British Journal of Nutrition (2011), **106**, 1638–1648
doi:10.1017/S0007114511005058

British Journal of Nutrition

Review – Systematic with Meta-Regression

A systematic review and meta-regression analysis of the vitamin D intake–serum 25-hydroxyvitamin D relationship to inform European recommendations

Kevin D. Cashman[1,2]*, Anthony P. Fitzgerald[3,4], Mairead Kiely[1] and Kelly M. Seamans[1]

[1]*School of Food and Nutritional Sciences, University College Cork, Cork, Republic of Ireland*
[2]*Department of Medicine, University College Cork, Cork, Republic of Ireland*
[3]*Department of Epidemiology and Public Health, University College Cork, Cork, Republic of Ireland*
[4]*Department of Statistics, University College Cork, Cork, Republic of Ireland*

(Received 8 March 2011 – Revised 25 July 2011 – Accepted 10 August 2011 – First published online 14 October 2011)

Abstract

The present study used a systematic review approach to identify relevant randomised control trials (RCT) with vitamin D and then apply meta-regression to explore the most appropriate model of the vitamin D intake–serum 25-hydroxyvitamin D (25(OH)D) relationship to underpin setting reference intake values. Methods included an updated structured search on Ovid MEDLINE; rigorous inclusion/exclusion criteria; data extraction; and meta-regression (using different model constructs). In particular, priority was given to data from winter-based RCT performed at latitudes >49·5°N (*n* 12). A combined weighted linear model meta-regression analyses of natural log (Ln) total vitamin D intake (i.e. diet and supplemental vitamin D) *v.* achieved serum 25(OH)D in winter (that used by the North American Dietary Reference Intake Committee) produced a curvilinear relationship (mean (95 % lower CI) serum 25(OH)D (nmol/l) = 9·2 (8·5) Ln (total vitamin D)). Use of non-transformed total vitamin D intake data (maximum 1400 IU/d; 35 μg/d) provided for a more linear relationship (mean serum 25(OH)D (nmol/l) = 0·044 × (total vitamin D) + 33·035). Although inputting an intake of 600 IU/d (i.e. the RDA) into the 95 % lower CI curvilinear and linear models predicted a serum 25(OH)D of 54·4 and 55·2 nmol/l, respectively, the total vitamin D intake that would achieve 50 (and 40) nmol/l serum 25(OH)D was 359 (111) and 480 (260) IU/d, respectively. Inclusion of 95 % range in the model to account for inter-individual variability increased the predicted intake of vitamin D needed to maintain serum 25(OH)D ≥ 50 nmol/l to 930 IU/d. The model used to describe the vitamin D intake–status relationship needs to be considered carefully when setting new reference intake values in the Europe.

Key words: Systematic reviews: Meta-regression: Vitamin D requirements: Serum 25-hydroxyvitamin D: European Micronutrient Recommendations Aligned (EURRECA)

Severe vitamin D deficiency has been shown to lead to rickets in children and osteomalacia in adults[1], while less severe vitamin D deficiency causes secondary hyperparathyroidism, increased bone turnover and bone loss[2–4], as well as being associated with increased risk of several non-skeletal chronic diseases[5,6]. Thus, ensuring adequate vitamin D status is important to human health and there is a consensus that serum 25-hydroxyvitamin D (25(OH)D) should be used to assess vitamin D status, as it reflects combined dietary supply and dermal production[7].

Serum 25(OH)D was used as a functional indicator of vitamin D status by the recent Institute of Medicine (IOM) dietary reference intake (DRI) committee on Ca and vitamin D in North America[8], as well as by the UK and the European Union authorities[1,9–11] during the 1990s, in establishing dietary requirements for vitamin D. To date, many, if not all, agencies briefed with establishing dietary requirements for vitamin D have used a cutoff of 25–30 nmol/l serum 25(OH)D as the lower threshold for vitamin D status (on the basis of rickets and osteomalacia)[1,8–11]. There has been

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; DRI, dietary reference intake; IOM, Institute of Medicine; Ln, natural log; RCT, randomised control trial; UVB, UV blue.

* **Corresponding author:** Professor K. D. Cashman, fax +353 21 4270244, email k.cashman@ucc.ie

an increasing body of data on the relationship between vitamin D status and a wide range of non-skeletal health outcomes, and some of these (mainly CVD or cancer outcomes) were reviewed in a comprehensive systematic review[12], commissioned by several US and Canadian federal government agencies, for use by the DRI committee during their deliberations. However, many of the studies of non-skeletal health effects provided often mixed and inconclusive results and led the DRI committee to question their reliability. The DRI committee instead prioritised bone health outcomes as the basis for establishing the new DRI values for vitamin D (and Ca)[8].

For individuals aged 1 year and older, the DRI committee choose serum 25(OH)D concentrations of 40 and 50 nmol/l as the median value (above which approximately half the population might meet vitamin D requirements for bone health and below which one-half might not) and that covering the needs of 97·5 % of a normal healthy population, respectively. These served as the target concentrations for an estimated average requirement and RDA for dietary vitamin D, respectively[8]. The DRI committee then used data from nine vitamin D intervention studies of individuals aged 6 to >60 years, performed at the northern latitudes in Europe (>49·5°N) and Antarctica (78°S) during their respective winter seasons (with minimal UV blue (UVB) sun exposure) to establish regression equations of the simulated response of serum 25(OH)D concentration to total vitamin D intake. A similar analysis on data from randomised control trials (RCT) conducted in the latitude band 40 to <49·5°N (all from the USA) yielded quite different regression equations, suggesting that UVB exposure during winter was not minimal[8]. The estimated average requirement and RDA for vitamin D of 10 and 15 μg/d (20 μg/d for those >70 years), respectively, were derived from the >49·5°N/S RCT regression analysis to approximate conditions of minimal UVB sun exposure[8]. The DRI committee, however, highlighted that the regression analysis had several assumptions and/or uncertainties: lack of age effect on the response of serum 25(OH)D to total vitamin D intake, large inter-study variance and uncertainties surrounding the predicted CI of the vitamin D intake–status relationship[8], which were used to estimate the DRI values for vitamin D. Furthermore, it is possible that European agencies briefed with the task of re-evaluation of DRI for vitamin D may decide to use a serum 25(OH)D target concentration other than 50 nmol/l, which may require a different regression model.

Thus, the aim of the present study was to use a systematic review approach to identify relevant RCT with vitamin D in children/adolescents, adults and older adults, and then apply meta-regression models (including that used by the DRI committee[8]) to the extracted data, as well as using individual data from two recent vitamin D RCT in the northern European adults and elderly[13,14] during winter, to explore the most appropriate model of the vitamin D intake–serum 25(OH)D relationship. In addition, whether latitude influenced this relationship was investigated, as much of Europe resides between 40 and >70°N.

Methods

Research questions to be addressed by the present analysis and their rationale

The following key research questions were addressed in the present regression analysis:

(1) Does latitude (between 40 and 49·5°N compared to ≥49·5°N (and 78°S)) influence the response of serum 25(OH)D to increased vitamin D intake during winter? Rationale: The rationale for choosing RCT that finished at (or at least reported data from) the end of winter, as outlined by the IOM, was due to uncertainties about the contribution of sunlight to overall serum 25(OH)D concentration and that vitamin D requirements cannot be based on an accepted or 'recommended' level of sun exposure due to potential skin damage and cancer[8]. Instead, the best remaining approach was to describe the relationship between total vitamin D intake and serum 25(OH)D levels under conditions of minimal sun exposure, as would be achieved in winter time[8], an approach we have also advocated in our recent studies of vitamin D dietary requirements[13–15]. The IOM used the two latitude bands (namely, 40 to <49·5°N and ≥49·5°N and 78°S) to test the assumption of minimal sun exposure during winter, which they found was met in RCT performed in the latter but not the former latitude region[8]. As much of Europe resides between 40 and >70°N, the two latitude bands used by the IOM seemed appropriate to test in the present analysis. Finally, the choice of which months should be designated as winter (during which there is insufficient UVB sunshine to allow for dermal synthesis of vitamin D) may differ in these two latitude bands, particularly at the lower latitude range. Therefore, in addition to testing the DRI committee's definition of winter as September to June or part thereof[8], we also used September to April as a shorter winter period, as it is highly likely that there is UVB sunshine of sufficient strength in May and June to allow for dermal synthesis of vitamin D and thus could contribute significantly to achieved serum 25(OH)D concentration.

(2) What is the most appropriate model for the relationship between total vitamin D intake and achieved serum 25(OH)D? Rationale: The DRI committee used an integrated bone health outcome approach (incorporating Ca absorption, bone mineral density, risk of rickets and osteomalacia) to define the estimated average requirement-like serum 25(OH)D concentration (40 nmol/l) and 50 nmol/l as the concentration of 25(OH)D covering the needs of nearly all in the population[8]. As mentioned previously, it is possible that European agencies briefed with the task of re-evaluation of population reference intake (PRI), or member state-specific equivalent dietary reference values, for vitamin D may decide to use a serum 25(OH)D target concentration other than 50 nmol/l. For example, should a European agency decide to use risk of rickets or osteomalacia as

the health outcome used to establish their dietary reference value in preference to the more integrated bone health outcome approach used by the DRI, they may well use 30 nmol/l, or even the more precautionary 40 nmol/l[8], as the target 25(OH)D concentration on which to base their intake requirement value. Therefore, we wished to explore the vitamin D intake–serum 25(OH)D relationship under different meta-regression model constructs, particularly at different serum 25(OH)D concentrations.

Systematic review of vitamin D intake–status relationship

The methodology used in the systematic review and meta-regression in the present study follows the general methodology for a recent series of systematic reviews in relation to markers of nutrient status[16] and in particular for our recent systematic review of existing and potentially novel functional markers of vitamin D status[17], with brief specific details as follows:

Inclusion criteria. Studies were RCT of vitamin D (D_3 with or without Ca) supplementation in apparently healthy human subjects or in patients in whom there is no underlying reason for altered vitamin D metabolism or response to vitamin D supplementation that fulfilled all of the following characteristics: (1) vitamin D_3 $\leq$2000 IU/d (50 μg/d; 1 μg = 40 IU) administered orally alone or with Ca on a daily basis (inclusion of vitamin D_3 and not D_2 was chosen on the basis that the IOM DRI committee used studies with vitamin D_3 in their regression analysis of the northern European RCT (and Antarctica), on which DRI values were set[8], and there is still some debate as to the relative potency of vitamin D_2 relative to vitamin D_3[18,19]; (2) reported serum or plasma 25(OH)D levels following supplementation in at least one intervention group and one control group; (3) no vitamin D metabolites (25(OH)D and 1,25$(OH)_2$D) and analogues (e.g. α-calcidol) co-administered; (4) minimum duration of 6 weeks (on the basis that following initiation of vitamin D supplementation, serum 25(OH)D concentrations reach equilibrium after at least 6–8 weeks in adults and elderly subjects[20,21]); (5) studies performed above 40°N (or 40°S) (these minimum latitudes were those used in RCT included in the regression analysis by the DRI committee[8]); (6) studies were carried out in children and adolescents (1–18 years), adults (18–64 years) and older adults (>64 years). (Infants and pregnant or lactating women are life-stage groups that have special considerations in relation to vitamin D and were excluded from the present analysis.)

Search strategy. In our recent systematic review of existing and potentially novel functional markers of vitamin D status[17], electronic searches were run (on Ovid MEDLINE (Ovid, http://www.ovid.com), EMBASE and Cochrane CENTRAL (http://www.thecochranelibrary.com)) from inception to 25 September 2007 by using a structured search strategy in the following format: ((vitamin D supplements) AND (supplementation or depletion studies in human subjects)) (details available online at http://www.ajcn.org/content/vol0/issue2009/images/data/ajcn.2009·27230D/DC1/AJCN_27230D_ST1.doc for full Ovid MEDLINE search strategy). As part of that systematic review, a vitamin D expert was contacted, reference lists of ten reviews drawn from electronic searches of reviews run on Ovid MEDLINE and all included studies were checked and additional articles collected and assessed for inclusion[17]. For the present study, an updated search from 26 September 2007 to 30 November 2010, using the same structured search strategy, was performed. In addition, there have been two major systematic reviews performed in the area of vitamin D (and Ca) and bone[12,22] and other health outcomes[12], as well as the IOM DRI report on Ca and vitamin D[8]. For completeness, we cross-referenced the RCT that were both included and excluded in these reviews against those identified for inclusion or exclusion in the present study.

Data collection for updated search. Screening of titles and abstracts for collection, screening full-text articles for inclusion and data extraction (including quality assessment) from included studies were all performed by two independent reviewers. Both reviewers for the updated search were the same individuals who performed the original electronic searches[17].

Data synthesis. A flowchart showing the number of studies assessed (original and updated search) and included in the review is shown in Fig. 1. We extracted the number of participants included (and assessed) in each arm of each RCT, plus mean values and standard deviations of the baseline and final values in the treatment and control arms at each time point and for each vitamin D dose. In cases in which there were greater than or equal to two intervention arms and one common control group within an RCT, the various arms (up to and including 2000 IU/d) *v.* control were included so long as the arms fell into different dose range subgroups.

Meta-regression of the response of serum 25-hydroxy-vitamin D to total vitamin D intake. Weighted linear model meta-regression analyses of total vitamin D intake (i.e. habitual intake of the vitamin plus the supplemental dose) *v.* achieved serum or plasma 25(OH)D concentration (i.e. the concentration at the end of the winter sampling point) were performed in SPSS for Windows version 15.0 (SPSS, Inc., Chicago, IL, USA). As per the analysis by the IOM DRI committee, the regression analysis in the present study was performed on data from all RCT that were conducted during the winter period (September to June or for part thereof, as defined in the IOM DRI report[8]) stratified by >40 to <49·5°N and ≥49·5°N and 78°S.

In situations in which the RCT did not assess and/or report the habitual vitamin D intake of the cohort(s) within their study, the appropriate age and sex group mean vitamin D intake value from the national nutrition survey relevant to the country in which the RCT was performed, where available (or where unavailable then from a published study in the relevant sex and age group) was used as a surrogate (Table 1). The habitual intake estimates were added to the supplemental vitamin D dose to generate total vitamin D intake estimates, which were then transformed to the natural log (Ln) before regression analysis, the approach used by the DRI committee[8]. As per the DRI committee approach,

British Journal of Nutrition

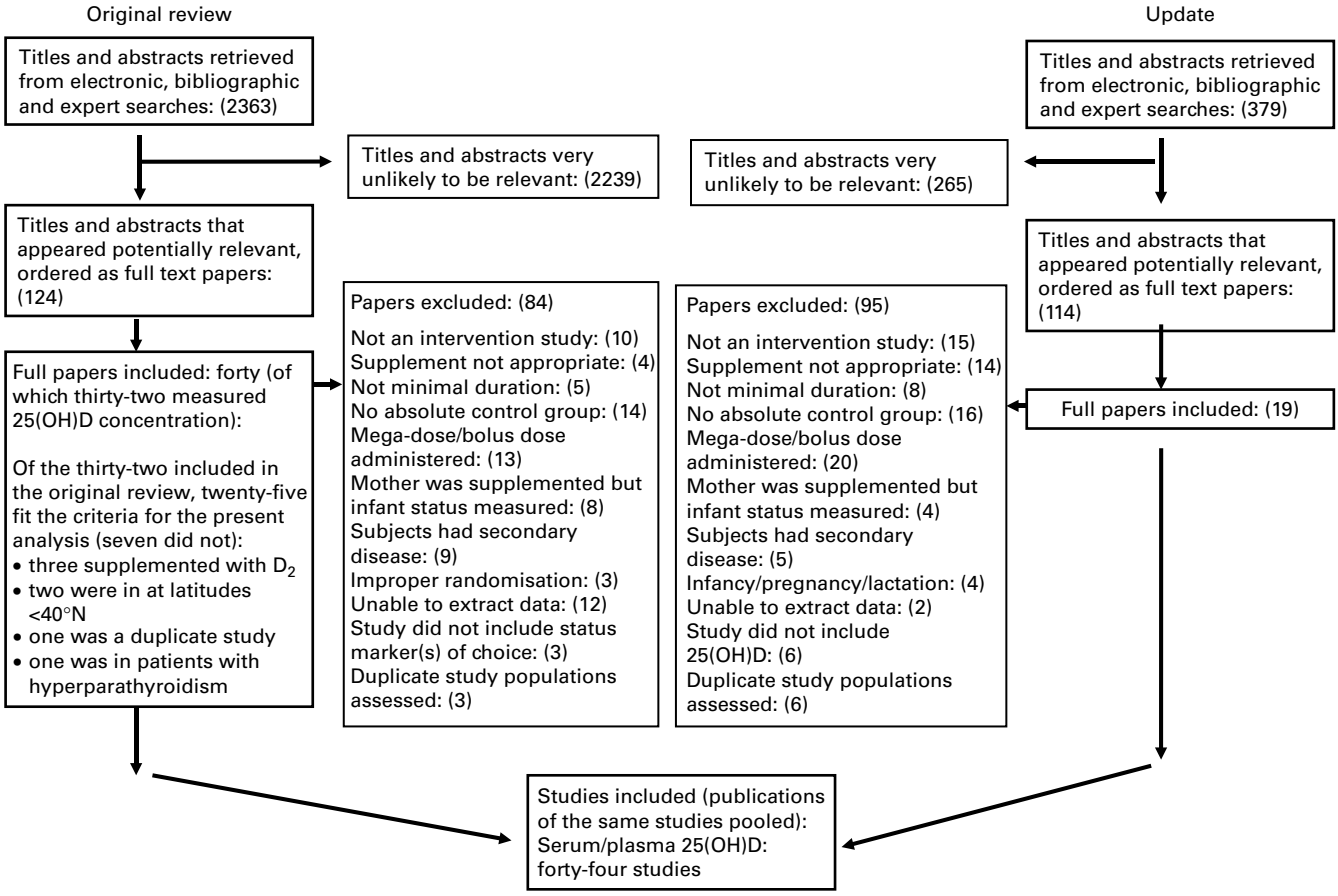

Fig. 1. Flow diagram for systematic review of vitamin D intake–status relationship. 25(OH)D, 25-hydroxyvitamin D; D_2, vitamin D_2.

the various vitamin D arms of an RCT were included as long as the arms fell into different dose range subgroups[8]. In addition, the regression was set for a y_0 intercept of 0 nmol of 25(OH)D/l of serum, on the basis that the DRI committee suggested that this is consistent with the biological reality preventing a negative value for achieved serum 25(OH)D levels[8]. In addition, regression models of achieved serum 25(OH)D concentration and total vitamin D intake were run without Ln transforming total intake, but limiting the total vitamin D intake data points to a maximum of 1400 IU/d, on the basis of Aloia *et al.*[23], who in their recent analysis of sixty-four vitamin D RCT using a spline-fit approach showed that the slope response of serum 25(OH)D to increasing dose becomes constant at a dose of 1400 IU/d. Thus, at doses above this level, the response of serum 25(OH)D is more blunted and would not be best described by a linear fit model.

Regression analysis of combined individual data from two winter-based randomised controlled vitamin D intervention trials in adults and older adults at latitudes >49·5°N. Regression analysis using the same model characteristics (total vitamin D intake with and without prior Ln transformation, setting y_0 intercept of 0 nmol of 25(OH)D/l of serum (Ln model) or allowing model predict y_0 intercept (non-Ln model)) was performed on individual data from two recently published randomised, double-blind, placebo-controlled, vitamin D_3 intervention studies in northern European (≥52°N) 20- to 40-year-old adults (*n* 238[13]) and greater than 64-year-old adults (*n* 225[14]), which estimated the dietary requirement for vitamin D in these population subgroups during late winter.

Results

In total, 2742 (2363 in our previous review[17] and 379 in our updated search) titles and abstracts were screened (Fig. 1) and forty-four RCT were included[4,13–15,19,20,24–62], all of which met the inclusion criteria of the present study and provided extractable data on serum or plasma 25(OH)D. Details of the forty-four included studies (including some criteria of quality) have been presented in our previous systematic review[17] or in the Tufts systematic review group[12], the IOM DRI report[8] or are shown in Table 1. Of the forty-four studies, seven were in males and twenty-four in females (the remainder were mixed). Of the RCT, four were in children and adolescents (8–15 years), eleven in adults (18–64 years) and twenty-two in elderly (>65 years; some studies had more than one population subgroup). Among these, twenty-four studies gave vitamin D supplementation alone, twenty vitamin D plus Ca (some studies had both arms) and one study co-administered phylloquinone[30] and one alendronate[32]. In all, five studies gave ≤200, fifteen 201–400, thirty 401–1000 and six 1001–2000 IU/d of supplemental vitamin D (some studies provided multiple doses). Of the winter-based RCT,

NS British Journal of Nutrition

Table 1. Study characteristics of randomised controlled trials >49·5°N selected for the meta-regression analysis
(Mean values and standard deviations)

Source	Country	Latitude	Age (years)		Male (%)	Duration (weeks)	Participants (*n* per group)	Supplemental vitamin D_3 dose (IU/d)	Habitual vitamin D intake (IU/d)	Total vitamin D intake (IU/d)	Baseline 25(OH)D (nmol/l)		Achieved 25(OH)D (nmol/l)		Randomisation reported	Dose check*	Compliance reported	Analytical method†
			Mean	Range							Mean	SD	Mean	SD				
Ala-Houhala *et al.*[24]	Finland	61°N	9	10–15	45	56	24	400	200‡	600	49·2	19·0	77·9	23·7	Yes	No	No	CPBA
							27	0	200‡	200	45·9	15·5	43·2	19·5	–	–	–	–
Barnes *et al.*[26]	UK	55°N	21	18–27	50	8	15	600	65	665	47·9	16·0	85·6	24·5	Yes	No	No	EIA
							12	0	96	96	55·5	18·6	48·3	16·8	–	–	–	–
Cashman *et al.*[13]	Ireland	51–55°N	30	20–40	50	22	53	600	144	744	75·9	24·8	69·0	18·6	Yes	Yes	Yes	EIA
							57	400	140	540	72·2	26·8	60·0	13·4	–	–	–	–
							48	200	172	372	60·0	29·4	49·7	11·4	–	–	–	–
							57	0	136	136	65·7	26·5	37·4	12·2	–	–	–	–
Cashman *et al.*[14]	Ireland	51–55°N	71	64+	40	22	48	600	192	792	55·1	22·8	73·8	20·0	Yes	Yes	Yes	EIA
							53	400	168	568	54·3	21·8	69·5	17·0	–	–	–	–
							48	200	164	364	51·8	22·1	53·2	17·0	–	–	–	–
							55	0	188	188	58·8	25·9	58·8	17·1	–	–	–	–
Cashman *et al.*[15]	Finland and Denmark	58°N§	11·4‖		0	52	49	400	156	556	58·4	13·9	58·8	10·9	Yes	Yes	Yes	HPLC
							49	200	156	356	57·4	12·4	47·6	8·0	–	–	–	–
							46	0	148	148	54·5	14·8	31·0	10·0	–	–	–	–
Honkanen *et al.*[43]¶	Finland	63°N	70	67–72	0	11	30	1800	380‡	2180	42·8	19·6	80·7	15·4	Yes	No	No	HPLC
							27	0	380‡	380	36·0	13·3	23·3	13·3	–	–	–	–
Meier *et al.*[50]	Germany	50°N	56	33–78	33	26	27	500	128**	628	75·1	28·5	87·6	20·0	Yes	No	No	RIA
							16	0	128**	128	76·9	23·2	51·2	21·2	–	–	–	–
Pfeifer *et al.*[55]	Germany	52°N	74	70+	0	8	74	800	128**	928	25·7	13·6	64·8	27·4	Yes	No	Yes	RIA
							77	0	128**	128	24·6	12·1	44·4	27·4	–	–	–	–
Smith *et al.*[58]	Antarctica	78°S	42‖		59	20	18	2000	302	2302	45·0	14·0	71·0	23·0	Yes	Yes	Yes	RIA
							19	1000	329	1329	44·0	19·0	63·0	25·0	–	–	–	–
							18	400	356	756	44·0	18·0	57·0	15·0	–	–	–	–
							7	0	334	334	36·0	17·0	34·0	12·0	–	–	–	–
Viljakainen *et al.*[21]	Finland	61°N	71	65–85	0	12	13	800	388	1188	44·1	13·5	70·2	13·5	Yes	No	No	HPLC
							11	400	424	824	46·5	10·2	60·4	10·2	–	–	–	–
							13	200	388	588	46·0	10·2	55·0	10·2	–	–	–	–
							12	0	436	436	52·2	19·9	43·9	19·9	–	–	–	–
Viljakainen *et al.*[62]	Finland	61°N	29	21–49	100	25	16	800	344	1144	60·3	11·6	90·1	11·6	Yes	Yes	Yes	EIA
							16	400	304	704	62·3	13·6	75·6	13·6	–	–	–	–
							16	0	264	264	64·7	18·5	52·2	18·5	–	–	–	–

25(OH)D, 25-hydroxyvitamin D; CBPA, competitive binding protein assay; EIA, enzyme-linked immunoassay.
* Dose of vitamin D confirmed independently by analysis.
† Analytical methods for analysing circulating 25(OH)D levels: EIA; CBPA; HPLC.
‡ Intake estimated from Andersen *et al.*[76].
§ An average latitude was taken from Helsinki, Finland (61°N) and Copenhagen, Denmark (55°N).
‖ Mean age is given where range is not available.
¶ Data was extracted from the 'outpatient' group of the study only.
** Intake estimate was obtained from Flynn *et al.*[71].

eleven studies were carried out in northern Europe (>49·5°N) and one in Antarctica (78°S), seven studies were in latitudes between 40 and 49·5°N, of which six were in the USA and one from Europe (Switzerland). Six of the twelve RCT in >49·5°N and four of the seven RCT at >40°N to <49·5°N were included in the comprehensive systematic review by the Ottawa group[22] and all had a Jadad score ≥3. The remaining RCT were not within the timeframe of that systematic review and thus do not have a Jadad score, but were all included in the IOM analysis[8].

Meta-regression of the response of serum 25-hydroxyvitamin D to total vitamin D intake

The listing of winter-based RCT at ≥49·5°N and 78°S (*n* 12), identified through the search strategy and data collection of the present study, differed modestly from those (*n* 9) used in the IOM DRI committee's meta-regression analyses: seven RCT[13,14,21,24,58,61,62] were common to both analyses, three RCT[63–65], which were used by the DRI committee, were excluded from the present analysis as they were too short in duration (4–5 weeks), whereas we included five RCT[26,43,50,51,55] that fit with the inclusion criteria of the present study, but were not included in the DRI committee's analysis (possibly because Ca was co-administered, but this has not been shown to influence the response of serum 25(OH)D to vitamin D in a meta-analysis[17] or experimentally in an intervention study[66], or because the RCT was after the IOM's timeframe[51]). In the case of two RCT, combined data from 11-year-old girls in the RCT by Viljakainen *et al.*[61] and Mølgaard *et al.*[51], who were on the vitamin D intervention during September/October to March/April (a subset of the entire group) were presented recently by Cashman *et al.*[15].

We also checked the literature for any relevant studies between 1 December 2010 and 28 February 2011, but none of the three studies published during this time frame[67–69] could be included in the present regression analysis.

Weighted linear model meta-regression analyses of Ln total vitamin D intake *v.* achieved serum or plasma 25(OH)D concentration (and setting y_0 at 0 nmol/l serum 25(OH)D) from winter-time-only RCT stratified by latitude showed that in those performed at latitudes >40 to <49·5°N or ≥49·5°N and 78°S, the interaction term between age and the Ln of total vitamin D intake (P=0·922 and 0·472, respectively), as well as the main effect of age (P=0·652 and 0·325, respectively) were non-significant. Therefore, because there was no age effect in the response of serum 25(OH)D level to Ln total intake of vitamin D, a single, combined regression analysis was carried out on the data from RCT at both latitude groupings separately.

With RCT at >40 to <49·5°N (*n* 7), the present analysis yielded the predictive equation of achieved serum 25(OH)D in nmol/l = 12·6 Ln (total vitamin D intake) (Table 2). These RCT were conducted during the winter period, as defined broadly by the DRI committee as September to June or part thereof[8]. The regression analysis was also run after omitting RCT whose end point was beyond April (*n* 2), which yielded the predictive equation of achieved serum 25(OH)D in nmol/l = 11·4 Ln (total vitamin D intake).

A single, combined regression analysis was also carried out with data from winter-time-only (April, the latest end date) RCT at latitudes ≥49·5°N and 78°S, and resulted in the predictive equation of achieved serum 25(OH)D in nmol/l = 9·2 Ln (total vitamin D intake) (Table 2 and Fig. 2). Using the combined regression predicted 95 % lower CI of y = 8·5 Ln (total vitamin D intake) and inputting a total intake value of 600 IU/d (the RDA for those aged 1–70 years[8]) would predict an achieved serum 25(OH)D of 54·4 nmol/l. Using the same equation, but in reverse, to predict the total intake of vitamin D that would achieve a serum 25(OH)D of 50 nmol/l (the concentration that would meet the needs of 97·5 % of the population[8]), the required total intake of vitamin D dropped dramatically to 359 IU/d.

As an alternative to the curvilinear relationship arising from the Ln-transformed intake data, if non-transformed total vitamin D intake (and limiting it to a maximum of 1400 IU/d on the basis of Aloia *et al.*[23]) was used, a more linear relationship resulted (Fig. 2). The interaction term between age and total vitamin D intake (P=0·213), as well as the main effect of age (P=0·196) were non-significant, allowing for a single, combined regression analysis that resulted in the predictive equation of achieved serum

Table 2. Predictive regression equations of achieved winter serum 25-hydroxyvitamin D (s25(OH)D) as a function of natural log (Ln) and linear total vitamin D intake

Study grouping	Predicted mean (95 % lower CI) equations	Predicted s25(OH)D (nmol/l) at 600 IU/d*	Predicted RDA (IU/d) at s25(OH)D of 50 nmol/l*	Predicted RDA (IU/d) at s25(OH)D of 40 nmol/l*
Ln model				
Winter-based RCT >40 to <49·5°N (*n*† 7)	y = 12·6 Ln (total vitamin D intake) (y = 11·7 Ln (total vitamin D intake))	74·8	72	31
Winter-based RCT ≥49·5°N and 78°S (*n*† 12)	y = 9·2 Ln (total vitamin D intake) (y = 8·5 Ln (total vitamin D intake))	54·4	359	111
Linear model				
Winter-based RCT ≥49·5°N and 78°S (*n*† 12)	y = 0·044 (total vitamin D intake) + 33·035‡	55·2	480	260

RCT, randomised controlled trials; *y*, achieved serum 25(OH)D (nmol/l) in winter.
* Using the 95 % lower CI regression equation.
† Refers to number of RCT.
‡ Maximum total vitamin D intake was limited to 1400 IU/d (35 μg/d).

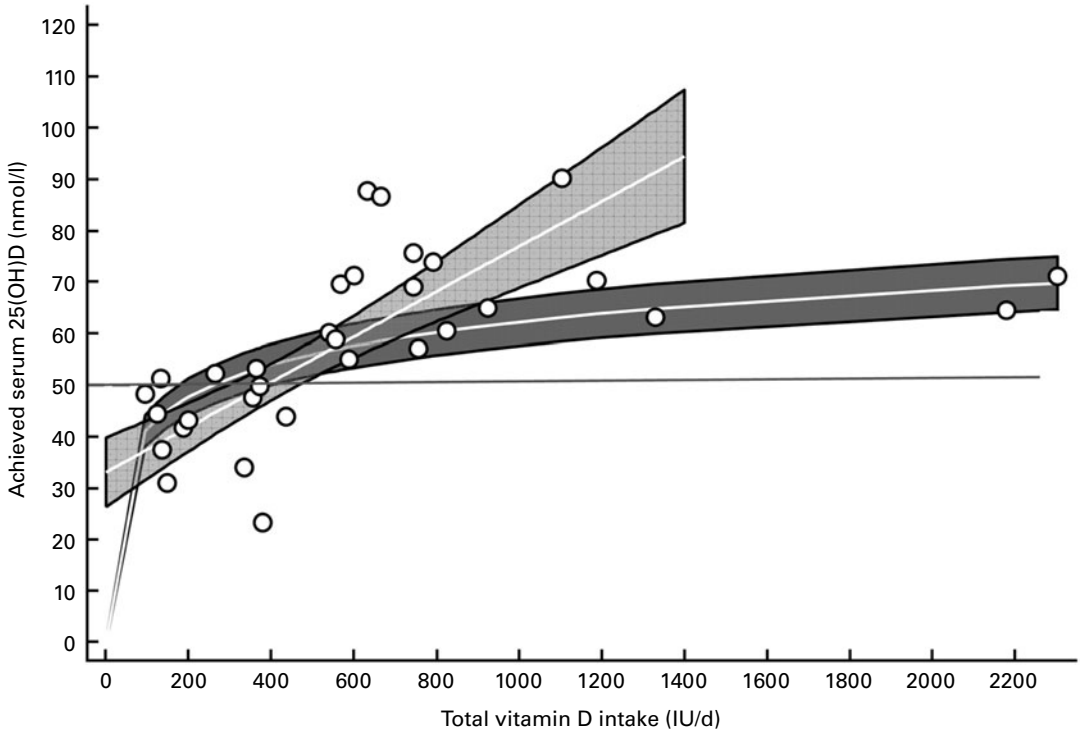

Fig. 2. Response of serum 25-hydroxyvitamin D (25(OH)D) level to total intake of vitamin D in northern latitudes in Europe (>49·5°N) and Antarctica (78°S) during their respective winter seasons, when effective sun exposure for endogenous vitamin D synthesis is minimal. Mean responses (white lines) with 95 % CI using a weighted linear meta-regression model following either a natural logarithmic transformation (dark gray shading, curvilinear model) or no transformation (pale gray shading, linear model) of total vitamin D intake data. The maximum total intake data point in the linear model was <1400 IU/d (35 μg/d). A line is plotted at 50 nmol/l serum 25(OH)D for illustrative purposes.

25(OH)D in nmol/l = 0·044 (total vitamin D intake) + 33·035. The 95 % lower CI predictive regression values from the linear intake–status relationship predicted an achieved serum 25(OH)D of 55·2 nmol/l, at a total intake value of 600 IU/d. The lower CI regression equations predicted the total intake of vitamin D that would achieve a serum 25(OH)D of 50 nmol/l of 480 IU/d (Table 2).

Using the Ln and linear lower CI regression equations, it was predicted that the total intake of vitamin D would achieve a serum 25(OH)D of 40 nmol/l at 111 and 260 IU/d, respectively.

Regression analysis using the combined data from two vitamin D randomised control trials

The predicted RDA estimates for vitamin D at two target serum 25(OH)D concentrations (i.e. 40 and 50 nmol/l) using the 95 % lower CI meta-regression analysis with group means (*n* 8) and regression analysis with 95 % lower CI and 95 % range of individual combined data (total *n* 463; maximum total vitamin D intake = 1310 IU/d; hence, all data were included) from the two RCT with adults[13] and older adults[14] are shown in Table 3. The RDA estimates from the meta-regression and regression of individual data, which both used the 95 % lower CI, were dramatically lower than that from the regression model of individual data that used the 95 % range.

After seeing the magnitude of the difference in the requirement estimates arising from use of 95 % lower CI and 95 % range, we also went back and for comparative purposes applied a 95 % range to the linear meta-regression analysis of twelve winter-based RCT at >49·5°N, although caution is warranted when applying a 95 % range when the number of data points are relatively low (*n* 30). This analysis predicted that the total intake of vitamin D that would achieve a serum 25(OH)D of 50 nmol/l was 930 IU/d (*v.* 480 IU/d with the 95 % lower CI).

Table 3. Predicted RDA estimates using linear regression models of group means and individual data from two winter-based vitamin D randomised controlled trials (RCT) at ≥52°N

Regression approach	RDA estimate (IU/d) at 50 nmol/l 25(OH)D	RDA estimate (IU/d) at 40 nmol/l 25(OH)D
Combined meta-regression analysis of group means from two RCT (*n** 8) and use of 95 % lower CI	395	235
Regression analysis of individual data (*n*† 463) and use of 95 % lower CI	320	140
Regression analysis of individual data (*n*† 463) and use of 95 % range	1040	830

25(OH)D, 25-hydroxyvitamin D.
* Refers to eight group means from two RCT (young adults, Cashman *et al.*[13]; and older adults, Cashman *et al.*[14]).
† Refers to number of combined individuals within the two RCT.

NS British Journal of Nutrition

Discussion

The new IOM DRI values for vitamin D[8] relied heavily on data from eight northern European (plus one from Antarctica) winter-based vitamin D RCT and thus would appear to be highly relevant to Europe in terms of a re-evaluation of its vitamin D dietary reference values (i.e. population reference intakes; PRI) and indeed those of its constituent member states/regions. Using the systematic review approach of the present study, we identified and extracted data from eleven relevant European-based RCT (plus one RCT from Antarctica) with vitamin D_3, which were conducted at latitudes ≥49·5°N during winter, in line with the approach used by the IOM DRI committee. Despite some differences in the final collection of RCT included in the present regression analysis relative to that in the DRI committee's analysis (as outlined previously), the 95 % lower CI predictive equations of achieved 25(OH)D *v.* Ln total vitamin D intake were very close.

The DRI committee choose to apply the curvilinear Ln model to the intake–status data from the RCT to account for the more blunted response of serum 25(OH)D to high intakes of vitamin D[8]. This non-linear response of serum 25(OH)D to vitamin D intake is to be expected on the basis of metabolic kinetics. Heaney *et al.*[70] showed that the relationship between serum vitamin D_3 and 25(OH)D concentrations is biphasic due to the fact that hepatic 25-hydroxylase becomes saturated and the reaction switches from first to zero order. Even though the lower limit CI predictive equations arising from the Ln model overshoots the target serum 25(OH)D mark of 50 nmol/l at the 600 IU/d vitamin D intake level (e.g. 56 nmol/l in the DRI analysis[8], 54 nmol/l in the present analysis), the committee used this intake estimate to allow for some uncertainties and limitations within the analysis[8]. Should one choose to use 50 nmol/l as the target concentration of serum 25(OH)D in these equations, then the dietary requirement estimate is 313 IU/d (359 IU/d in the present analysis), and alternatively, if one uses 40 nmol/l, it is only 99 IU/d (111 IU/d in the present analysis). The Ln model has a steep decline in achieved serum 25(OH)D concentrations at total vitamin D intakes, particularly at the lower end of intakes, and at zero intake, the achieved serum 25(OH)D was 0 nmol/l due to a forcing of the model to avoid a negative predicted value for achieved serum 25(OH)D levels[8]. However, it is also worth considering whether it is likely that someone with no vitamin D intake during winter (if that were possible) might still have a serum 25(OH)D concentration greater than zero as a consequence of tissues stores? For example, the adult subject with lowest total vitamin D intake (24 IU/d) in our vitamin D intervention study in 20- to 40-year-old adult group[13] had a winter serum 25(OH)D of 31·6 nmol/l.

Others, including ourselves[17], have reported serum 25(OH)D response estimates to vitamin D supplementation from RCT based on a linear analysis[22,41], but with doses up to 2000 IU/d[22] and even 10 000 IU/d[41]. This clearly does not take account of the smoothening in the response of serum 25(OH)D to higher intakes of vitamin D. Therefore, in the present analysis, we also performed a linear analysis of the intake–status data, but excluded intake data points (*n* 2) in excess of 1400 IU/d on basis of Aloia *et al.*[23], who showed that the response slope of serum 25(OH)D becomes constant at this level. In Ireland and the UK, as well as in several other European member states[71], the 95th percentile of total vitamin D intake in national nutrition surveys is generally less than 600 IU/d; thus, a range of 0–1400 IU/d brackets the nutritional intake of vitamin D seen in the population. Using an intake of 600 IU/d in the lower limit CI predictive linear regression equation, the predicted serum 25(OH)D concentration was 55 nmol/l – similar to that from the Ln models, and providing further support to the DRI committee's findings. However, an intake of 260 and 480 IU/d was required to achieve serum 25(OH)D concentrations of 40 and 50 nmol/l, respectively (2·3- and 1·4-fold higher than that predicted from the Ln model, respectively).

Clearly, the shape of the intake–status relationship has an important bearing on the predicted RDA estimates for vitamin D at serum 25(OH)D target concentrations ≤50 nmol/l. However, and maybe more importantly, irrespective of whether a Ln or linear model is applied in these meta-regression analyses, estimates of 359 or 480 IU/d vitamin D requirements, respectively, to cover the needs of 97·5 % of the population in terms of maintaining serum 25(OH)D >50 nmol/l does not fit well with our estimates from experimental studies that suggest that 988–1120 IU/d would be required[13,14]. The use of CI in meta-regression analyses provides some estimate of the variability about the fitted response line, but does not provide any estimate of the variability between individuals in terms of dietary intake of vitamin D needed to achieve a serum 25(OH)D concentration (i.e. an estimate of the range). This was illustrated in the present study, wherein RDA estimates from either the meta-regression or regression of individual data that used the 95 % lower CI were dramatically lower than the model that used the 95 % range. The former variability term gives 95 % surety that the average serum 25(OH)D level in the adult population is above 50 nmol/l at a certain intake of vitamin D, whereas the latter can be used to take account of inter-individual variability on intake required to reach a chosen serum 25(OH)D cutoff. The importance of this inter-individual variability term (95 % range) can also be seen if one compares the estimates from our RCT in young adults[13] and older adults[14], which incorporated the range, and suggest that 346 IU/d of vitamin D are required to keep winter time serum 25(OH)D levels >25 nmol/l in 97·5 % of the population, whereas the model with lower 95 % CI predicts that 0 IU/d intake will suffice. We have reported previously that if one tests the former estimate within the nationally representative UK National Diet and Nutrition Survey databases of adults and older adults, there was a 11–18·4 % prevalence of serum 25(OH)D below 25 nmol/l during late winter/early spring in those with intakes below 346 IU/d and only 0–2·9 % prevalence in those with intakes above this estimate[72], suggesting that this intake did indeed protect the vast majority of the adult population from vitamin D deficiency.

The present regression analysis also predicted a higher response of serum 25(OH)D to total vitamin D intake at

lower (40–49·5°N) than at higher latitudes (≥49·5°N/S), in line with the DRI committee's findings[8]. Although the reason(s) for this could not be explored in the present analysis, it may relate to differences in the capacity for dermal synthesis during extended winter in these different regions and/or stores of vitamin D arising from previous summer UVB sun exposure. Nevertheless, it may be relevant to Europe, much of which resides between 40 and >70°N. The present analysis had a number of limitations arising from the available data on which to base the meta-regression. Most of the subjects in the twelve RCT that met with the inclusion criteria of the present study were Caucasians, and thus does not reflect the ethnic diversity that exists in many European member states. Recent data from national nutrition and health surveys in the USA and Canada clearly show that risk of serum 25(OH)D concentrations below 30 and 50 nmol/l was higher in non-white than in white persons[73,74]. The DRI committee highlighted the need for a greater understanding of how skin pigmentation influences vitamin D synthesis, and highlighted that South Asian and Middle Eastern immigrant groups may be a particular concern[8]. Finally, the quality of vitamin D intake data in the meta-regression analysis may be a limitation, as in some cases national intake vitamin D data had to be used as a surrogate in those studies that did not measure/report intake data. Furthermore, there can be considerable differences in food compositional data for vitamin D across countries[75].

In conclusion, although the relation of serum 25(OH)D to vitamin D intake is critical to the establishment of dietary requirements for vitamin D, the model used to describe this relationship needs to be configured to take into account important considerations such as target serum 25(OH)D concentration, range of intakes of vitamin D within the population and inter-individual variability. There may be additional benefit from use of individual data from vitamin D RCT, if these were available, to augment the meta-analyses approach.

Acknowledgements

The present study was carried out with partial financial support from the Commission of the European Communities, specific RTD Programme 'Quality of Life and Management of Living Resources', within the 6th Framework Programme (contract no. FP6-036196-2 EURRECA: EURopean micronutrient RECommendations Aligned). This paper does not necessarily reflect the views of the Commission and in no way anticipates future policy in this area. K. M. S. and K. D. C. assessed the studies for inclusion, extracted data and assessed validity; K. M. S., A. P. F. and K. D. C. conducted meta-regression and regression; K. M. S. and K. D. C. tabulated data; K. M. S., M. K. and K. D. C. wrote the manuscript. The authors declare no conflicts of interest.

References

1. UK Department of Health (1998) *Nutrition and Bone Health: With Particular Reference to Calcium and Vitamin D. Report on Health and Social Subjects (49)*. London: The Stationery Office.
2. Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* **22**, 477–501.
3. Parfitt AM, Gallagher JC, Heaney RP, *et al.* (1982) Vitamin D and bone health in the elderly. *Am J Clin Nutr* **36**, 1014–1031.
4. Ooms ME, Lips P, Roos JC, *et al.* (1995) Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. *J Bone Miner Res* **10**, 1177–1184.
5. Zittermann A (2003) Vitamin D in preventive medicine: are we ignoring the evidence? *Br J Nutr* **89**, 552–572.
6. Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers and cardiovascular disease. *Am J Clin Nutr* **80**, 1678S–1688S.
7. Cashman KD (2007) Calcium and vitamin D. *Novartis Found Symp* **282**, 123–138.
8. Institute of Medicine Food and Nutrition Board (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press.
9. Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects (41)*. London: Her Majesty's Stationery Office.
10. Nordic Council of Ministers (2004) *Nordic Nutrition Recommendations 2004. Integrating Nutrition and Physical Activity*. Copenhagen: Nordic Council of Ministers.
11. Commission of the European Communities (1993) Vitamin D. In *Nutrient and Energy Intakes of the European Community. Report of the Scientific Committee for Food (31st series)*, pp. 132–139. Brussels: Commission of the European Communities.
12. Agency for Healthcare Research and Quality (AHRQ) (2009) Vitamin D and calcium: a systematic review of health outcomes. AHRQ Publication No. 09-E015. http://www.ahrq.gov/clinic/tp/vitadcaltp.htm (accessed 28 October 2010).
13. Cashman KD, Hill TR, Lucey AJ, *et al.* (2008) Estimation of the dietary requirement for vitamin D in healthy adults. *Am J Clin Nutr* **88**, 1535–1542.
14. Cashman KD, Wallace JM, Horigan G, *et al.* (2009) Estimation of the dietary requirement for vitamin D in free-living adults ≥64 y of age. *Am J Clin Nutr* **89**, 1366–1374.
15. Cashman KD, Fitzgerald AP, Viljakainen HT, *et al.* (2011) Estimation of the dietary requirement for vitamin D in healthy adolescent white girls. *Am J Clin Nutr* **93**, 549–555.
16. Hooper L, Ashton K, Harvey LJ, *et al.* (2009) Assessing potential biomarkers of micronutrient status by using a systematic review methodology: methods. *Am J Clin Nutr* **89**, 1953S–1959S.
17. Seamans KM & Cashman KD (2009) Existing and potentially novel functional markers of vitamin D status: a systematic review. *Am J Clin Nutr* **89**, 1997S–2008S.
18. Cashman KD (2011) The role of vitamers and dietary-based metabolites of vitamin D in prevention of vitamin D deficiency. *Food Nutr Res* (In the Press).
19. Lanham-New S, Vieth R & Heaney R (2010) Vitamin D_2 and vitamin D_3 comparisons: fundamentally flawed study methodology. *Am J Clin Nutr* **92**, 999.
20. Harris SS & Dawson-Hughes B (2002) Plasma vitamin D and 25OHD responses of young and old men to supplementation with vitamin D_3. *J Am Coll Nutr* **21**, 357–362.

British Journal of Nutrition

21. Viljakainen HT, Palssa A, Kärkkäinen M, *et al.* (2006) How much vitamin D_3 do the elderly need? *J Am Coll Nutr* **25**, 429–435.
22. Cranney A, Horsley T, O'Donnell S, *et al.* (2007) Effectiveness and safety of vitamin D in relation to bone health. In *Evidence Report/Technology Assessment No. 158 (Prepared by the University of Ottawa Evidence-based Practice Center (UO-EPC) under Contract No. 290-02-0021*, AHRQ Publication No. 07-E013: Agency for Healthcare Research and Quality Rockville, MD.
23. Aloia JF, Patel M, Dimaano R, *et al.* (2008) Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. *Am J Clin Nutr* **87**, 1952–1958.
24. Ala-Houhala M, Koskinen T, Koskinen M, *et al.* (1988) Double blind study on the need for vitamin D supplementation in prepubertal children. *Acta Paediatr Scand* **77**, 89–93.
25. Andersen R, Molgaard C, Skovgaard LT, *et al.* (2008) Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. *Br J Nutr* **100**, 197–207.
26. Barnes MS, Robson PJ, Bonham MP, *et al.* (2006) Effect of vitamin D supplementation on vitamin D status and bone turnover markers in young adults. *Eur J Clin Nutr* **60**, 727–733.
27. Biancuzzo RM, Young A, Dibuld D, *et al.* (2010) Fortification of orange juice with vitamin D(2) or vitamin D(3) is less effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr* **91**, 1621–1626.
28. Bischoff HA, Stähelin HB, Dick W, *et al.* (2003) Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res* **18**, 343–351.
29. Blum M, Dallal GE & Dawson-Hughes B (2008) Body size and serum 25 hydroxy vitamin D response to oral supplements in healthy older adults. *J Am Coll Nutr* **27**, 274–279.
30. Bolton-Smith C, McMurdo ME, Paterson CR, *et al.* (2007) Two-year randomized controlled trial of vitamin K_1 (phylloquinone) and vitamin D_3 plus calcium on the bone health of older women. *J Bone Miner Res* **22**, 509–519.
31. Brazier M, Grados F, Mathieu M, *et al.* (2005) Clinical and laboratory safety of one year's use of a combination calcium + vitamin D tablet in ambulatory elderly women with vitamin D insufficiency: results of a multicenter, randomized, double-blind, placebo-controlled study. *Clin Ther* **27**, 1885–1893.
32. Brazier M, Kamel S, Lorget F, *et al.* (2002) Biological effects of supplementation with vitamin D and calcium in postmenopausal women with low bone mass receiving alendronate. *Clin Drug Investig* **22**, 849–857.
33. Chapuy MC, Arlot ME, Duboeuf F, *et al.* (1992) Vitamin D_3 and calcium to prevent hip fractures in the elderly women. *N Engl J Med* **327**, 1637–1632.
34. Chel V, Wijnhoven HA, Smit JH, *et al.* (2008) Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int* **19**, 663–671.
35. Dawson-Hughes B, Harris SS, Krall EA, *et al.* (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* **337**, 670–676.
36. Dawson-Hughes B, Dallal GE, Krall EA, *et al.* (1991) Effect of vitamin D supplementation on wintertime and overall bone loss in healthy postmenopausal women. *Ann Intern Med* **115**, 505–512.
37. Fedirko V, Bostick RM, Flanders WD, *et al.* (2009) Effects of vitamin D and calcium on proliferation and differentiation in normal colon mucosa: a randomized clinical trial. *Cancer Epidemiol Biomarkers Prev* **18**, 2933–2941.
38. Grados F, Brazier M, Kamel S, *et al.* (2003) Prediction of bone mass density variation by bone remodeling markers in postmenopausal women with vitamin D insufficiency treated with calcium and vitamin D supplementation. *J Clin Endocrinol Metab* **88**, 5175–5179.
39. Grant AM, Avenell A, Campbell MK, *et al.* (2005) Oral vitamin D_3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomized Evaluation of Calcium Or vitamin D, RECORD): a randomized placebo-controlled trial. *Lancet* **365**, 1621–1628.
40. Heikkinen A, Parviainen MT, Tuppurainen MT, *et al.* (1998) Effects of postmenopausal hormone replacement therapy with and without vitamin D_3 on circulating levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D. *Calcif Tissue Int* **62**, 26–30.
41. Heaney RP, Davies KM, Chen TC, *et al.* (2003) Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* **77**, 204–210.
42. Himmelstein S, Clemens TL, Rubin A, *et al.* (1990) Vitamin D supplementation in elderly nursing home residents increases 25(OH)D but not $1,25(OH)_2D$. *Am J Clin Nutr* **52**, 701–706.
43. Honkanen R, Alhava E, Parviainen M, *et al.* (1990) The necessity and safety of calcium and vitamin D in the elderly. *J Am Geriatr Soc* **38**, 862–866.
44. Karkkainen MK, Tuppurainen M, Salovaara K, *et al.* (2010) Does daily vitamin D 800 IU and calcium 1000 mg supplementation decrease the risk of falling in ambulatory women aged 65–71 years? A 3-year randomized population-based trial (OSTPRE-FPS). *Maturitas* **65**, 359–365.
45. Kenny AM, Biskup B & Robbins B (2003) Effects of vitamin D supplementation on strength, physical function, and health perception in older, community-dwelling men. *J Am Geriatr Soc* **51**, 1762–1767.
46. Krieg MA, Jacquet AF, Bremgartner M, *et al.* (1999) Effect of supplementation with vitamin D_3 and calcium on quantitative ultrasound of bone in elderly institutionalized women: a longitudinal study. *Osteoporos Int* **9**, 483–488.
47. Lappe JM, Travers-Gustafson D & Davies KM (2007) Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr* **85**, 1586–1591.
48. Li-Ng M, Aloia JF, Pollack S, *et al.* (2009) A randomized controlled trial of vitamin D_3 supplementation for the prevention of symptomatic upper respiratory tract infections. *Epidemiol Infect* **137**, 1396–1404.
49. Lips P, Graafmans WC, Ooms ME, *et al.* (1996) Vitamin D supplementation and fracture incidence in elderly persons. A randomized, placebo-controlled clinical trial. *Ann Intern Med* **124**, 400–406.
50. Meier C, Woitge HW, Witte K, *et al.* (2004) Supplementation with oral vitamin D_3 and calcium during winter prevents seasonal bone loss: a randomized controlled open-label prospective trial. *J Bone Miner Res* **19**, 1221–1230.
51. Mølgaard C, Larnkjaer A, Cashman KD, *et al.* (2010) Does vitamin D supplementation of healthy Danish Caucasian girls affect bone turnover and bone mineralization? *Bone* **46**, 432–439.
52. Nelson ML, Blum JM, Hollis BW, *et al.* (2009) Supplements of 20 μg/d cholecalciferol optimized serum 25-hydroxyvitamin D concentrations in 80% of premenopausal women in winter. *J Nutr* **139**, 540–546.

NS British Journal of Nutrition

53. Orwoll ES, Weigel RM, Oviatt SK, *et al.* (1988) Calcium and cholecalciferol: effects of small supplements in normal men. *Am J Clin Nutr* **48**, 127–130.
54. Patel R, Collins D, Bullock S, *et al.* (2001) The effect of season and vitamin D supplementation on bone mineral density in healthy women: a double-masked crossover study. *Osteoporos Int* **12**, 319–325.
55. Pfeifer M, Begerow B, Minne HW, *et al.* (2001) Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. *J Clin Endocrinol Metab* **86**, 1633–1637.
56. Schaafsma A, Muskiet FA, Storm H, *et al.* (2000) Vitamin D(3) and vitamin K(1) supplementation of Dutch postmenopausal women with normal and low bone mineral densities: effects on serum 25-hydroxyvitamin D and carboxylated osteocalcin. *Eur J Clin Nutr* **54**, 626–631.
57. Schleithoff SS, Zittermann A, Tenderich G, *et al.* (2006) Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* **83**, 754–759.
58. Smith SM, Gardner KK, Locke J, *et al.* (2009) Vitamin D supplementation during Antarctic winter. *Am J Clin Nutr* **89**, 1092–1098.
59. Sorva A, Risteli J, Risteli L, *et al.* (1991) Effects of vitamin D and calcium on markers of bone metabolism in geriatric patients with low serum 25-hydroxyvitamin D levels. *Calcif Tissue Int* **49** (Suppl.), S88–S89.
60. Talwar SA, Aloia JF, Pollack S, *et al.* (2007) Dose response to vitamin D supplementation among postmenopausal African American women. *Am J Clin Nutr* **86**, 1657–1662.
61. Viljakainen HT, Natri AM, Kärkkäinen M, *et al.* (2006) A positive dose–response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *J Bone Miner Res* **21**, 836–844.
62. Viljakainen HT, Vaisanen M, Kemi V, *et al.* (2009) Wintertime vitamin D supplementation inhibits seasonal variation of calcitropic hormones and maintains bone turnover in healthy men. *J Bone Miner Res* **24**, 346–352.
63. Schou AJ, Heuck C & Wolthers OD (2003) A randomized, controlled lower leg growth study of vitamin D supplementation to healthy children during the winter season. *Ann Hum Biol* **30**, 214–219.
64. Larsen ER, Mosekilde L & Foldspang A (2004) Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. *J Bone Miner Res* **19**, 370–378.
65. van der Klis FR, Jonxis JH, van Doormaal JJ, *et al.* (1996) Changes in vitamin-D metabolites and parathyroid hormone in plasma following cholecalciferol administration to pre- and postmenopausal women in the Netherlands in early spring and to postmenopausal women in Curaçao. *Br J Nutr* **75**, 637–646.
66. McCullough ML, Bostick RM, Daniel CR, *et al.* (2009) Vitamin D status and impact of vitamin D_3 and/or calcium supplementation in a randomized pilot study in the Southeastern United States. *J Am Coll Nutr* **28**, 678–686.
67. Cherniack EP, Florez HJ, Hollis BW, *et al.* (2011) The response of elderly veterans to daily vitamin D_3 supplementation of 2,000 IU: a pilot efficacy study. *J Am Geriatr Soc* **59**, 286–290.
68. Kärkkäinen M, Tuppurainen M, Salovaara K, *et al.* (2010) Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65–71 years: a 3-year randomized population-based trial (OSTPRE-FPS). *Osteoporos Int* **21**, 2047–2055.
69. Austin N, Devine A, Bruce D, *et al.* (2010) A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. *J Am Geriatr Soc* **58**, 2063–2068.
70. Heaney RP, Armas LA, Shary JR, *et al.* (2008) 25-Hydroxylation of vitamin D_3: relation to circulating vitamin D_3 under various input conditions. *Am J Clin Nutr* **87**, 1738–1742.
71. Flynn A, Hirvonen T, Mensink GB, *et al.* (2009) Intake of selected nutrients from foods, from fortification and from supplements in various European countries. *Food Nutr Res* **12**, 53.
72. Cashman K & Kiely M (2009) Author reply to Letter to editor 'Experimentally observed vitamin D requirements are higher than extrapolated one'. *Am J Clin Nutr* **90**, 1115–1116.
73. Looker AC, Johnson CL, Lacher DA, *et al.* (2011) Vitamin D status: United States, 2001–2006. *NCHS Data Brief* **59**, 1–8.
74. Whiting SJ, Langlois KA, Vatanparast H, *et al.* (2011) The vitamin D status of Canadians relative to the 2011 Dietary Reference Intakes: an examination in children and adults with and without supplement use. *Am J Clin Nutr* **94**, 128–135.
75. EuroFIR (2011) Food Composition Databases. http://www.eurofir.net/eurofir_knowledge/european_databases (accessed 25 July 2011).
76. Andersen R, Mølgaard C, Skovgaard LT, *et al.* (2005) Teenage girls and elderly women living in northern Europe have low winter vitamin D status. *Eur J Clin Nutr* **59**, 533–541.

NS British Journal of Nutrition