3 SD from mean) were examined.

Energy adjusted total vitamin D intake was calculated based on the following equation

$$\frac{\text{(crude average total vitamin D intake derived from 24-h recall 1 and 2)}}{\text{(average energy intake derived from 24-h recal 1 and 2}} \times 10000.$$

Differences of means were examined using Student's t-test or Wilcoxon signed-rank tests for normally or non-normally distributed variables, respectively, with normality being confirmed using qq-plots and Shapiro-Wilk statistics. Pearson's or Spearman rank correlations were calculated, depending on the distribution of the data. The Bland-Altman method, defined as the limit of agreements (LoA) (± 2 SD of the mean difference), assessed agreement between FFQ 2 and 24-h dietary recalls 1 and 2. The mean difference ((mean 24-hrecalls 1 and 2)-FFQ 2) was plotted against the mean ((mean 24-h recall 1 and 2) + FFQ 2)/2) for dietary vitamin D and calcium intake. The plot of the difference against the mean was used to investigate potential relationship between measurement error and true value and how much vitamin D (IU/d) and calcium intake differed between the FFQ and 24-h recalls. The upper and lower 95% CI were calculated [15]. To examine relative agreement, contingency (cross), classification by tertiles [16] were performed by Chi-square and KW was calculated for vitamin D and calcium. KW > 0.80 indicates very good agreement, between 0.61 to 0.80 good agreement, between 0.41 to 0.60 moderate agreement, between 0.21 to 0.40 fair agreement, and < 0.20 poor agreement [17].

Results

Of the 53 preschoolers included in the validation study, 26 (49.0%) were boys, and the median age was 4.1 years (Table 1). The majority (n = 31) were studied in the vitamin D non-synthesizing period (November 1st- March 31st). Plasma 25(OH)D concentration was significantly higher in the vitamin D synthesizing period (77.6 ± 27.9 nmol/L, n = 22) than in the non-synthesizing period (67.0 ± 19.2 nmol/L, n = 31, p = 0.05). However, mean vitamin D intake from both 24-h recalls was not different among vitamin D synthesizing (231.0 ± 85.0 IU) and non-synthesizing periods (266.1 ± 93.3 IU, p=0.16). Similarly, mean vitamin D intake from both FFQ was not different among vitamin D synthesizing (399.8 ± 145.6 IU) and non-synthesizing periods (432.3 ± 163.4 IU, p = 0.45).

Characteristics	
Age, y	4.1 (3.4-4.9)
White[1], %	58.5
Household income[2] ≥ 45,000 $, %	64.2
Maternal education, university degree or higher, %	66.0
Boys, %	49.0
Weight, kg	16.6 (14.6-18.3)
Weight for age-z-score[3]	0.07 (-0.35-0.42)
Height, cm	103.5 (96.2-107.5)
Height for age-z-score[3]	-0.14 (-0.88-0.25)
BMI, kg/m^2	16.0 (15.0-16.7)
BMI for age-z-score[3]	0.5 (-0.2-0.8)
Plasma 25(OH)D, nmol/L	71.2 (57.7-80.2)
Vitamin D synthesizing period, nmol/L[4]	73.2 (60.2-82.9)
Vitamin D non-synthesizing period, nmol/L	67.8 (53.7-79.2)

Table 1: *Selected characteristics of preschoolers attending day care in Montreal area Values are percent or median (IQR).*

[1] 1 missing value.

[2] 5 missing values.

[3] According to the World Health Organization.

[4] Synthesizing vitamin D period in Canada extends from April 1st to Oct 31st (29).

There were no significant differences in median calcium and vitamin D intakes (home, day care, total dietary, supplemental, or total) from both FFQs 1 and 2. An interval of 2 to 21 days separated the first and the second dietary data collection, with a median of 9 days. A significant positive correlation was observed for calcium and vitamin D intake ranging from 0.42 to 0.78 between FFQ 1 and 2 (Table 2). Similarly no differences were observed in median calcium and vitamin D intakes (home, day care, total dietary) between the first and repeated 24-h recalls. For instance, median day care calcium intake was 381 (320-539) mg from 24-h recall 1, while it was 369 (290-557) mg from 24-h recall 2, p = 0.89. Similarly, median day care vitamin D intake was 97.6 (55.4-125.6) IU from 24-h recall 1, while it was 84.0 (39.3-104.6) IU from 24-h recall 2, p = 0.10.

Food Frequency Assessment				
Nutrients[1]	**FFQ1**	**FFQ2**	**Mean Difference(FFQ1-FFQ2)**	**Correlation coefficient[2]**
Calcium (mg)				
Home	766 (581-1050)	814 (612-1071)	-59 (-196-151)	0.56*
Day care	355 (211-464)	354 (214-467)	-1 (-104-102)	0.48*
Total[3]	1161 (897-1472)	1225 (969-1500)	44 (-316-190)	0.50*
Supplemental	0 (0-0)	0 (0-0)	0 (0-0)	0.64*
Total intake[4]	1160 (893-1486)	1225 (969-1492)	44 (-332-190)	0.52*

Food Frequency Assessment				
Vitamin D (IU)				
Home	272.2 (185.4-402.4)	261.0 (176.5-414.5)	2.1 (-58.3-52.9)	0.62*
Day care	95.5 (65.7-122.5)	(67.5-124.1)	-3.7 (-27.1-27.9)	0.42*
Total[3]	396.8 (298.7-497.5)	403.0 (296.5-523.5)	-5.6 (-77.3-55.8)	0.56*
Supplemental	0.0 (0.0-24.4)	0.0 (0.0-0.0)	0 (0.0-0.0)	0.78*
Total intake[4]	396.8 (298.7-547.3)	410.0 (296.5-560.5)	-1.4 (-77.4-78.4)	0.64*
24-h recall Assessment				
Nutrients	**24-h recall 1**	**24-h recall 2**	**Mean Difference (24-h recall 1-24-h recall 2)**	**Correlation coefficient[2]**
Dietary Intake				
Calcium (mg)				
Home[1]	530 (318-841)	503 (337-753)	54 (-260-292)	0.31*
Day care[1]	381 (320-539)	369 (290-557)	9 (-111-120)	0.12
Total[3, 5]	1040 ± 386	1032±425	30 (-282-306)	0.29*
Vitamin D (IU)				
Home[1]	140.5 (76.2-233.4)	125.8 (76.4-197.7)	11.8 (-25.3-44.6)	0.39**
Day care[1]	97.6 (55.4-125.6)	84.0 (39.3-104.6)	20.1 (-24.6-77.4)	-0.07
Total[1,3]	252.0 (186.0-319.8)	212.8 (137.3-288.3)	42.5 (-28.1-103)	0.19

Table 2: *Calcium and vitamin D intake (dietary, supplemental, and total) and correlation coefficient between food frequency 1 and 2*
FFQ: food frequency questionnaire; there were no significant differences between mean intakes (dietary, supplemental, total) between FFQ1 and FFQ2 There were no significant differences between mean intakes (home, day care and total) between 24 hour recall 1 and 2.
[1]Wilcoxon signed-rank.
[2]Pearson or Spearman correlations coefficient based on normality of the data.
[3]Total = Day care + home.
[4]Total intake = dietary + supplemental.
[5]Student's paired t-test.
$*p < 0.01$ $**p \leq 0.01$.
Values presented as median (interquartile range).

Calcium (home, day care, total dietary) and vitamin D (home and total) intakes from FFQ 2 were significantly higher than those of 24-h recalls (1 and 2). However, day care vitamin D intake from FFQ 2 was similar to that of the 24-h recalls (1 and 2) intake. Correlation coefficients were significant and ranged from 0.33 to 0.53 (Table 3). Similar trends were observed when FFQ 1 was compared to 24-h recalls 1 and 2.

There was no significant correlation between the crude total dietary vitamin D from the 24-h recalls 1 and 2 and 25(OH)D concentration, overall and in the vitamin D non-synthesizing period. However, in the vitamin D synthesizing period, home vitamin D intake from 24-h recalls 1 and 2 was positively correlated with 25(OH)D ($r = 0.50$, $p = 0.02$) and crude total dietary vitamin D intake from 24-h recalls 1 and 2 was positively correlated with 25(OH)D ($r = 0.49$, $p = 0.02$). A significant positive correlation was observed between the energy adjusted total vitamin D intake from the 24-h recalls 1 and 2 and 25(OH)D concentration overall ($r = 0.30$, $p = 0.03$) and in the vitamin D -synthesizing period ($r = 0.6$, $p < 0.01$), but not in the vitamin D non-synthesizing period.

Nutrients	FFQ 2	Mean 24-h recall 1 and 2	Correlation coefficient
Calcium (mg)			
Home	814 (612-1071)[a]	533 (485-799)[b]	0.48**
Day care	354 (214-467)[a]	459 (309-538)[b]	0.33*
Total[1]	1234 ± 446[a]	1036 ± 327[b]	0.49**
Vitamin D (IU)			
Home	261.0 (176.5-414.5)[a]	145.7 (82.8-214.7)[b]	0.38**
Day care	103.0 (67.5-122.0)	102.0 (74.9-121.3)	0.53**
Total[1]	417.2 ± 117.2[a]	251.5 ± 90.7[b]	0.48**

Table 3: *Dietary intakes and correlation coefficients between FFQ and mean dietary 24 hour recall 1 and 2*

FFQ: *food frequency questionnaire*

Pearson or Spearman correlations coefficient based on normality of the data explored relationship between nutrients estimated by FFQ2 and 24-h recalls; Student's paired t-test or Wilcoxon signed-rank test based on normality of the data compared nutrient intake between FFQ2 and 24-h recalls.

[1]Total = Day care + home.

$^{**}p \leq 0.01$.

$^{*}p \leq 0.05$.

Values presented as median (interquartile range) (non-normal data) or mean±SD (normal data).

Using FFQ 2, significant positive correlations with 25(OH)D were observed with home, total dietary, supplement intake and total dietary and supplemental vitamin D intake ($p < 0.05$) (Table 4). In the vitamin D synthesizing period, vitamin D supplement intake from FFQ 2 was positively correlated with 25(OH)D ($r = 0.45$, $p = 0.03$) and total vitamin D intake from FFQ 2 was positively correlated with 25(OH)D ($r = 0.44$, $p = 0.05$). In the vitamin D non-synthesizing period, only vitamin D supplement intake from FFQ 2 was positively correlated with 25(OH)D ($r = 0.42$, $p = 0.02$).

	FFQ 2 vs. 25(OH)D	p-value	Mean 24-h recall 1 and 2 vs. 25(OH)D	p-value
Dietary				
Home	0.39	< 0.01	0.18	0.20
Day care	0.06	0.69	0.10	0.94
Total	0.41	< 0.01	0.18	0.21
Supplement	0.48	< 0.01	-	-
Total intake	0.48	< 0.01	-	-

Table 4: *Pearson and spearman correlation coefficients between vitamin D intake (IU) from FFQ and mean 24 hour dietary recall 1 and 2 with plasma 25-hydroxyvitamin D (25(OH)D) concentration.*

The Bland-Altman plot between FFQ 2 and mean 24-h recall 1 and 2 for vitamin D and calcium intake showed a positive mean difference (Figure 1). Vitamin D intake from both dietary methods and 25(OH)D concentration were divided into tertiles (same, adjacent, opposite) and cross-classification analyses were conducted (Table 5).

Between the FFQ 2 and 24-h dietary recall 1 and 2, percentage of preschoolers classified into the same or adjacent tertile was 92.4% for dietary vitamin D and 94.3% for calcium. KW indicated fair agreement for calcium and vitamin D intake ($p < 0.05$). Between FFQ 2 and 25(OH)D concentration, preschoolers classified into the same or adjacent tertile was 86.7% and KW indicated poor agreement.

Between vitamin D intake from 24-h dietary recalls 1 and 2 and 25(OH)D concentration, preschoolers classified into the same or adjacent tertile was 81.1% and KW indicated fair agreement ($p < 0.05$).

	FFQ 2 vs. mean 24h recall 1 and 2				FFQ 2 vs. 25(OH)D				Mean24-h recall 1 and 2 vs. 25(OH)D			
	Same	Adjacent	Opposite	Kw	Same	Adjacent	Opposite	Kw	Same	Adjacent	Opposite	Kw
Vitamin D	50.9	41.5	7.5	0.26**	41.5	45.2	13.2	0.12	47.2	33.9	18.9	0.21*
Calcium	56.6	37.7	5.7	0.35**	-	-	-	-	-	-	-	-

Table 5: *Cross-classification of vitamin D and calcium intake between FFQ 2 or mean 24 hour recall 1 and 2 and 25-hydroxyvitamin D (25(OH)D) concentration into the same, adjacent, and opposite tertiles with weighted Kappa statistics.*
**p < 0.05.*
***p ≤ 0.01.*

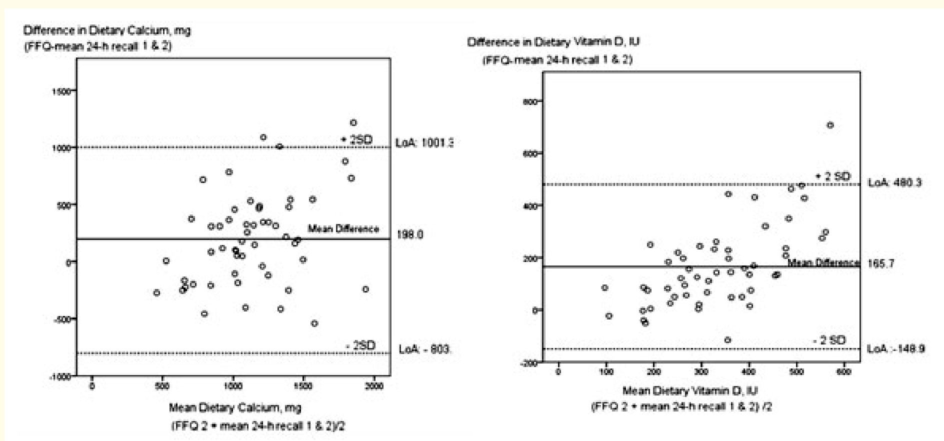

Figure 1: *Bland-Altman plot showing agreement between FFQ and mean 24-h recall 1 and 2 for absolute dietary vitamin D and calcium intakes. Solid line is the mean difference; dashed lines are plus or minus 2 SD; LoA is the limits of agreement. Negative mean difference indicates overall underestimation by FFQ and positive mean difference indicates overall overestimation by FFQ, n = 53.*

Discussion

To the best of our knowledge, this is the first study to validate a semi-quantitative FFQ assessing vitamin D intake in Canadian preschoolers attending day care, by using both a dietary method and biochemical comparator. Previously, FFQs have been validated, for vitamin D intake in Canadians, among young adults [5], women [19,20] and only one study used a biochemical indicator [5].

Dietary and total vitamin D intakes were similar between FFQ1 and FFQ2. Correlation coefficients in the current study varied between 0.42 to 0.78. Similar positive correlations have been observed in several studies among children [21] and adults [5]. Correlation coefficients varied between 0.66 for total calcium intake [5] to 0.80 for total vitamin D [21]. In the current study, correlation coefficients between FFQs were higher than 0.50 when total intake was considered for calcium and higher than 0.56 for vitamin D intake. Accordingly, these results suggest that the current semi-quantitative FFQ has acceptable reproducibility.

Comparison of median values, in the current study, revealed higher home, day care and total calcium intakes and higher home and total vitamin D intakes from FFQ 2 compared to the 24- h dietary recalls 1 and 2. Overestimation of vitamin D [22] and calcium [23,24] intake from FFQ has been demonstrated in previous studies among children and adolescents of 2 to 16 years age and among adults [20] compared to other dietary assessments methods. For instance, vitamin D intake from a semi-quantitative 14-day FFQ of 39 questions was 260 (IQR: 176-372) IU compared to 156 (IQR: 64-292) IU from a 7-day weighed food records in children 2 years of age (n = 187) [22]. Overestimation of vitamin D and calcium intake by the FFQ, at home, and calcium intake by FFQ, at day care, could be attributed to underreporting during 24-h recall or difficulty in comparing standardized FFQ portion sizes to usual portion consumed, in addition to rounding and averaging effects.

Significant positive correlations between the 2 dietary methods varied between 0.33 to 0.49 for calcium and between 0.38 to 0.53 for vitamin D intakes. Similar to the current study, a correlation coefficient of 0.52 has been observed for calcium intakes from a 1-year FFQ compared to 4-day food records [23]. Also, the correlation coefficient for vitamin D intake from a 14 day FFQ compared to a 7-day weighed food records of 0.41 was in concordance with the current study [22].

Correlation coefficients for vitamin D intake from food using both 24-h recalls were not significantly related to plasma 25(OH)D concentration. Few FFQ validation studies have used both dietary assessment methods and a clinical biomarker [5,25,26]. Similarly, in a sample of 68 American preschool children, authors found that correlation coefficients were weak for vitamin D intake, derived from four 24-h recalls (0.2) compared to plasma 25(OH)D concentration [25].

On the other hand, positive correlation coefficients for home, dietary total, supplemental and dietary and supplemental total vitamin D intake was observed with plasma 25(OH)D ($p \le 0.05$). Similarly, a Canadian study showed a positive correlation between vitamin D intake from the FFQ (37 items) and 25(OH)D concentration ($r = 0.48$, $p < 0.001$) in young adults [5]. Plasma 25(OH)D concentration is considered the best indicator for assessing vitamin D status as it reflects both endogenous and exogenous sources [27]. Accordingly, 25(OH)D concentration will not be a reflection of nutrient intake only and high agreement between 25(OH)D and vitamin D intake is unlikely except in winter months at northern latitudes or if supplement use is common. In the current study, only vitamin D supplement intake was significantly correlated in the vitamin D non-synthesizing period ($r = 0.42$, $p = 0.02$). In a sample of more than 900 participants, researchers reported a significant positive correlation between serum 25(OH)D and vitamin D intake derived from a 3 month FFQ (134 items) ranging between 0.26 to 0.54 in European men and women, respectively. Correlation coefficients were not significant anymore once vitamin D supplement users were excluded [26]. It is possible that high doses of vitamin D, derived from supplement intake, are more likely to be correlated with plasma 25(OH)D concentration than more moderate daily intakes from food.

The use of correlation coefficients in validation studies is no longer advised, but is important to enable comparison to previously published studies. Correlations measure the strength of a relation between two methods, rather than agreement [15]. Other methods are suggested for measuring agreement, including Bland-Altman plots, cross-classification and KW. The positive mean difference for dietary vitamin D and calcium intake indicates overestimation by the FFQ compared to mean 24-h recall 1 and 2. The mean difference from the Bland-Altman plot was higher than that reported among post-menopausal women (165.7; LoA:-148.9 to 480.3 IU) for vitamin D, and lower (198; LoA:-803 to 1001 mg) for calcium [20]. In the current study, agreement between FFQ and the 24-h recall was fairly good with only 1 (1.9%) observation, for vitamin D, and with 3 (5.7%) observations, for calcium, occurring outside the LoA, but the wide LoA suggest some bias at higher intakes. The agreement between the 2 dietary methods was higher than that reported for both calcium and vitamin D (6.6%) derived from a 1-month FFQ, including 161 items and a 5-day diet record among postmenopausal women [20]. Unfortunately, we were not able to identify vitamin D and calcium validation studies using Bland-Altman method among preschoolers. However, a study among adolescents with anorexia nervosa and control girls (n = 75) have shown that a 4-day weighed food record and one-year FFQ (40 items) have resulted in 6.7% of observations, for vitamin D and 8% of the observations for calcium occurring outside the LoA [28].

The FFQ was able to place 92.4% participants into the same or adjacent tertile for dietary vitamin D and 94.3% for dietary calcium intake in comparison with 24-h recalls 1 and 2. Even though some misclassifications, occurred the overall classification is satisfactory and KW indicated fair agreement for both nutrients ($p < 0.05$). Differences in agreement between the 2 dietary methods could be due to the inability of the 24-h recall to capture vitamin D food sources that are not consumed on a daily basis. Likewise, previous data has demonstrated that the proportion of participants in the same or within one quartile, between a 1-month FFQ (40 items) and a 4-day food record, in a sample of healthy 12 to 18 years girls (n = 39), was 85% for vitamin D and 82% for calcium. However, in contrast to the present study, the work by Taylor *et al.* [28] reported fair agreement only for calcium intake and poor agreement for vitamin D intake. Similar trends to the current study for vitamin D among 81 Scottish adults (19-58 years) were found with KW = 0.39 for calcium and KW = 0.23 between a 2 to 3 month semi-quantitative FFQ (150 items) and a 4-day weighed diet records [16].

In the current study, KW indicated fair (24-h recalls) to poor (FFQ) agreement with plasma 25(OH)D concentration. Similarly, poor agreement between 25(OH)D and vitamin D intake derived from a 3 month FFQ was reported in men, yet fair agreement in women [26]. It could be that better agreement was observed in women since 17% of women were taking vitamin D supplements while only 7% of men did.

This validation study is not without limitations. First, the sample size was small which would limit the generalizability of the findings. However the sample was powered to detect differences between the mean of 24-h recalls 1 and 2 and FFQ 2. The power for calcium analysis was 0.95 and the power for vitamin D analysis was 1. Plasma 25(OH)D concentration was derived from a non-fasting capillary sample and studies have shown that capillary samples overestimate 25(OH)D concentration [29] which might have limited our ability to find a correlation to dietary vitamin D intake. However, this study provides novel pilot data for prospective validation studies among Canadian preschoolers attending day care.

Although the validation results from the current study were moderate, this semi-quantitative FFQ could be a promising tool to assess dietary and supplemental calcium and vitamin D intakes of Canadian preschoolers attending day care. It could be a screening tool that would provide a quick assessment of both calcium and vitamin D intakes in a clinical setting.

Authors' Contributions

All authors contributed to the manuscript preparation; JEH, TTP, SF, TJH, CV and HW collected the samples, data and contributed to analysis of data; and HW and SF designed the study and secured grant funding. JEH and HW have been involved in writing and revising critically the manuscript. JEH and HW had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. All authors read and approved the final manuscript.

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