



# A validated FFQ to determine dietary intake of vitamin D

Stephanie Watkins, Ellen Freeborn and Sohail Mushtaq\*

Department of Clinical Sciences and Nutrition, University of Chester, Parkgate Road, Chester CH1 4BJ, UK

Submitted 29 May 2020: Final revision received 23 October 2020: Accepted 3 November 2020: First published online 6 November 2020

## Abstract

**Objective:** The aim of the present study was to develop and validate a vitamin D 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 (4 d-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 4 d-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 4 d-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.

## Keywords

FFQ  
Vitamin D  
Dietary intake  
Validation

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

Vitamin D deficiency is becoming more common in developed countries, with 30–40 % of the general population in the UK being classed as vitamin D deficient during the winter<sup>(9)</sup>. Currently, vitamin D sufficiency is classified as plasma 25(OH)D levels at 50–125 nmol/l, vitamin D insufficiency at 25–50 nmol/l and vitamin D deficiency at below 25 nmol/l<sup>(10)</sup>. Vitamin D deficiency is the most common cause of rickets with hospitalisation in England at its highest in five decades<sup>(11)</sup>.

In 2016, the Scientific Advisory Committee on Nutrition proposed a reference nutrient intake for vitamin D of 10 µg/d (400 IU) in adults over 4 years old in the UK (including pregnant and lactating women)<sup>(10)</sup>. 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/d, and adults between the ages of 18–64

have a mean vitamin D intake of 2.7 µg/d<sup>(9)</sup>. This alarmingly equates to only 20 and 27 % of the reference nutrient intake, respectively.

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

Health and disease are intricately linked to diet and nutrition; therefore, assessing food intake is crucial<sup>(16)</sup>. There are many methods available, including weighed and non-weighed food diaries, 24-h recalls and FFQ<sup>(17)</sup>. The gold standard recommendation is the weighed food record, which has the benefit of not relying on memory to record foods eaten. However, completion of such records is time-consuming, labour-intensive and relies on participant motivation and written capabilities. A FFQ is designed to capture habitual food intake with a lower

\*Corresponding author: Email s.mushtaq@chester.ac.uk

© The Author(s), 2020. Published by Cambridge University Press on behalf of The Nutrition Society

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<sup>(18,19)</sup>.

Although there have been previous vitamin D FFQ validation studies<sup>(18,20-22)</sup>, these have been carried out in countries including Northern Ireland, the USA, Canada and Qatar. The dietary habits of those populations are likely to differ from 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 18–64 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 (4 d-FD) followed by a 30-d interval before completing the alternative method of dietary assessment (Fig. 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 4 d-FD, both food sources and supplemental vitamin D were included in total vitamin D intakes.

### Four-day food diary

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

### Food frequency questionnaire

The FFQ (see Supplementary Material) was designed using information from McCance and Widdowson's, 'The composition of Foods'<sup>(15)</sup> and 'Food Portion Sizes'<sup>(23)</sup>. 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/week, 2/week, 3/week, 4–5/week, daily or 2/d. Participants were asked to indicate which frequency of consumption best fit each food according to their habitual dietary habits. Vitamin D intake ( $\mu\text{g}/\text{d}$ ) 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 4 d-FD and FFQ, a fingerprick blood test was performed on all participants. Blood was collected into a blood collection tube treated with EDTA. Blood samples were centrifuged at  $2680 \times g$  for 10 min at  $4^\circ\text{C}$ . The plasma was removed and stored at  $-80^\circ\text{C}$ . Concentrations of total 25(OH)D ( $\text{D}_2$  and  $\text{D}_3$ ) were analysed by an automated immunoanalyser (VIDAS<sup>®</sup>; Biomérieux) using the enzyme-linked fluorescent assay technique. Quality and accuracy of the method were monitored by participation in the Vitamin D External Quality Assessment Scheme, with intra- and inter-assay CV of 2.0 and 7.3%, respectively. If samples were outside the range of detection (vitamin D concentration  $< 20.3 \text{ nmol}/\text{l}$ ) they were analysed by ELISA (ELISA; Calbiotech VD220B). The intra- and inter-assay CV for this ELISA is 11.3 and 3.6%, respectively.

### Sample size and statistical analysis

Sample sizes for validation studies are wide ranging from 3 to 3750 with a median of 110. A previous vitamin D FFQ validation study used a sample size of 50<sup>(18)</sup>. 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<sup>(24)</sup>. When using biomarkers as validation for FFQ, the gold standard is fifty participants<sup>(25)</sup>;

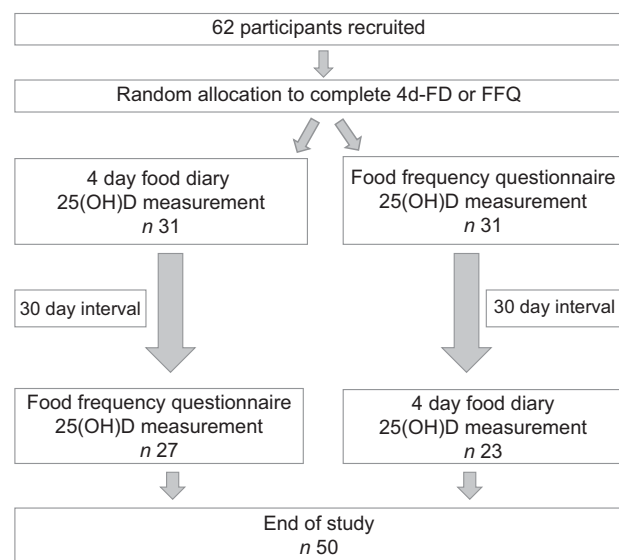


Fig. 1 Flow chart of the study protocol

therefore, sixty-two participants were recruited to account for dropouts leading to a final number of fifty. 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 were 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<sup>(26)</sup> provides a visual representation of the agreement between the FFQ and 4 d-FD. Spearman's correlation coefficients were calculated between the FFQ, 4 d-FD and plasma 25(OH)D concentration.

## Results

A total of sixty-two participants were recruited to the study with twelve dropouts resulting in a final sample number of fifty 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;  $31 \pm 8$  years,  $n = 16$ , female;  $37 \pm 13$  years,  $n = 34$ ,  $P = 0.212$ ). There was no significant difference in plasma 25(OH)D concentration before the FFQ or 4 d-FD (FFQ;  $39.8 \pm 24.2$  nmol/l, 4 d-FD;  $38.3 \pm 19.9$  nmol/l,  $P = 0.668$ ). Using the thresholds described by Scientific Advisory Committee on Nutrition<sup>(10)</sup>, in the present study, twelve participants (24%) were classed as vitamin D sufficient (50–125 nmol/l); twenty-five participants (50%) were

vitamin D insufficient (25–50 nmol/l) and thirteen participants (26%) were vitamin D deficient (below 25 nmol/l).

### FFQ v. four-day food diary

As shown in Table 2, a strong, significant correlation was observed between vitamin D intake recorded by the FFQ and the 4 d-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 4 d-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 (Fig. 2) shows the difference in vitamin D intakes between the FFQ and 4 d-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 4 d-FD and FFQ was  $-1.8$  (SD 3.8) (Fig. 2).

### FFQ and four-day food diary v. 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 4 d-FD ( $r = 0.360$ ,  $n = 50$ ,  $P = 0.01$ ).

The mean dietary intake of vitamin D recorded by the FFQ and 4 d-FD was 17.3 and 16.0  $\mu\text{g}/\text{d}$  for males including supplements and 4.4 and 3.6  $\mu\text{g}/\text{d}$  excluding supplements. For females mean dietary intake of vitamin D recorded by the FFQ and 4 d-FD was 6.1 and 4.1  $\mu\text{g}/\text{d}$  including supplements and 4.96 and 2.9  $\mu\text{g}/\text{d}$  excluding supplements. There was no significant difference in dietary intake of vitamin D between males and females when measured by the FFQ ( $P = 0.603$ ) and 4 d-FD ( $P = 0.442$ ).

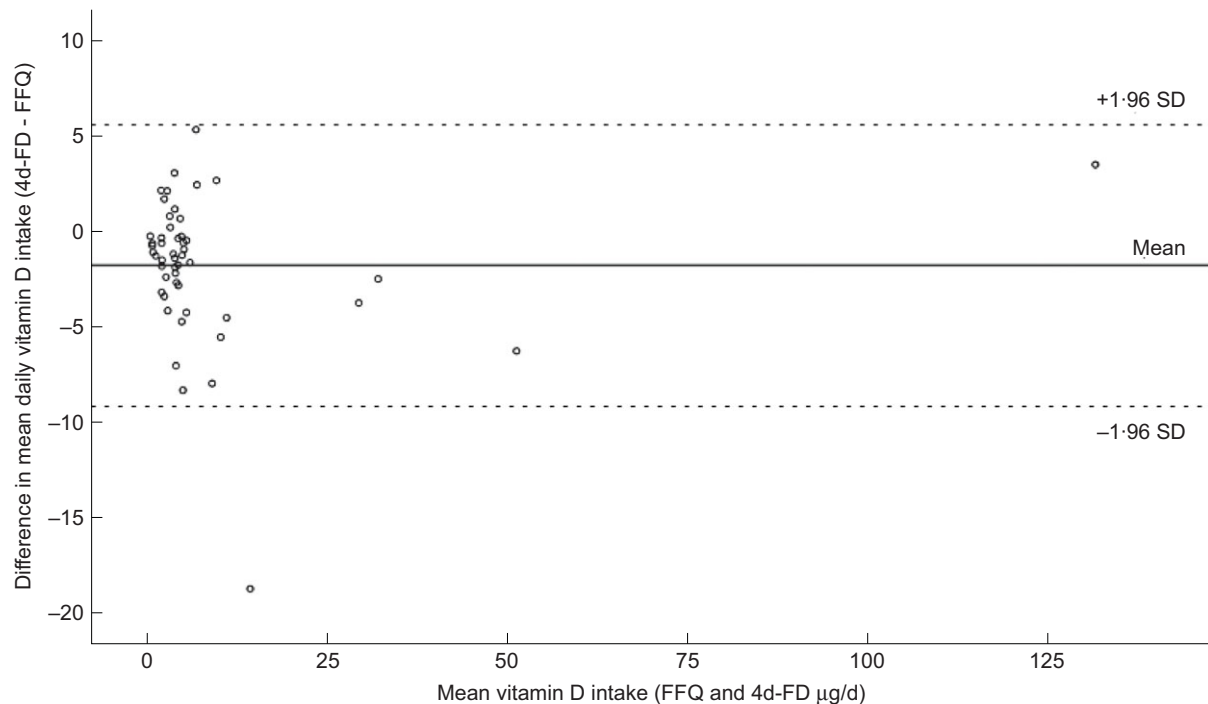
**Table 1** Participant characteristics ( $n = 50$ )

	Mean	sd	Median	Minimum	Maximum
Age (years)	35.0	12.0	31.0	19.0	62.0
Vitamin D intake ( $\mu\text{g}/\text{d}$ )					
Including supplements					
FFQ	9.7	19.7	4.8	0.5	129.5
4 d-FD	7.9	19.9	3.3	0.3	133.0
Excluding supplements					
FFQ	4.8	3.7	4.4	0.5	23.6
4 d-FD	3.1	2.3	3.0	0.2	10.2
Plasma 25(OH)D ( $\text{nmol l}^{-1}$ )					
FFQ	39.8	24.2	32.1	7.3	122.0
4 d-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 4 d-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  $P < 0.05$ .



**Fig. 2** Bland–Altman plot showing the difference in mean daily vitamin D intake between the four-day food diary (4 d-FD) and FFQ. The solid line represents the mean difference in daily vitamin D intake ( $-1.8 \mu\text{g}/\text{d}$ ), the dashed lines show the 95 % limits of agreement ( $1.96 \text{ SD}$ )

## 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 4 d-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 4 d-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<sup>(27)</sup>. 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 4 d-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<sup>(18)</sup>.

The mean vitamin D intake reported from the FFQ was 9.7 and 7.9  $\mu\text{g}/\text{d}$  from the 4 d-FD. The NDNS reported a mean dietary vitamin D intake of 2.7  $\mu\text{g}/\text{d}$  for adults aged 19–64 years<sup>(9)</sup>. The difference in intakes from the NDNS compared with the current 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 4 d-FD without including supplements is closer to the data recorded from the NDNS at 4.8  $\mu\text{g}/\text{d}$  for the FFQ and 3.1  $\mu\text{g}/\text{d}$  for the 4 d-FD. The mean vitamin D intakes reported in the present study are low compared with the reference nutrient intake for vitamin D of 10  $\mu\text{g}/\text{d}$ <sup>(10)</sup>. These findings are not entirely unexpected, as the mean UK dietary vitamin D intake for adults aged 19–64 is  $< 3 \mu\text{g}/\text{d}$ <sup>(9)</sup> 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, Scientific Advisory Committee on Nutrition recommends that year-round supplementation with 10  $\mu\text{g}/\text{d}$  of vitamin D is required to compensate for low dietary intakes<sup>(10)</sup>. 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 the current study support previous findings that showed a mean intake of 7.36  $\mu\text{g}/\text{d}$  as measured by a FFQ and 5.74  $\mu\text{g}/\text{d}$  when measured using a four day weighed food record<sup>(18)</sup>. However, Weir *et al.*<sup>(18)</sup> 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<sup>(28)</sup>. In the present study, there was no difference in dietary intake of vitamin D between males and females. Furthermore, significant correlations were found between the FFQ and 4 d-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<sup>(29)</sup>. The strengths of the current study include the crossover study design and adequate sample size. The 4 d-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 BMI were not collected. It is suggested that further studies assess the BMI of participants.

Additionally, all participants included in the analysis completed the FFQ and 4 d-FD within 1 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 4 d-FD for assessing dietary intake of vitamin D in healthy adults. While there is some difference between the two methods, FFQ 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 healthcare professionals and researchers alike.

## Acknowledgements

*Acknowledgements:* The authors would like to thank the participants for their time and effort in taking part in the study. *Financial support:* This research received no specific grant from any funding agency, commercial or not-for-profit sectors. *Conflict of interest:* None. *Authorship:* S.M. designed the study, E.F. carried out data collection,

S.W. carried out data analysis and S.W. and S.M. prepared the manuscript. *Ethics of human subject participation:* The current study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Faculty of Medicine, Dentistry and Life Sciences Research Ethics Committee, University of Chester; 1129/15/EF/CSN. Written informed consent was obtained from all subjects prior to enrolment on the study.

## Supplementary material

For supplementary material accompanying this paper visit <https://doi.org/10.1017/S136898002000453X>

## References

1. Verstuyf A, Carmeliet G, Bouillon R *et al.* (2010) Vitamin D: a pleiotropic hormone. *Kidney Int* **78**, 140–145.
2. Bellavia D, Costa V, De Luca A *et al.* (2016) Vitamin D level between calcium-phosphorus homeostasis and immune system: new perspective in osteoporosis. *Curr Osteoporos Rep.* Published online: 13 October 2016. doi: 10.1007/s11914-016-0331-2.
3. Christakos S, Dhawan P, Porta A *et al.* (2011) Vitamin D and intestinal calcium absorption. *Mol Cell Endocrinol* **347**, 25–29.
4. Li X, Zheng W & Li YC (2003) Altered gene expression profile in the kidney of vitamin D receptor knockout mice. *J Cell Biochem* **89**, 709–719.
5. Anjum I, Jaffery SS, Fayyaz M *et al.* (2018) The role of vitamin D in brain health: a mini literature review. *Cureus.* Published online: 10 July 2018. doi: 10.7759/cureus.2960.
6. Kiani A, Abedini A, Adcock IM *et al.* (2018) Association between vitamin D deficiencies in sarcoidosis with disease activity, course of disease and stages of lung involvements. *J Med Biochem* **37**, 103–109.
7. Baeke F, Korf H, Overbergh L *et al.* (2010) Human T lymphocytes are direct targets of 1, 25-dihydroxyvitamin D<sub>3</sub> in the immune system. *J Steroid Biochem Mol Biol* **121**, 221–227.
8. Holick MF (2002) Vitamin D. *Clin Rev Bone Miner Metab* **1**, 181–207.
9. Public Health England (2016) National Diet and Nutrition Survey. <https://www.gov.uk/government/collections/national-diet-and-nutrition-survey> (accessed February 2020).
10. Scientific Advisory Committee on Nutrition S (2016) Vitamin D and Health Report. <https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report> (accessed February 2020).
11. Goldacre M, Hall N & Yeates DG (2014) Hospitalisation for children with rickets in England: a historical perspective. *Lancet* **383**, 597–598.
12. Webb AR & Engelsen O (2006) Calculated ultraviolet exposure levels for a healthy vitamin D status. *Photochem Photobiol* **82**, 1697–1703.
13. Webb AR, Kline L & Holick MF (1988) Influence of season and latitude on the cutaneous synthesis of vitamin D<sub>3</sub>: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D<sub>3</sub> synthesis in human skin. *J Clin Endocrinol Metab* **67**, 373–378.
14. Webb A, DeCosta B & Holick M (1989) Sunlight regulates the cutaneous production of vitamin D<sub>3</sub> by causing its photodegradation. *J Clin Endocrinol Metab* **68**, 882–887.



15. McCance RA & Widdowson EM (2014) *McCance and Widdowson's the Composition of Foods*. Cambridge: Royal Society of Chemistry.
16. Adamson AJ & Mathers JC (2004) Effecting dietary change. *Proc Nutr Soc* **63**, 537–547.
17. Dao MC, Subar AF, Warthon-Medina M *et al.* (2019) Dietary assessment toolkits: an overview. *Public Health Nutr* **22**, 404–418.
18. Weir R, Carson E, Mulhern MS *et al.* (2016) Validation of a food frequency questionnaire to determine vitamin D intakes using the method of triads. *J Hum Nutr Diet* **29**, 255–261.
19. Fallaize R, Forster H, Macready AL *et al.* (2014) Online dietary intake estimation: reproducibility and validity of the Food4Me food frequency questionnaire against a 4-day weighed food record. *J Med Internet Res*. Published online: 11 August 2014. doi: 10.2196/jmir.3355.
20. Taylor C, Lamparello B, Kruczek K *et al.* (2009) Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. *J Am Diet Assoc* **109**, 479–485.
21. Wu H, Gozdzik A, Barta JL *et al.* (2009) 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. *Nutr Res* **29**, 255–261.
22. Ganji V, Abu-Dbaa R, Othman H *et al.* (2020) Validation of vitamin D-specific food frequency questionnaire against food records for Qatari women. *Foods* **9**, 195.
23. Crawley H, Mills A & Patel S (2002) Food standards agency. *Food Portion Sizes*. London: The Stationery Office.
24. Cade J, Thompson R, Burley V *et al.* (2002) Development, validation and utilisation of food-frequency questionnaires – a review. *Public Health Nutr* **5**, 567–587.
25. Serra-Majem L, Andersen LF, Henríque-Sánchez P *et al.* (2009) Evaluating the quality of dietary intake validation studies. *Br J Nutr* **102**, S3–S9.
26. Bland JM & Altman D (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **327**, 307–310.
27. Chen TC, Turner AK & Holick MF (1990) Methods for the determination of the circulating concentration of 25-hydroxyvitamin D. *J Nutr Biochem* **1**, 315–319.
28. Lee H, Kang M, Song WO *et al.* (2016) Gender analysis in the development and validation of FFQ: a systematic review. *Br J Nutr* **115**, 666–671.
29. Forster H, Fallaize R, Gallagher C *et al.* (2014) Online dietary intake estimation: the Food4Me food frequency questionnaire. *J Med Internet Res* Published online: 9 June 2014. doi: 10.2196/jmir.3105.