A validated food frequency questionnaire to determine dietary intake of vitamin D

Abstract

Objective

The aim of the present study was to develop and validate a vitamin D food frequency questionnaire (FFQ) for assessment of dietary vitamin D intake in healthy adults in England, UK.

Design:

The current study assessed the agreement between a four-day food diary (4d-FD) and a new vitamin D FFQ to measure dietary intake of vitamin D. Dietary intake was estimated using Nutritics dietary analysis software and Spearman's and Bland-Altman tests were utilised to assess correlation and agreement, respectively. Participants also provided a blood sample for plasma analysis of vitamin D concentrations.

Setting:

Home setting.

Participants:

Fifty participants were recruited to the study from the University of Chester and vicinity.

Results:

Results showed a strong correlation between vitamin D intake recorded by the FFQ and the 4d-FD ($r = 0.609$; $P < 0.0001$) within 95% limits of agreement. Furthermore, a significant correlation between plasma 25(OH)D concentrations and vitamin D intake measured by the FFQ ($r = 0.290$, $P = 0.041$) and the 4d-FD ($r = 0.360$, $P = 0.01$) was observed.

Conclusion:

Our analysis suggests this FFQ is a useful and rapid tool for researchers and health professionals to assess vitamin D dietary intakes in healthy adults in the UK.

Introduction

Vitamin D is a secosteroid hormone [1] with the essential role of maintaining calcium and phosphorus homeostasis to support bone metabolism [2]. Furthermore, vitamin D also has important functions in the intestine [3], kidney [4], brain [5], lung [6] and immune system [7], due to the widespread distribution of the vitamin D receptor [8].

Vitamin D deficiency is becoming more common in developed countries, with 30-40% of the general population in the United Kingdom (UK) being classed as vitamin D deficient during the winter [9]. Currently, vitamin D sufficiency is classified as plasma 25(OH)D levels at $50 - 125$ nmol L^{-1}, vitamin D insufficiency at $25 - 50$ nmol L^{-1} and vitamin D deficiency at below 25 nmol L^{-1} [10]. Vitamin D deficiency is the most common cause of rickets with hospitalisation in England at its highest in five decades [11].

In 2016, the Scientific Advisory Committee on Nutrition (SACN) proposed a reference nutrient intake (RNI) for vitamin D of 10 µg day^{-1} (400 international units (IU)) in adults over 4 years old in the UK (including pregnant and lactating women) [10]. The National Diet and Nutrition Survey (NDNS) Rolling Programme shows children between the ages of 4 and 18 have a mean vitamin D intake of 2 µg day^{-1}, and adults between the ages of 18 - 64 have a mean vitamin D intake of 2.7 µg day^{-1} [9]. This alarmingly equates to only 20% and 27% of the RNI, respectively.

In humans, vitamin D can be sourced endogenously from sunlight and exogenously from dietary sources. Availability of ultraviolet (UV)B radiation affects skin synthesis of vitamin D [12] therefore, during the winter months in the UK the availability of UVB sunlight is not sufficient to synthesise biologically relevant quantities of vitamin D [13; 14]. Therefore, vitamin D synthesis is reliant on dietary sources with the main dietary sources being fish, eggs, meat and fortified foods [15].

Health and disease are intricately linked to diet and nutrition therefore, assessing food intake is crucial [16]. There are many methods available including: weighed and non-weighed food diaries, 24-hour recalls and food frequency questionnaires (FFQs) [17]. The gold standard recommendation is the weighed food record (WFR), which has the benefit of not relying on memory to record foods eaten. However, completion of such records is time-consuming, labour-intense and relies on participant motivation and written capabilities. A FFQ is designed to capture habitual food intake with a lower burden on the participant due to removal of the need to weigh or record food at the

time of intake. Validation of a FFQ requires comparison of nutrient biomarkers with another method of dietary assessment to determine reliability of the FFQ [18; 19].

Although there have been previous vitamin D FFQ validation studies[18; 20; 21; 22], these have been carried out in countries including Northern Ireland, The United States of America, Canada and Qatar. The dietary habits of those populations are likely to differ to those of English participants. Therefore, the present questionnaire is the first to be validated in England.

The aim of the present study was to develop and validate a rapid vitamin D FFQ that can be used to accurately estimate vitamin D intake in adults in England, UK.

Experimental methods

Study sample

Fifty participants aged 18 to 64 years were recruited from the University of Chester (excluding students or academic staff in the department of clinical sciences and nutrition) and surrounding areas, Northern England, UK. The final study population included both students and non-students. A pre-test questionnaire was used to assess eligibility for the study. Those that had been on a sun holiday in the month previous to taking part in the study or during the study, those that use sun beds and those using medications were excluded from participation in the study.

Study design

Participants were randomly allocated to receive either the FFQ or a four-day food diary (4d-FD) followed by a 30 day interval before completing the alternative method of dietary assessment (Figure 1). All participants completed both methods of dietary assessment during the months of November - April. Participants were asked not to change their diet during the study. For the FFQ and 4d-FD, both food sources and supplemental vitamin D were included in total vitamin D intakes.

Four-day food diary

Participants were provided with a 4d-FD and asked to record all food and drinks consumed on three weekdays and one weekend day. Before completing the 4d-FD, participants were provided with guidance on recording food and drink consumed along with portion sizes. Diet information obtained from the 4d-FD was analysed using Nutritics dietary analysis software (Research Edition, v5.096, Dublin, Nutritics, 2019) to give an average vitamin D intake (μg day^{-1}).

Food frequency questionnaire

The FFQ (see supplementary material) was designed using information from McCance and Widdowson's, 'The composition of Foods' [15] and 'Food Portion Sizes' [23]. Foods were grouped into sections within the questionnaire along with portion sizes. Frequency of consumption was measured using the following options: never/less than monthly, monthly, 1 per week, 2 per week, 3 per week, 4-5 per week, daily or 2 per day. Participants were asked to indicate which frequency of consumption best fit each food according to their habitual dietary habits. Vitamin D intake (μg day^{-1}) was calculated by multiplying frequency of consumption by the amount of vitamin D in the specified portion size of each food.

Biomarker analysis

Before filling out both the 4d-FD and FFQ, a fingerprick blood test was performed on all participants. Blood was collected into a blood collection tube treated with ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged at 2680 x g for 10 mins at 4˚C. The plasma was removed and stored at -80˚C. Concentrations of total 25(OH)D (D_2 and D_3) were analysed by an automated immunoanalyser (VIDAS®; Biomérieux) using the enzyme linked fluorescent assay technique. Quality and accuracy of the method was monitored by participation in the Vitamin D External Quality Assessment Scheme (DEQAS), with intra and inter-assay CV of 2.0 % and 7.3 % respectively. If samples were outside the range of detection (vitamin D concentration < 20.3 nmol L^{-1}) they were analysed by enzyme-linked immunosorbent assay (ELISA; Calbiotech VD220B). The intra and inter-assay CV for this ELISA are 11.3 % and 3.6 % respectively.

Sample size and Statistical analysis

Sample sizes for validation studies are wide ranging from 3 – 3750 with a median of 110. A previous vitamin D FFQ validation study used a sample size of 50 [18]. For use of Bland Altman method, the size of at least 50 is preferable to allow a large enough sample size to allow the limits of agreement to be estimated [24]. When using biomarkers as validation for FFQ, the gold standard is 50 participants [25] therefore, 62 participants were recruited to account for drop outs leading to a final number of 50. A post-hoc power calculation using G Power (3.1) based on the results of the present study with an effect size of 0.6 indicates a sample size of 25 is required for 95% power ($\alpha = 0.05$).

Statistical analysis was carried out using IBM SPSS version 26. Statistical significance was accepted at $P < 0.05$. Data was not normally distributed therefore, nonparametric tests were used throughout. Mean plasma 25(OH)D concentrations were compared using Mann-Whitney tests. A Bland-Altman plot [26] provides a visual representation of the agreement between the FFQ and four-day food diary. Spearman's correlation coefficients were calculated between the FFQ, 4d-FD and plasma 25(OH)D concentration.

Results

A total of 62 participants were recruited to the study with 12 dropouts resulting in a final sample number of 50 participants included in the analysis. General participant characteristics are detailed in Table 1, there was no significant difference in mean (SD) age between male and female participants (male; 31 ± 8 years, N = 16, female; 37 ± 13 years, N = 34, P = 0.212). There was no significant difference in plasma 25(OH)D concentration before the FFQ or 4d-FD (FFQ; 39.8 ± 24.2 nmol L^{-1}, 4d-FD; 38.3 ± 19.9 nmol L^{-1}, P = 0.668). Using the thresholds described by SACN [10], in the present study, 12 participants (24%) were classed as vitamin D sufficient (50 – 125 nmol L^{-1}); 25 participants (50%) were vitamin D insufficient (25 – 50 nmol L^{-1}) and 13 participants (26%) were vitamin D deficient (below 25 nmol L^{-1}).

FFQ vs. 4d-FD

As shown in Table 2, a strong, significant correlation was observed between vitamin D intake recorded by the FFQ and the 4d-FD (r = 0.609, N = 50, P < 0.0001). When split by gender, there was a significant correlation between vitamin D intake recorded by the FFQ and 4d-FD in males (r = 0.727, N = 16, P = 0.001) and females (r = 0.538, N = 34, P = 0.001).

The Bland-Altman plot (Figure 2) shows the difference in vitamin D intakes between the FFQ and 4d-FD plotted against the mean, including 95% limits of agreement (1.96 SD) of the mean difference. All but one of the differences fell within the limits of agreement. The mean (SD) difference between the 4d-FD and FFQ was -1.8 (3.8) (Figure 2).

FFQ and 4d-FD vs. 25(OH)D concentration

Additionally, a significant correlation was observed between plasma 25(OH)D concentrations and vitamin D intake measured by the FFQ (r = 0.290, N = 50, P = 0.041) and the 4d-FD (r = 0.360, N = 50, P = 0.01).

The mean dietary intake of vitamin D recorded by the FFQ and 4d-FD was 17.3 µg day^{-1} and 16.0 µg day^{-1} for males including supplements and 4.4 µg day^{-1} and 3.6 µg day^{-1} excluding supplements. For females mean dietary intake of vitamin D recorded by the FFQ and 4d-FD was 6.1 µg day^{-1} and 4.1 µg day^{-1} including supplements and 4.96 µg day^{-1} and 2.9 µg day^{-1} excluding supplements. There was not a significant difference in dietary intake of vitamin D between males and females when measured by the FFQ (P = 0.603) and 4d-FD (P = 0.442).

Discussion

The aim of the present study was to develop and validate a FFQ to determine dietary intakes of vitamin D in healthy adults in England, UK. The significant correlation strongly suggests that this FFQ is an accurate tool to assess individual vitamin D dietary intakes. Furthermore, to our knowledge this is the first vitamin-D FFQ to be validated based upon the diet in England, UK.

A significant correlation was shown between measuring dietary vitamin D intakes using the FFQ and the 4d-FD. The results from the Bland-Altman plot further support the validity of the FFQ. All participants except one fell within the 95% limits of agreement and clustering of participants around the mean was present. This shows agreement between the FFQ and 4d-FD in assessing dietary intake of vitamin D.

The widely used biomarker for vitamin D status is plasma 25(OH)D concentration, which reflects both endogenous and exogenous production of vitamin D [27]. However, due to sunlight being a major source of vitamin D for humans this presents a confounding factor. This confounding may explain the weak correlations found between plasma 25(OH)D concentrations and vitamin D intake measured by both the FFQ and 4d-FD. Although both correlations were statistically significant, therefore the validity of the FFQ is not compromised. The vitamin D status of the participants in the present study was consistent with previously reported data, which classified 28% of the study population as vitamin D sufficient, 45% as vitamin D insufficient and 27% as vitamin D deficient [18].

The mean vitamin D intake reported from the FFQ was 9.7 µg day^{-1} and 7.9 µg day^{-1} from the 4d-FD. The NDNS reported a mean dietary vitamin D intake of 2.7 µg day^{-1} for adults aged 19 – 64 years [9]. The difference in intakes from the NDNS compared with this study is largely accounted for by participants being recruited from the university population which may therefore not be representative of the whole population. Furthermore, the mean vitamin D intake reported from the FFQ and 4d-FD without including supplements is closer to the data recorded form the NDNS at 4.8 µg day^{-1} for the FFQ and 3.1 µg day^{-1} for the 4d-FD. The mean vitamin D intakes reported in the present study are low compared to the RNI for vitamin D of 10 µg day^{-1} [10]. These findings are not entirely unexpected, as the mean UK dietary vitamin D intake for adults aged 19 – 64 is less than 3 µg day^{-1} [9] as mentioned. Together these data show that both the study population and the wider population are not meeting recommended nutrient intakes for vitamin D. Due to this, SACN

recommend that year-round supplementation with 10 µg day^{-1} of vitamin D is required to compensate for low dietary intakes [10]. This is particularly important when considering the ongoing COVID19 pandemic and potentially limited sunlight exposure due to lockdown restrictions. The data on mean daily dietary intakes of vitamin D collected in this study support previous findings that showed a mean intake of 7.36 µg day^{-1} as measured by a FFQ and 5.74 µg day^{-1} when measured using a four day weighed food record [18]. However, Weir et al. (2016) did not report data on vitamin D dietary intake excluding supplements.

A limitation of previous FFQ validation studies has included the lack of consideration of gender which could cause inaccuracy in dietary intake assessment [28]. In the present study, there was no difference in dietary intake of vitamin D between males and females. Furthermore, significant correlations were found between using the FFQ and 4d-FD in both men and women suggesting this FFQ is valid for use in both genders.

A potential improvement to the current FFQ would be the inclusion of photographs to aid participants with portion size estimation as used in the Food4Me FFQ [29]. The strengths of the current study include the cross-over study design and adequate sample size. The 4d-FD is the method of dietary analysis utilised for the NDNS, which adds further validity. Although we believe the questionnaire is valid for age groups beyond those included in the present study, future studies could assess validation in those under 18 years and over 64 years. Additionally, there was a lack of ethnic minorities in the study population largely due to the local demographic, and future studies may be required to validate the study for ethnic minority populations. In the present study, data to calculate body mass index (BMI) was not collected. It is suggested that further studies assess the BMI of participants.

Additionally, all participants included in the analysis completed the FFQ and 4d-FD within one month of the other to minimize any changes in dietary patterns. The nature of dietary assessment studies means that there is reliance on self-reporting therefore, over or under estimation of vitamin D intake may be present. Further validation is required to determine the validity of the FFQ in children and older adults.

Conclusions

To our knowledge, this is the first FFQ that has been validated to assess dietary intake of vitamin D in healthy adults in England, UK. The FFQ shows strong agreement with a 4d-FD for assessing dietary intake of vitamin D in healthy adults. While there is some difference between the two methods, FFQs are not commonly used to measure absolute intakes, therefore, the agreement with another method of dietary assessment and the ease of use make the vitamin D FFQ a rapid tool for assessment of dietary intake of vitamin D by health care professionals and researchers alike.

Tables

Table 1 Participant characteristics (N = 50)

	Mean	SD	Median	Minimum	Maximum
Age (years)	35.0	12.0	31.0	19.0	62.0
Vitamin D intake (µg day^{-1})					
Including supplements					
FFQ	9.7	19.7	4.8	0.5	129.5
4d-FD	7.9	19.9	3.3	0.3	133.0
Excluding supplements					
FFQ	4.8	3.7	4.4	0.5	23.6
4d-FD	3.1	2.3	3.0	0.2	10.2
Plasma 25(OH)D (nmol L^{-1})					
FFQ	39.8	24.2	32.1	7.3	122.0
4d-FD	38.3	19.9	32.3	10.6	94.3

Table 2 Summary of correlations for vitamin D intake recorded by the FFQ and 4d-FD and plasma 25(OH)D and vitamin D intake.

Measure	Food Record		Plasma 25OHD		FFQ	
	r	P value*	r	P value*	r	P value*
FFQ	0.609	< 0.0001	0.290	0.041		
Plasma 25OHD	0.360	0.01				

* significance accepted at P < 0.05

Figure legends

Figure 1. Flow chart of the study protocol.

Figure 2. Bland-Altman plot showing the difference in mean daily vitamin D intake between the four-day food diary (4d-FD) and food frequency questionnaire (FFQ). The solid line represents the mean difference in daily vitamin D intake (-1.8 µg day^{-1}), the dashed lines show the 95% limits of agreement (1.96 SD).

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