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Publication date	2014-04-29
Original Citation	Cashman, K. D., Kinsella, M., McNulty, B. A., Walton, J., Gibney, M. J., Flynn, A. and Kiely, M. (2014) 'Dietary vitamin D2 – a potentially underestimated contributor to vitamin D nutritional status of adults?', British Journal of Nutrition, 112(2), pp. 193-202. doi:10.1017/S0007114514000725
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1017/S0007114514000725
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Download date	2026-11-07 23:14:05
Item downloaded from	https://hdl.handle.net/10468/3733



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British Journal of Nutrition (2014), **112**, 193–202
doi:10.1017/S0007114514000725
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Dietary vitamin D_2 – a potentially underestimated contributor to vitamin D nutritional status of adults?

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(Submitted 23 October 2013 – Final revision received 4 March 2014 – Accepted 4 March 2014 – First published online 29 April 2014)

Abstract

It has been suggested that vitamin D_2 is not very prevalent in the human food chain. However, data from a number of recent intervention studies suggest that the majority of subjects had measurable serum 25-hydroxyvitamin D_2 (25(OH)D_2) concentrations. Serum 25(OH)D_2, unlike 25(OH)D_3, is not directly influenced by exposure of skin to sun and thus has dietary origins; however, quantifying dietary vitamin D_2 is difficult due to the limitations of food composition data. Therefore, the present study aimed to characterise serum 25(OH)D_2 concentrations in the participants of the National Adult Nutrition Survey (NANS) in Ireland, and to use these serum concentrations to estimate the intake of vitamin D_2 using a mathematical modelling approach. Serum 25(OH)D_2 concentration was measured by a liquid chromatography–tandem MS method, and information on diet as well as subject characteristics was obtained from the NANS. Of these participants, 78·7 % (*n* 884) had serum 25(OH)D_2 concentrations above the limit of quantification, and the mean, maximum, 10th, 50th (median) and 90th percentile values of serum 25(OH)D_2 concentrations were 3·69, 27·6, 1·71, 2·96 and 6·36 nmol/l, respectively. To approximate the intake of vitamin D_2 from these serum 25(OH)D_2 concentrations, we used recently published data on the relationship between vitamin D intake and the responses of serum 25(OH)D concentrations. The projected 5th to 95th percentile intakes of vitamin D_2 for adults were in the range of 0·9–1·2 and 5–6 μg/d, respectively, and the median intake ranged from 1·7 to 2·3 μg/d. In conclusion, the present data demonstrate that 25(OH)D_2 concentrations are present in the sera of adults from this nationally representative sample. Vitamin D_2 may have an impact on nutritional adequacy at a population level and thus warrants further investigation.

Key words: Serum 25-hydroxyvitamin D_2: Vitamin D_2: Distribution: Adults: Dietary sources

There is growing awareness about the discordance between currently identifiable sources of vitamin D and circulating 25-hydroxyvitamin D (25(OH)D) levels, which has led to the focus on the concentrations of 25(OH)D in meat and poultry as a potential additional food source of vitamin D activity[1]. We have recently provided data from a randomised controlled trial (RCT) which suggests that each microgram of orally consumed 25(OH)D_3 was about five times more effective in raising serum 25(OH)D concentrations in older adults in winter than an equivalent amount of vitamin D_3[2].

Food composition databases provide detailed information on the levels of nutritionally important components in foods and are essential for epidemiological research, public health nutrition and education, clinical practice, and the food industry[3]. Accurate food composition data underpin the analysis of dietary intake surveys by converting food consumption data to nutrient intake data; such data remain central in elucidating the role of food components in health and disease[4]. The UK nutrient databank, maintained by the Department of Health (formerly by the Food Standards Agency) and described in *McCance and Widdowson's The Composition of Foods*[5], is widely used in nutrition surveys in the UK and Ireland, and nutrient data from the UK databank are widely borrowed internationally. *The Composition of Foods* includes data on the 25(OH)D_3 content of certain foods (including meat and egg yolk) and applies a factor of 5 to generate total vitamin D activity for these foods[5]. It is not surprising, therefore, that together with the flesh of fatty fish (such as salmon, tuna and mackerel), meat and eggs are listed as meaningful sources of vitamin D_3[5,6]. In contrast, it

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; EAR, estimated average requirement; LC, liquid chromatography; LoD, limit of detection; LoQ, limit of quantification; MDI, mean daily intake; NANS, National Adult Nutrition Survey; RCT, randomised controlled trial; RMP, reference measurement LC–MS procedure.

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has been widely reported that vitamin D_2 is not very prevalent in the human food chain, with a few exceptions[6–8]. Some supplements and fortified foods can contain vitamin D_2, while mushrooms provide vitamin D_2 in variable amounts[7,8]. In recent times, mushrooms and baker's yeast with enhanced levels of vitamin D_2 have been developed with exposure to UVB light under controlled conditions[9,10], but as yet, these are not very common in the European Union.

Despite the widely held assumption that dietary exposure to vitamin D_2 is relatively limited in the population, baseline mean serum 25(OH)D_2 concentrations have been reported to be in the range of 1·5 to 3·0 nmol/l in several RCT, which have used chromatographic analytical techniques capable of delineating 25(OH)D_2 and 25(OH)D_3[11–15]. However, these RCT are generally of small sample size (typically *n* 34–40 per study), and a variety of different liquid chromatography (LC)–MS methods with likely different detection limits were used, which might have an impact on the validity of these baseline mean serum 25(OH)D_2 data. However, it is possible that serum 25(OH)D_2 concentrations in this range in the general population, if confirmed by an appropriate analytical platform, could represent nutritionally relevant intakes of vitamin D_2 (approximately 1–2·5 μg/d). Mean daily vitamin D intakes from nationally representative surveys are generally in the range of 2 to 7 μg/d[16]. Unfortunately, and unlike the case with vitamin D_3, the ability to estimate vitamin D_2 intake by traditional dietary assessment means is impeded by the fact that food composition data are extremely sparse for this vitamer[5,7].

Thus, the aim of the present study was to characterise serum 25(OH)D_2 concentrations in the participants of the recent National Adult Nutrition Survey (NANS) in Ireland, and use these serum concentrations to estimate vitamin D_2 intake using a mathematical modelling approach. Potential determinants for serum 25(OH)D_2 concentrations were also explored in an attempt to help identify the sources of dietary vitamin D_2.

Subjects and methods

Subject sampling and recruitment procedures and methods of data collection

A detailed description of the methodology used in the NANS, including the sampling procedure as well as sample recruitment, has been reported elsewhere[17,18]. Briefly, the fieldwork phase of the NANS was carried out between October 2008 and April 2010, providing a seasonal balance to the data and biological sample collection. To achieve a nationally representative sample of community-dwelling adults aged 18 years and over, a quota sampling approach was adopted using data from the 2006 Census[19]. A sample of 1500 free-living adults representing a population of approximately 4·2 million participated in the dietary survey. There were few exclusion criteria other than pregnancy/lactation and inability to complete the survey due to disability. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, University College Cork and the Human Ethics Research Committee of University College Dublin. Written informed consent was obtained from all subjects.

The analysis of demographic features revealed that the sample was representative of Irish adults with respect to age, sex, social class and geographical location when compared with the Census data[17–19]. While provision of a blood sample was not an eligibility criterion for participating in the survey, all participants were asked whether they were willing to provide a blood sample. Of the total group of respondents, 75·5 % (*n* 1132) provided a blood sample. The demographic features of the group of participants who provided a blood sample and those in the entire sample have been described elsewhere[17,18]. Seasonality was based on the date the respondents provided the blood sample (November to March (representing the 'winter' period) or April to October (representing the 'summer' period)), which is consistent with studies based on the National Health and Nutrition Examination Survey[20] and the recent analysis of the Canadian Health Measures Survey for vitamin D status[21].

The approach to food intake data collection, food quantification and estimation of vitamin D intake in the NANS has been provided in detail elsewhere[17,18]. Of note, food intake data were analysed using WISP© V3.0 (Tinuviel Software), which uses data from *McCance and Widdowson's The Composition of Foods*, sixth and fifth editions plus all nine supplemental volumes to generate nutrient intake data, as described elsewhere[17,22]. Although *The Composition of Foods* includes data on the 25(OH)D_3 content of certain foods and applies a factor of 5 to generate total vitamin D activity, it has no data on vitamin D_2 content of foods[5,7]. Information on social class, education level, smoking status, alcohol intake and medication use (including those that contain nutrients) was also collected[17,18]. Anthropometric measures including height, weight, waist and hip circumferences and measures of body composition were taken in the respondents' homes, as described previously[17]. The approach towards the assessment of the consumption of vitamin D-containing supplements has been provided elsewhere[17,18]. In brief, current supplement use was assessed by the respondents' answer to the question 'Do you take any vitamin, mineral or food supplements?', which was included in the self-administered health and lifestyle questionnaire. The respondents also entered each supplement as it was consumed into a 4 d food diary. Researchers checked the respondents who had reported using supplements in the questionnaire, which was administered at the start of the recording period, and those who entered the supplements as they were consumed into the food diary. By accounting for the contribution of vitamin D from supplemental forms, vitamin D intake data taken from all sources (food and supplements) as well as from food sources only were generated. Consumption of vitamin D-containing supplements did not distinguish whether the supplements contained vitamin D_2 or D_3. This was also the case for vitamin D-containing fortified

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foods (including ready-to-eat breakfast cereal, margarine and fat spreads, milk and yogurt).

Blood collection

Blood samples were collected by venepuncture into a vacutainer tube by a qualified nurse at designated centres within the survey area or in the respondent's home if the respondent could not travel. The samples were transported to the laboratory for further processing, and serum was stored at −80°C until required for analysis.

Analysis of serum 25-hydroxyvitamin D_2 and other 25-hydroxyvitamin D metabolites

Concentrations of total 25(OH)D (i.e. 25(OH)D_2 plus 25(OH)D_3) in serum samples were measured by the Vitamin D Research Group at University College Cork using a LC–tandem MS method. This LC–tandem MS method measures the 3-epimer of 25(OH)D_3 (3-epi-25(OH)D_3), which is not chromatographically resolved from 25(OH)D_3 by most routine LC–tandem MS methods, in addition to 25(OH)D_2 and 25(OH)D_3 in serum (see Fig. 1). The presence of 3-epimers of 25(OH)D can pose problems for LC–tandem MS methods because the mass and fragmentation patterns are the same as those for 25(OH)D, thus failure to account for these metabolites can result in the overestimation of 25(OH)D_2 and 25(OH)D_3 concentrations[23]. The present method does not measure the 3-epimer of 25(OH)D_2.

All solvents and mobile-phase additives were of MS grade and purchased from Sigma-Aldrich. Zinc sulphate was sourced from Merck, while the stable isotope-labelled D3-25(OH)D_2, D3-25(OH)D_3 and D3-3-epi-25(OH)D_3 were purchased from Isosciences. Certified calibrators for 25(OH)D_2 and 25(OH)D_3 were bought from the National Institute of Standards and Technology (SRM 2972), while a CertiMass reference standard for 3-epi-25(OH)D_3 was sourced from Isosciences. Low and high serum quality control materials were commercially available from Chromsystems. The chromatographic column was a Supelco Ascentis Express F5 available from Sigma-Aldrich.

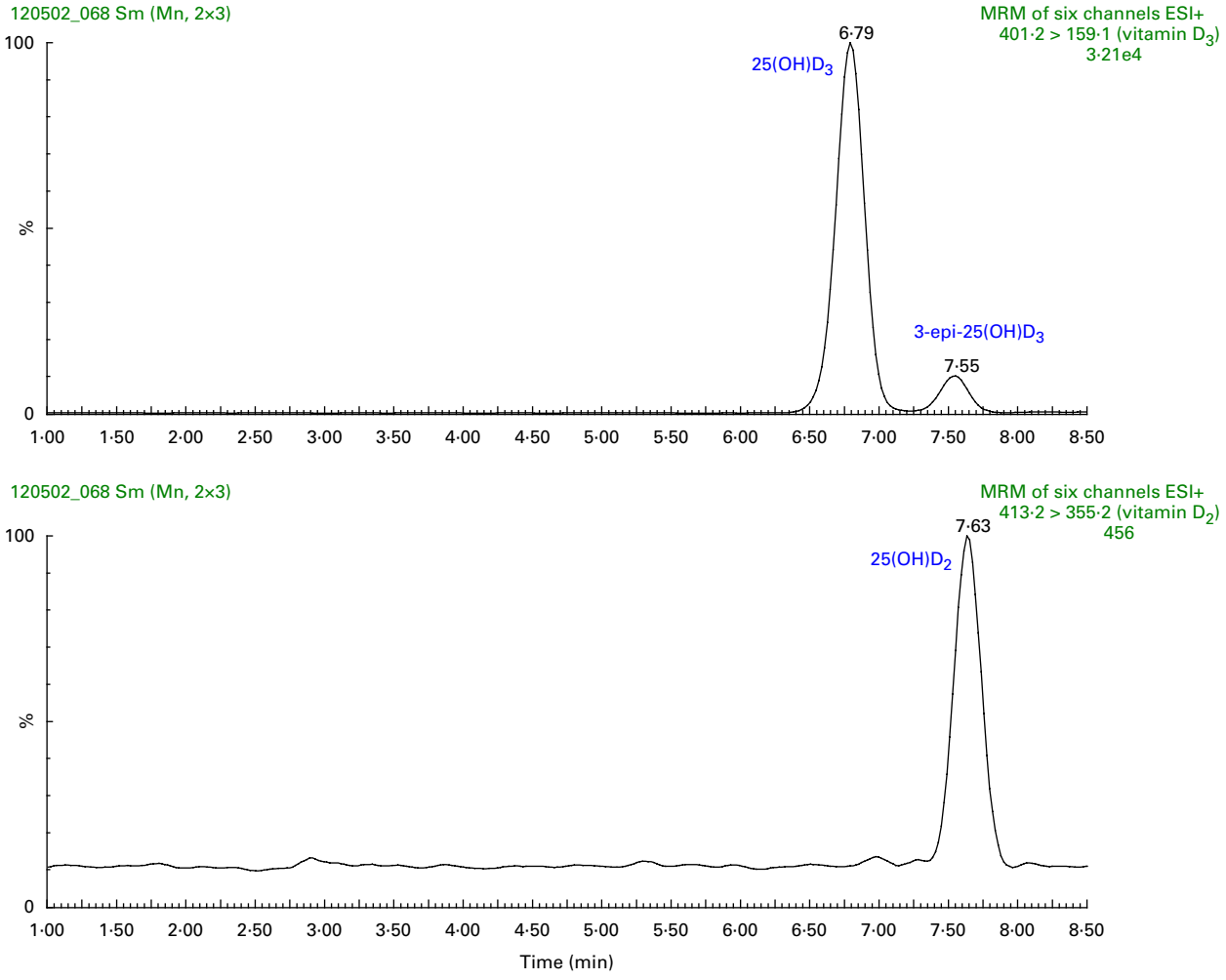


Fig. 1. Liquid chromatography–positive-ion electrospray ionisation (ESI) tandem MS chromatograms of 25-hydroxyvitamin D_3 (25(OH)D_3), 3-epimer of 25(OH)D_3 (3-epi-25(OH)D_3) and 25(OH)D_2 in a serum sample. MRM, multiple reaction monitoring. A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

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The liquid–liquid extraction method was favoured over other more complex sample extraction procedures due to its simplicity of operation and the ability to extract 100 serum samples over approximately 5–6 h. Briefly, following the addition of an internal standard to the sample and adequate mixing, an aqueous solution of zinc sulphate was added to aid in the release of analytes from vitamin D-binding proteins. Methanol was used to precipitate these proteins, while hexane was used as the extraction solvent. Following extraction, samples were dried and reconstituted in the ultra-performance LC mobile phase. Then, 20 μl of the samples were injected into the ultra-performance LC–MS/MS system (Waters Acquity UPLC (Triple Quadrupole) TQD® System; Waters). Chromatographic resolution of 25(OH)D_3 and its isobaric 3-epi-25(OH)D_3 was achieved on an Ascentis Express F5 column using an isocratic mixture of the mobile phases A and B (32:68), where A consists of 0·1 % formic acid + 2 mM-ammonium acetate in water, while B is composed of 0·1 % formic acid + 2 mM-ammonium acetate in methanol. Very good peak separation of 25(OH)D_3 and 3-epi-25(OH)D_3 was achieved under isocratic conditions at 0·45 ml/min, while a gradient with increased organic composition was applied towards the end of the 10 min chromatographic run to wash out organic materials followed by re-equilibration to initial conditions. MS detection was achieved on the Waters Acquity triple quadrupole detector through electrospray ionisation in a positive-ion multiple-reaction monitoring mode. N_2 generated from a peak scientific nitrogen generator acted as the desolvation gas, and high-purity Ar (BOC Limited) was used as the collision gas.

The ion multiple-reaction monitoring transitions were *m/z* 416·3 → *m/z* 358·2 for D3-25(OH)D_2 and *m/z* 404·3 → *m/z* 162 for D3-25(OH)D_3. The quantifier transition for both 25(OH)D_3 and 3-epi-25(OH)D_3 was *m/z* 401·3 → *m/z* 159, with *m/z* 401·3 → *m/z* 365·2 being used as a confirmatory transition. In the case of 25(OH)D_2, *m/z* 413·4 → *m/z* 355·2 was the quantifier transition, while *m/z* 413·4 → *m/z* 83 served as the qualifier ion. Commercially available Chromsystems quality control samples were extracted and analysed in parallel to the serum samples, and were strategically placed close to the beginning, middle and end of the analysis on the LC–MS/MS instrument. The inter-assay CV of the method for 25(OH)D_2 were 9·6 and 6·7 % at concentrations about 1·5–2·5 and 2·5–7·5 nmol/l, respectively, while the intra-assay CV was < 6 % at both concentrations. The limit of detection (LoD) and the limit of quantification (LoQ), with a signal: noise ratio of 10, for 25(OH)D_2 in serum were found to be 0·44 and 1·43 nmol/l, respectively, using the LC–tandem MS method by the Vitamin D Research Group at University College Cork. The quality and accuracy of serum 25(OH)D analysis using the LC–tandem MS method in our laboratory was monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital); however, this scheme was for total serum 25(OH)D and does not delineate 25(OH)D compounds. In addition, the Vitamin D Research Group is a participant in the Centre for Disease Control's Vitamin D Standardization Certification Program[24] that reports total 25(OH)D as well as 25(OH)D_2 and 25(OH)D_3 concentrations. The Vitamin D Research Group is also a participant of the Vitamin D Standardization Program (VDSP)'s inter-laboratory comparison study in which fifty especially commissioned patients' serum samples (covering a wide range of serum 25(OH)D concentrations) were analysed by each national survey laboratory as well as by two higher-order reference laboratories that assigned values for total 25(OH)D as well as for 25(OH)D_2 and 25(OH)D_3 using a reference measurement LC–MS procedure (RMP)[25]. Of the fifty serum samples analysed, seventeen had serum 25(OH)D_2 concentrations above the LoQ, which ranged from 1·8 to 19·7 nmol/l. The analysis of the relationship between serum 25(OH)D_2 concentrations using the RMP (University of Ghent)[26] and the LC–tandem MS method (University College Cork) is shown in Fig. 2. While there was a very close relationship between the two methods, the mean bias for the LC–tandem MS method was −5·1 % (using a difference plot), suggesting an underestimation on average.

Although data on the concentrations of serum total 25(OH)D (i.e. 25(OH)D_2 plus 25(OH)D_3) in the NANS have been reported previously[23], there are no data available for concentrations of serum 25(OH)D_2, which forms the basis of the present analysis.

Estimation of vitamin D_2 intake from serum 25-hydroxyvitamin D_2 distribution data and assessment of the adequacy of vitamin D intake

The ability to estimate the intake of vitamin D_2 by dietary assessment is not possible as food composition data are extremely sparse for this vitamer[5,7,17]. Therefore, we used two approaches to estimate the intake of vitamin D_2 in the NANS subsample (i.e. those whose serum 25(OH)D_2 concentrations were above the LoQ, representing 78·7 % of the entire NANS

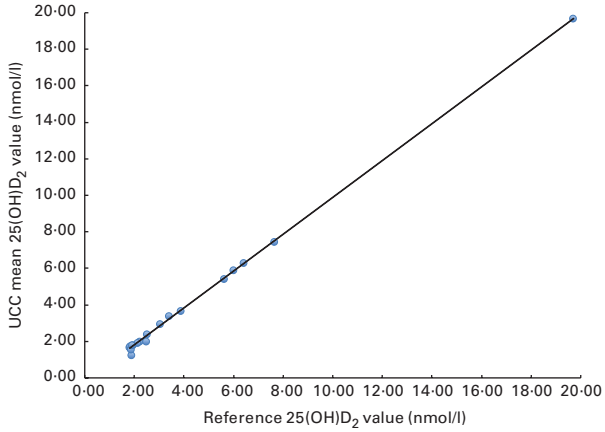


Fig. 2. Relationship between 25-hydroxyvitamin D_2 (25(OH)D_2) concentrations in the sera of seventeen patients from the inter-laboratory comparison study analysed using the reference measurement procedure at the University of Ghent (*x*-axis) and using the liquid chromatography–tandem MS method by the Vitamin D Research Group at University College Cork (UCC) (*y*-axis). $Y = X \times 1{\cdot}0083$ (95 % CI 0·9939, 1·0277) − 0·1704 (95 % CI −0·2579, −0·0827); r^2 0·999; *n* 17. A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

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sample with serum 25(OH)D_2 distribution data). The first approach used recent meta-analysis data on the relationship between vitamin D_3 intake and serum 25(OH)D concentrations during wintertime at latitudes >49·5°N[27], and assumed that the increase in serum 25(OH)D_2 concentrations arising from each microgram of vitamin D_2 ingested would be of a similar magnitude to the increase in the concentrations of serum 25(OH)D_3 per μg of vitamin D_3 ingested[13,28]. Therefore, we applied this meta-analysis estimate of a mean increment of serum 25(OH)D_3 concentrations of 1·76 (95 % CI 1·28, 2·24) nmol/l per μg of vitamin D_3 ingested[27] to that of serum 25(OH)D_2 distribution in the subsample of the NANS to calculate the approximate intake of vitamin D_2.

The second approach took account of emerging evidence that the rise in serum 25(OH)D_2 concentrations per μg of vitamin D_2 ingested may be less than that achieved in serum 25(OH)D_3 concentrations per μg of vitamin D_3 ingested[12,14,15]. Logan *et al.*[15] conducted a winter-based vitamin D supplementation study in adults, aged 18–50 years, in New Zealand at a latitude of 46°S. Subjects were supplemented with 25 μg/d of vitamin D_2 or vitamin D_3, or received a placebo, daily for 25 weeks, and serum 25(OH)D_2 and 25(OH)D_3 concentrations were measured by the LC–MS method. Compared with the placebo group at the end of winter, it can be estimated that the mean increase in serum 25(OH)D_3 concentrations per μg of supplemental vitamin D_3 was 1·72 nmol/l, which was comparable with the estimate that we applied from the meta-analysis (1·76 nmol/l). The mean increase in serum 25(OH)D_2 concentrations per μg of supplemental vitamin D_2 was lower at 1·28 (95 % CI 0·94, 1·62) nmol/l[15]. Houghton & Vieth[29], in their review of the available data and evidence, provided a very succinct overview of several biologically plausible mechanisms that could contribute to differential responses to the two vitamers, and reported that serum 25(OH)D_2 has a lower affinity for vitamin D-binding proteins and results in a shorter circulating half-life than 25(OH)D_3, and also suggested a higher affinity of hepatic 25-hydroxylase for vitamin D_3 than for vitamin D_2, and potential differences in the production of metabolites and deactivation. However, there is an ongoing debate in this area[30]. Therefore, as equivalent meta-analysis data do not exist for the response of serum 25(OH)D_2 concentrations to the intake of vitamin D_2, we used the estimate of 1·28 (95 % CI 0·94, 1·62) nmol/l per μg of vitamin D_2 ingested to calculate the intake of vitamin D_2 in the subsample of the NANS under these more conservative intake-status response conditions.

The two calculated estimates of vitamin D_2 intake arising from each individual's serum 25(OH)D_2 concentrations were added to their previously measured mean daily intake (MDI) of vitamin D (arising from vitamin D_3 and 25(OH)D_3 concentrations) in the subgroup not taking vitamin D supplements (*n* 733). These intake estimates from food sources, with and without the inclusion of vitamin D_2, were then compared against the suggested estimated average requirement (EAR) values so that percentage adequacy of intake (i.e. at or above the EAR) at the population level was estimated. We used the EAR of vitamin D for adults (10 μg/d) proposed by the Institute of Medicine[31] as well as an EAR estimate (5 μg/d) of vitamin D in adults reported previously in our RCT[32], in which we re-analysed serum 25(OH)D concentrations by the LC–tandem MS method (original analysis was carried out by the enzyme immunoassay) and estimated the intake of vitamin D required to maintain serum 25(OH)D concentrations above 40 nmol/l in half of the population during winter (KD Cashman, JY Zhang and M Kiely, unpublished results).

Data interpretation and statistical analyses

Data and statistical analyses were conducted using SPSS© version 20.0 for Windows™ (SPSS, Inc.). Descriptive statistics (frequencies, means, medians and percentiles) were generated for serum 25(OH)D_2 data. Linear regression analysis was performed to identify independent predictors of serum 25(OH)D_2 concentration. The following categorical variables were included: vitamin D-containing supplement use (coded as 0 (no supplements) and 1 (taking supplements)); vitamin D-containing fortified food use (coded as 0 (not taking) and 1 (taking)); season (coded as 0 (summer/autumn) and 1 (winter/spring)); sex (0 (female) and 1 (male)). The following continuous numerical variables were included: age (years); BMI (kg/m^2); MDI of Mg (mg/d, Mg intake has been recently suggested as a determinant of serum total 25(OH)D concentration[33]); total meat intake (g/d, which included fresh and processed meat and meat dishes such as beef, pork, lamb and poultry); dark chocolate (g/d, dark chocolate has also been shown to possess vitamin D due to the drying of cocoa beans in the sun and the associated fungal synthesis of vitamin D_2[34]); mushroom (g/d, which included raw, boiled and fried mushrooms, mushroom soup and garlic mushrooms). Statistical significance was defined at $P<0·05$.

Results

Distribution of serum 25-hydroxyvitamin D_2 concentrations in participants of the National Adult Nutrition Survey

Of the entire NANS population for which serum 25(OH)D_2 samples were available (*n* 1123), only two subjects had serum 25(OH)D_2 concentrations below the LoD, 237 subjects had concentrations between the LoD and LoQ and 884 subjects (78·7 %) had serum 25(OH)D_2 concentrations above the LoQ. The frequency distribution of serum 25(OH)D_2 concentrations in the subsample of the NANS for which serum 25(OH)D_2 samples were available is shown in Fig. 3 . In the remainder of the analysis, priority was placed on those subjects with serum 25(OH)D_2 concentrations above the LoQ, as too much uncertainty was associated with projected vitamin D_2 intake in subjects with serum 25(OH)D_2 concentrations below the LoQ but above the LoD. The mean, 10th, 50th (median) and 90th percentile values of serum 25(OH)D_2 concentrations in subjects with serum 25(OH)D_2 concentrations above the LoQ were 3·69, 1·71, 2·96 and 6·36 nmol/l, respectively, whereas the maximum serum 25(OH)D_2 concentration was 27·64 nmol/l.

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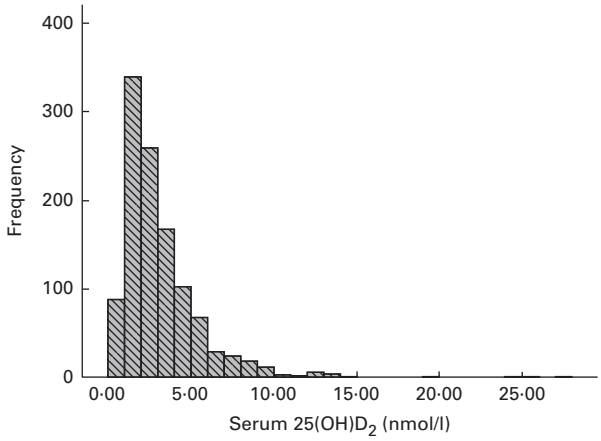


Fig. 3. Frequency distribution of 25-hydroxyvitamin D_2 ($25(OH)D_2$) concentrations present in the sera of adult participants of the National Adult Nutrition Survey (*n* 1123; mean 3·13 (SD 2·50) nmol/l).

Estimation of vitamin D_2 intake using serum 25-hydroxyvitamin D_2 distribution data

The distribution of serum $25(OH)D_2$ concentrations in the present analysis showed that vitamin D_2 is being consumed by the majority of adults in Ireland. As direct estimation of vitamin D_2 intake was not possible due to the absence of food composition data for this vitamer, we applied recent meta-analysis data on the relationship between vitamin D_3 intake and serum 25(OH)D concentrations during wintertime at latitudes >49·5°N[27], as well as data on serum $25(OH)D_2$ concentrations from a RCT with vitamin D_2 and D_3 during winter[15] to calculate the approximate intake of vitamin D_2 arising from serum $25(OH)D_2$ concentrations in the subsample of the NANS. Using the meta-analysis data and the assumption that the increase in serum $25(OH)D_2$ concentrations arising from each microgram of vitamin D_2 ingested would be of a similar magnitude to the increase in serum $25(OH)D_3$ concentrations per μg of vitamin D_3 ingested, the approximated intakes of vitamin D_2 in those who had serum $25(OH)D_2$ concentrations above the LoQ are shown in Table 1. The projected 5th to 95th percentile intakes of vitamin D_2 were in the range of approximately 0·9 to approximately 5 μg/d, with a median intake of 1·7 μg/d. Table 1 also shows the distribution of vitamin D_2 intakes calculated using the data derived from the vitamin D_2 and D_3 winter-based RCT conducted by Logan *et al.*[15], which suggested that the increase in serum $25(OH)D_2$ concentrations arising from each microgram of vitamin D_2 ingested was smaller than the increase in serum $25(OH)D_3$ concentrations per μg of vitamin D_3 ingested (1·28 and 1·72 nmol/l per μg, respectively). The projected 5th to 95th percentile intakes of vitamin D_2 were in the range of approximately 1·2 to approximately 6 μg/d, with a median intake of 2·3 μg/d.

Exploration of dietary and other determinants of serum 25-hydroxyvitamin D_2 concentrations – possible clues to the food sources of vitamin D_2

Regression analysis showed that while the overall model was significant ($P<0·0001$), it only explained about 8 % of the variance (R^2 8·3) in serum $25(OH)D_2$ distribution data. The significant positive predictors ($P<0·05$) of serum $25(OH)D_2$ concentrations within the model were age, vitamin D supplement use, season of sampling (winter serum $25(OH)D_2$ higher than that in summer) and vitamin D-fortified food use, whereas Mg intake was a borderline negative predictor ($P=0·070$), and sex, BMI, supplement use, mushroom consumption, dark chocolate consumption and total meat intakes were non-significant predictors (all $P>0·17$) (Table 2).

Table 1. Distribution of serum 25-hydroxyvitamin D_2 ($25(OH)D_2$) concentrations in adult participants of the National Adult Nutrition Survey and projected intakes of vitamin D_2 from these serum concentrations using a mathematical modelling approach*

(Projected mean values and 95 % confidence intervals)

		Projected vitamin D_2 intake (μg/d)†			
Distribution of serum $25(OH)D_2$		Assuming $D_2 = D_3$‡		Assuming $D_2 < D_3$§	
Cut-off	nmol/l	Mean	95 % CI	Mean	95 % CI
Mean	3·69	2·10	1·65, 2·88	2·88	2·28, 3·93
5th percentile	1·58	0·90	0·71, 1·23	1·23	0·98, 1·68
10th percentile	1·71	0·97	0·76, 1·34	1·34	1·06, 1·82
25th percentile	2·09	1·19	0·93, 1·63	1·63	1·29, 2·22
50th percentile	2·96	1·68	1·32, 2·31	2·31	1·83, 3·15
75th percentile	4·44	2·52	1·98, 3·47	3·47	2·74, 4·72
90th percentile	6·36	3·61	2·84, 4·97	4·97	3·93, 6·77
95th percentile	8·19	4·65	3·66, 6·40	6·40	5·06, 8·71
Maximum	27·64	15·70	12·34, 21·59	21·59	17·06, 29·40

* Data obtained from 884 subjects for whom serum $25(OH)D_2$ concentrations were above the limit of quantification.
† Based on serum $25(OH)D_2$ concentration distribution data and conversion factors (see footnotes ‡ and §).
‡ Projected intake assuming that the response of serum $25(OH)D_2$ concentrations to the intake of vitamin D_2 is equivalent to the response of total serum 25(OH)D concentrations to the intake of vitamin D_3 (applying a conversion factor of 1·76 (95 % CI 1·28, 2·24) nmol/l per μg[27]).
§ Projected intake using randomised controlled trial data suggesting that the response of serum $25(OH)D_2$ concentrations to the intake of vitamin D_2 is less than the response of serum $25(OH)D_3$ concentrations to the intake of vitamin D_3 (applying a conversion factor of 1·28 (95 % CI 0·94, 1·62) nmol/l per μg[15]).

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Table 2. Linear regression analysis of serum 25-hydroxyvitamin D_2 ($25(OH)D_2$) concentrations in adult participants of the National Adult Nutrition Survey*

(*B*-Coefficient values with their standard errors)

	B	SE	*β*	*P*
Age (years)	0·028	0·005	0·182	0·000
Vitamin D-fortified food intake	0·591	0·182	0·116	0·001
Vitamin D supplement intake	0·669	0·228	0·099	0·003
Season (sampled in winter)	0·393	0·168	0·077	0·020
Mg intake (mg/d)	−0·001	0·001	−0·065	0·070
Mushroom intake† (g/d)	−0·007	0·005	−0·045	0·168
Sex (female)	−0·213	0·188	−0·042	0·258
Dark chocolate intake (g/d)	−0·023	0·032	−0·024	0·469
Total meat intake‡ (g/d)	−0·001	0·001	−0·023	0·522
BMI (kg/m^2)	−0·002	0·014	−0·005	0·884

β, Standardised coefficient.

* Data obtained from 884 subjects for whom serum $25(OH)D_2$ concentrations were above the limit of quantification.

† Included raw, boiled and fried mushrooms, mushroom soup and garlic mushrooms.

‡ Included fresh and processed meat and meat dishes such as beef, pork, lamb and poultry.

Impact of food-based vitamin D_2 on nutritional intake and adequacy of vitamin D

To explore the impact of food-derived vitamin D_2 on the adequacy of vitamin D intake in the subsample of the NANS, the subsample was stratified by 'vitamin D supplement use' as a means of separating out any subjects potentially taking vitamin D_2 in supplemental form, even unknowingly. In addition, data on medicine use by these subjects were reviewed and no medications contained vitamin D_2. Among those not taking 'vitamin D-containing supplements' (*n* 733), the mean, 10th, 50th (median) and 90th percentile values of serum $25(OH)D_2$ concentrations were 3·55, 1·69, 2·96 and 6·19 nmol/l, respectively, whereas the maximum serum $25(OH)D_2$ concentration was 13·62 nmol/l. For the subjects taking 'vitamin D-containing supplements' (*n* 151), the mean, 10th, 50th (median) and 90th percentile values of serum $25(OH)D_2$ concentrations were 4·36, 1·78, 3·02 and 8·00 nmol/l, respectively, whereas the maximum serum $25(OH)D_2$ concentration was 27·64 nmol/l.

For each 'non-vitamin D supplement user' in the NANS subsample (*n* 733), we added the two estimates of individual vitamin D_2 intake (applying the response factors of 1·78 and 1·28 nmol/l per μg of vitamin D_2 to their individual serum $25(OH)D_2$ concentration) to their MDI of vitamin D as estimated previously[18], which would have contained no vitamin D_2. The percentage of subjects failing to achieve the EAR suggested by the Institute of Medicine for vitamin D (i.e. 10 μg/d) with no estimate of vitamin D_2 included (i.e. current MDI) was 96·3 %, and this decreased to 92·3 (95 % CI 89·2, 93·3) % when the estimate of vitamin D_2 (arising from 1·76 (95 % CI 1·28, 2·24) nmol/l per μg) was added to the MDI of each individual, and decreased further to 89·2 (95 % CI 84·5, 91·8) % when the estimate of vitamin D_2 (arising from 1·28 (95 % CI 0·94, 1·62) nmol/l per μg) was included. The percentage of subjects failing to achieve the lower EAR for vitamin D (i.e. 5 μg/d, arising from our RCT data for adults) with no estimate of vitamin D_2 included (i.e. current MDI) was 76·1 %, and this decreased to 54·9 (95 % CI 45·7, 60·9) % when the estimate of vitamin D_2 (arising from 1·76 (95 % CI 1·28, 2·24) nmol/l per μg) was added to the MDI of each individual, and decreased further to 45·7 (95 % CI 36·5, 52·9) % when the estimate of vitamin D_2 (arising from 1·28 (95 % CI 0·94, 1·62) nmol/l per μg) was included.

Discussion

To our knowledge, the present study is the first description of the distribution of serum $25(OH)D_2$ in a nationally representative sample of adults. Essentially, all subjects had serum $25(OH)D_2$ concentrations above the LoD, and 78·7 % had concentrations above the LoQ, and among these, the median serum $25(OH)D_2$ concentration was 2·96 nmol/l. Therefore, while the general assumption is that vitamin D_2 is only present in populations consuming sun-dried and UVB light-exposed mushrooms or vitamin D_2-containing supplements or fortified foods, the serum $25(OH)D_2$ data from the present nationally representative sample of Irish adults would suggest that vitamin D_2 is present in the diet of the majority of adults, at variable but possibly nutritionally relevant levels.

While, on average, serum $25(OH)D_2$ concentrations represented only 6·8 % of the total serum 25(OH)D concentrations (i.e. serum $25(OH)D_2$ plus serum $25(OH)D_3$), serum $25(OH)D_2$, unlike serum $25(OH)D_3$ and serum total 25(OH)D, is unaffected by dermal synthesis upon exposure to UVB sunlight in summer. Therefore, there is a direct relationship between the intake of vitamin D_2 and serum $25(OH)D_2$ concentrations throughout the year. Of note, serum $25(OH)D_2$ concentrations might suggest median intakes of vitamin D_2 ranging between 1·7 and 2·3 μg/d in the adult population, with a comparable median intake of vitamin D_3 of 3·1 μg/d in the same population[18]. While the intake estimates of vitamin D_3 in the NANS were derived by traditional dietary assessment methodology[18], the intake estimates of vitamin D_2 reported in the present subsample had to be derived using the serum $25(OH)D_2$ distribution data together with published data on the relationship between the intake of vitamin D_2 and serum $25(OH)D_2$ concentrations due to a paucity of food composition data on vitamin D_2. In the present study, although the two estimates used for the nmol/l increase in serum $25(OH)D_2$ concentrations per μg/d of vitamin D_2 ingested were taken from a meta-analysis[27] and an RCT in which sufficient data were available to calculate and compare the changes in two 25(OH)D metabolites upon respective supplementation with the two vitamers during winter[15], they appeared to be robust. For example, a number of RCT that were conducted at lower-latitude regions, or did not include vitamin D_3, reported a mean change in serum $25(OH)D_2$ concentrations per μg/d of vitamin D_2 ingested, in the form of supplements or fortified foods, ranging from 0·72 to 2·20 nmol/l per μg[11–15], and an average of 1·34 nmol/l per μg reported in studies that used foods[11,13,14]. A potential limitation of the modelling in the present study is that the lower-slope response estimate of serum $25(OH)D_2$ concentrations to vitamin D_2 intake was taken from a RCT that

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used 25 μg of supplemental vitamin D_2[15]. Thus, the response to naturally occurring food sources of vitamin D_2 may be different from that to supplemental vitamin D_2. However, in one study in which the response of serum 25(OH)D_2 concentrations to food-derived vitamin D_2 (via UVB-irradiated mushrooms) was compared with supplemental vitamin D_2, the authors concluded that the efficacy of conversion to 25(OH)D_2 was similar[11]. There is little information available to explore the impact of doses at lower dietary levels of vitamin D_2; however, in general, the response of total serum 25(OH)D concentrations to increasing vitamin D_3 status is less at higher doses (>25 μg/d) than at lower doses (<25 μg/d)[31]. While the debate will continue as to whether vitamin D_2 has equal[14,35] or lower potency[11,15] compared with an equal amount of vitamin D_3 in terms of raising serum total 25(OH)D concentrations and will only be resolved with further data, the present analysis used both possibilities in terms of its projections for vitamin D_2 intake in the adult population.

Regression analysis was used in the present study to explore the determinants of serum 25(OH)D_2 status, and in this way, tried indirectly to identify a select number of potential dietary sources of vitamin D_2. The present analysis showed that age and season were two significant predictors of serum 25(OH)D_2 status, and from a dietary perspective, vitamin D-containing supplement use and vitamin D-fortified foods were significant positive predictors. Sex, BMI, intake of mushrooms, Mg intake and dark chocolate consumption as well as total meat intakes were not significant determinants.

Unfortunately, the form of vitamin D in the supplements used by the participants in the NANS could not be defined, but based on product information, the majority of supplements available in Ireland contain vitamin D_3. Nevertheless, the finding that supplementation was a positive predictor of serum 25(OH)D_2 status is likely to be related to the fact that of the total of 151 subjects, an unquantified number are taking supplemental vitamin D_2. Likewise, the relative proportion of vitamin D-fortified foods containing vitamin D_3 or vitamin D_2 was not known, but the use of vitamin D-fortified foods, in general, was a significant determinant of serum 25(OH)D_2 status, presumably reflecting the fortification of some of these foods with vitamin D_2. Some manufacturers have used vitamin D_2 to cater for vegans and vegetarians. Despite vitamin D_2-containing supplements and fortified foods being the most likely dietary exposure routes, 43 % of the sample did not use vitamin D_2-containing supplements or regularly consume fortified foods (based on 4 d intake data), and thus there must be other dietary sources of vitamin D_2. The relatively simple regression analysis of the present study suggested that neither mushroom nor total meat consumption was a significant predictor of serum 25(OH)D_2 status. In relation to mushrooms, this is not surprising in light of the very low vitamin D_2 content of cultivated mushrooms (<0·1 μg/100 g fresh weight[6,8]), which are grown indoors without the benefit of UV light.

While rarely referred to as potential dietary sources of vitamin D_2, beef, pork, lamb and chicken could potentially contribute vitamin D_2 as well as possibly 25(OH)D_2. The content of vitamin D in the food of animal origin depends on what the animal has been fed. In relation to animal feed, Article 9t (b) of Council Directive 70/524/EEC allows a maximum content of vitamin D per kg of complete feeding stuff for beef cattle, pigs, lambs and chickens and other fowls for fattening, and make no distinction between vitamin D_2 or vitamin D_3[36]. Vitamin D_2 consumed by these animals will also be converted into 25(OH)D_2, and while not known to be the case for 25(OH)D_2, recent data from an RCT suggest that each microgram of orally consumed 25(OH)D_3 was about five times more effective in raising serum 25(OH)D concentrations in older adults in winter than an equivalent amount of vitamin D_3[2]. In addition, vitamin D_2 can be found in plants (including grasslands and hay) contaminated with fungi, which can then synthesis vitamin D_2 when exposed to sunlight[37]. Interestingly, Jäpelt *et al.*[38] recently showed that the ergosterol and vitamin D_2 contents of perennial ryegrass increased by a factor of 10 during the summer season, which they suggested, based on principal component analysis, was linked to conditions such as sun/temperature and precipitation. Thus, animals grazing during summer may have exposure to vitamin D_2. However, the seasonal effect in the present regression analysis suggested higher concentrations in winter months than that during summer months (e.g. median serum 25(OH)D_2 concentrations: January–March, 2·96 nmol/l; July–September, 1·94 nmol/l), possibly pointing to vitamin D_2 arising from wintertime use of animal feeds and/or vitamin D_2 from yeast contamination in stored fodder. The finding that meat consumption *per se* did not show up as a significant predictor of serum 25(OH)D_2 concentrations in the present analysis may relate to the way the data on meat consumption were used. Meat consumption included all fresh, processed and meat dishes, which included beef, pork, lamb and poultry, and in this way, may not have been discriminating enough and may require a more detailed analysis by type of meat.

Irrespective of individual dietary sources, the serum 25(OH)D_2 distribution data suggest that vitamin D_2 is ubiquitous in the diet. Inclusion of the projected vitamin D_2 intake estimates in the total vitamin D intake estimates lowered the percentage inadequacy by about 4 and 7 percentage points, although over 89 % still had intakes below the EAR of 10 μg/d suggested by the Institute of Medicine[31]. These trends may take on more significance if one accepts that the EAR may be closer to 5 μg/d; in which case, percentage inadequacy dropped from 76·1 to 45·7–54·9 %. In this context, dietary-derived vitamin D_2 could play an important role in ensuring vitamin D nutritional adequacy in the population, and is possibly quantitatively much more important than 25(OH)D in food. This potential underestimation of total vitamin D intake would also have implications for the vitamin D intake–serum 25(OH)D response relationship, which is so fundamental to the estimates of dietary requirements for vitamin D.

In conclusion, the present data not only demonstrate the presence of 25(OH)D_2 concentrations in the sera of adults in this nationally representative sample, but they further highlight an important research requirement flagged by our study group recently[39], i.e. investment in the provision of

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quality food composition data for vitamin D_3, vitamin D_2, 25(OH)D_2 and 25(OH)D_3 in order to support the assessment of vitamin D intake in national surveys and research in nutrition and health. Vitamin D_2 may have an impact on nutritional adequacy at a population level and thus warrants further investigation.

Acknowledgements

The authors acknowledge the support of the Higher Education Authority under its Programme for Research in Third Level Institutions Cycle 4 (Food Ireland: National Food and Health Collaborative Research Programmes) for funding the LC–tandem MS, and Dr Mike Kinsella. The Vitamin D Research Group at University College Cork is a partner in the VDSP, which is coordinated by the Office of Dietary Supplements, National Institutes of Health.

The present study was funded by the Irish Department of Agriculture, Food and the Marine under its Food for Health Research Initiative (2007-12).

The authors' contributions were as follows: M. J. G., K. D. C. and A. F. were involved in the conception of work and were grant holders; B. A. M., J. W., M. J. G. and A. F. contributed to the study design and execution of the study; M. Kinsella, M. Kiely and K. D. C. contributed to the sample analysis. All authors contributed to the data analysis and the writing of the manuscript.

None of the authors has a conflict of interest to declare.

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