Vitamin D₃ supplementation in healthy adults: a comparison between capsule and oral spray solution as a method of delivery in a wintertime, randomised, open-label, cross-over study

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Abstract

Vitamin D is typically supplied in capsule form, both in trials and in clinical practice. However, little is known regarding the efficacy of vitamin D administered via oral sprays – a method that primarily bypasses the gastrointestinal absorption route. This study aimed to compare the efficacy of vitamin D₃ liquid capsules and oral spray solution in increasing wintertime total 25-hydroxyvitamin D (25(OH)D) concentrations. In this randomised, open-label, cross-over trial, healthy adults (n=22) received 3000 IU (75 µg) vitamin D₃ daily for 4 weeks in either capsule or oral spray form. Following a 10-week washout phase, participants received the opposite treatment for a final 4 weeks. Anthropometrics and fasted blood samples were obtained before and after supplementation, with samples analysed for total 25(OH)D, creatinine, intact parathyroid hormone and adjusted Ca concentrations. At baseline, vitamin D sufficiency (total 25(OH)D >50 nmol/l), insufficiency (31–49 nmol/l) and clinical deficiency (<30 nmol/l) were evident in 59, 23 and 18% of the participants, respectively. Overall, baseline total mean 25(OH)D concentration averaged 59·76 (sd 29·88) nmol/l, representing clinical sufficiency. ANCOVA revealed no significant difference in the mean and standard deviation change from baseline in total 25(OH)D concentrations between oral spray and capsule supplementation methods (26·15 (sd 17·85) vs. 30·38 (sd 17·91) nmol/l, respectively; F=1·044, adjusted r² 0·493, P=0·313). Oral spray vitamin D₃ is an equally effective alternative to capsule supplementation in healthy adults.

Key words: Oral spray: Capsules: Vitamin D: Supplementation: Cross-over study: Comparative effectiveness

Epidemiological studies have revealed that vitamin D insufficiency and deficiency, defined as total 25-hydroxyvitamin D (25(OH)D) concentrations <50 and 30 nmol/l, respectively, are endemic worldwide(¹,²). Such findings have led to significant investment in vitamin D research with many exploring the impact of vitamin D supplementation on skeletal health as well as potential extra-skeletal outcomes(³–⁶). Scientists investigating the pleotropic role of vitamin D in randomised-controlled trials often use capsules or tablets as a peroral method of nutrient delivery(⁷,⁸). However, despite being commercially available, little is known regarding the efficacy of oral spray vitamin D, which is primarily absorbed at the buccal, sublingual and palatal membranes in the oral cavity rather than the gastrointestinal tract(⁸). Emerging evidence also suggests that oral spray vitamin D may provide an accelerated route of absorption compared with capsules and may be advantageous in those with gastrointestinal malabsorption(⁹). Owing to the lipophilic nature of vitamin D, oral sprays containing this micronutrient typically contain a TAG carrier substance as well as solubilising excipients such as α-tocopherol and oleic acid, which promote passive absorption of the micro-emulsified solution into systemic circulation(⁹). This is achieved through dispersion across capillary beds in the oral submucosa(¹¹). Following entry into systemic circulation, vitamin D (including both ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃) compounds) is bound to vitamin D-binding proteins and is transported to the liver where it undergoes hydroxylation, catalysed by 25-hydroxylase. This process forms the biomarker of vitamin D status, 25(OH)D, which is subsequently hydroxylated into the biologically active vitamin D metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D) in the kidneys and by cells elsewhere that also express 1α-hydroxylase(¹²). Such cells are present throughout the

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.

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body including sites such as the skeleton, prostate and immune system.(15) It is 1,25(OH)2D that governs vitamin D-related mechanisms of action by binding to the vitamin D receptor, which has been identified in an array of cell types.(14) Indeed, researchers have compared the efficacy of vitamin D injections, tablets and capsules at increasing total 25(OH)D concentrations(15,16). Yet, to our knowledge, no study to date has directly compared the efficacy of vitamin D3 supplements in a Western population residing at a northerly latitude. Therefore, the aim of this study was to compare the efficacy of two forms of vitamin D3 supplements – liquid capsules and oral spray solution – at increasing total 25(OH)D concentrations during wintertime in healthy adults.

Methods
Study overview
This randomised, open-label, two-period, cross-over study was conducted at the University of Ulster Coleraine at a latitude of 55°N during wintertime when vitamin D synthesis is minimal at this latitude (October 2015 to March 2016). The study was approved by the University of Ulster Research Ethics Committee (REC/15/0083), registered at www.clinicaltrials.gov (NCT02608164) and was conducted in accordance with the declaration of Helsinki. The protocol comprised two 4-week interventions that were separated by a 10-week washout period, Fig. 1. Washout length was based on the US Food and Drug Administration guidelines, which state that a washout 5x the plasma half-life of the measured substance is required to achieve over 95% elimination from the body, and on evidence that the plasma half-life of total 25(OH)D is approximately 2 weeks.(17–19)

Subjects
A total of twenty-two, healthy adults (males n 10 and females n 12) were recruited from the university and local area through circular emails and online advertisements. Participants completed a screening questionnaire and were provided with an information sheet before enrolment. Inclusion criteria were as follows: aged over 18 years and apparently healthy. Exclusion criteria were as follows: intending to consume a supplement containing vitamin D at any point during the study, currently taking medication(s) known to influence vitamin D metabolism (calcium-channel blockers, anticonvulsants, cardiac glycosides, thiazide diuretics, isoniazid, statins, active vitamin D metabolites/calcitriol, laxatives (regular/continued use)), those following a vegan diet, sun bed users and those planning a sun holiday at any point during the study. Informed consent was obtained at the first appointment. All appointments took place at either the Human Intervention Studies Unit at the University of Ulster, Coleraine, or the Northern Ireland Clinical Research Facility in Belfast City Hospital.

Supplements and compliance
The order in which vitamin D3 oral sprays or capsules were provided was determined by the clinical trials manager using MINIM randomisation software with an allocation ratio of 1:1.(20) Participants were asked to consume their respective supplements at the same time each day (in the morning before breakfast). Those allocated to sequence allocation one received an oral spray solution containing 3000 IU (75 µg) vitamin D3 spray, and were instructed to self-administer a single spray targeting the buccal membrane on a daily basis for a period of 4 weeks. Those allocated to sequence allocation two were instructed to consume three 1000 IU (25 µg) vitamin D3 capsules/d with water for a period of 4 weeks. Following the washout period, participants completed a final 4-week supplementation phase following the opposite treatment. Capsules were provided in pill boxes to aid compliance. The vitamin D3 contents of a single oral spray bottle solution from the supplied batch and 50 g of capsule matrix were confirmed by an independent laboratory using HPLC. The oral spray solution tested contained 75 (sd 7.5) µg vitamin D3/spray, and the capsules contained 25 (so 5) µg D3/capsule. The 3000 IU (75 µg) daily dose chosen was below the 4000 IU (100 µg) daily tolerable upper limit for vitamin D specified by the European Food Safety Authority.(21) Participants were asked to return pill boxes and oral spray bottles at the end of each supplementation phase to enable estimation of compliance. Percentage compliance to capsule supplementation was determined by capsule counting after intervention and by dividing the actual number of days on intervention by the expected number of days and multiplying by a factor of 100. The method used to calculate percentage compliance to oral spray supplementation is described elsewhere.(22)

Blood collection and processing
Participants were recruited to fast from 22.00 hours the night before blood sampling and were encouraged to drink water as usual. Blood samples were obtained from the antecubital vein by a trained phlebotomist. Samples were processed within 1 h of collection. Following inversion, serum samples were allowed to clot for up to 60 min, and plasma samples were placed under refrigeration until centrifugation. Tubes were centrifuged at 2200 rpm for 15 min at 4°C. Separated fractions of serum and plasma were then transferred into 0.5-ml aliquots and stored at −80°C until further analysis.

Blood analysis
Total serum 25(OH)D concentrations (25(OH)D2 plus 25(OH)D3) were measured by liquid chromatography tandem MS (LC-MS/MS) using a commercially available kit (API 4000; AB SCIEX, Chromsystems Instruments and MassChrom 25-OH-vitamin D3/D2; Chromsystems Instruments and Chemicals GmbH). Vitamin D analysis was conducted at the Biochemistry Department of St. James’ Hospital, Dublin. This laboratory is fully accredited to the ISO 15189 standard and complies with the Vitamin D External Quality Assessment Scheme and use of the National Institute of Standards and Technology 972 vitamin D standard reference material. The respective inter- and intra-assay CV were 6.5 and 7.5%, respectively. Intact parathyroid hormone (PTH) concentrations were measured in duplicate using a
commercially available ELISA (MD Biosciences Inc.). The intra- and inter-assay CV were 4.52 and 6.18%, respectively. Serum Ca, albumin and creatinine concentrations were quantified, in duplicate, using an Ilab 650 clinical chemistry analyser (Instrumentation Laboratory). The intra-assay CV were 1.11, 0.80 and 1.19%, respectively. The following equation was applied to total Ca and albumin concentrations to account for protein-bound Ca: adjusted Ca = 0.044 + total Ca × (40 − albumin)\(^{24}\), with adjusted Ca concentrations used in analyses thereafter. To confirm healthy renal function, the Modification of Diet in Renal Disease equation\(^{25}\) was used in order to obtain estimated glomerular filtration rate from creatinine concentrations.

**Dietary vitamin D intake**

Participants completed a validated vitamin D FFQ to estimate habitual dietary vitamin D intake on one occasion, owing to the minimal contribution of dietary vitamin D to overall vitamin D status in the Western diet\(^{25}\). Researchers asked participants a series of questions regarding their consumption of foods containing vitamin D, and a food atlas was used to estimate portion sizes\(^{26}\).

**Statistical analysis**

An *a priori* power calculation with a two-sided significance level of 5% and power at 80% concluded that a total of twenty-two participants were required to observe a significant 9.4 nmol/l difference in the total 25(OH)D concentration between two different vitamin D\(_3\) supplementation strategies (GPower, version 3.1)\(^{16,27}\). This figure was inclusive of an estimated 40% dropout rate. All further statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, version 22.0; IBM Corp.), with significance set at *P* < 0.05. Normality of data was assessed using the Shapiro–Wilk test. Age and PTH concentrations were skewed, and therefore transformed using the logarithmic function to achieve a more normal distribution before further analysis. Missing data were subject to intention-to-treat (ITT) analysis in line with the Consolidated Standards for Reporting Trials guidelines\(^{28}\). As such, statistical analyses included all participants randomised at baseline (n 22). As data were deemed to be missing completely at random, ITT consisted of forty imputed data sets with minimum and maximum value constraints pre-specified using *per protocol* data. An overview of imputed data is provided in Fig. 1. Comparisons between sequence allocations at baseline were made using an independent sample *t* test. Potential carryover effects were ruled out using a paired *t* test that compared total 25(OH)D concentration at baseline and at the beginning of the second supplementation phase. Following this, a time-by-treatment interaction was ruled out using an independent *t* test that compared overall change in total 25(OH)D concentration according to sequence allocation. Data from both sequence allocations were then pooled into a single database, and the effect of oral spray *v.* capsule vitamin D\(_3\) supplementation on total 25(OH)D concentration was tested using ANCOVA controlling for pre-intervention total 25(OH)D concentration. Magnitude of change in total 25(OH)D concentration was calculated as the percentage change from baseline by dividing the change in total 25(OH)D concentration during the intervention by baseline concentration and multiplying by a factor of 100.

**Results**

The participant flow is detailed in Fig. 1. Overall, four participants did not complete the trial as a result of sun holidays (n 2), illness unrelated to the intervention (n 1) and undisclosed reasons (n 1). Among participants who returned their oral spray bottle (n 16) and pill boxes (n 19), the average compliance to both interventions exceeded 80%. Nevertheless, two participants did not respond to oral spray vitamin D supplementation, despite >80% compliance, and were considered outliers. Oral spray supplementation phase data for these participants were therefore included in ITT. At baseline, vitamin D sufficiency (>50 nmol/l), insufficiency (31–49 nmol/l) and clinical deficiency (<30 nmol/l) were evident in 59, 23 and 18% of the participants, respectively. Overall, baseline total mean 25(OH)D concentrations averaged 59·76 (SD 29·88) nmol/l, representing clinical sufficiency, whereas dietary vitamin D intake averaged 6·25 (SD 6·24) µg/d. Baseline characteristics of the participants in each sequence allocation are provided in Table 1. There was no evidence of a carryover effect from the first supplementation phase with respect to mean total 25(OH)D concentration (59·76 ± 29·88 nmol/l (baseline)) *v.* 59·90 (SD 19·86) nmol/l (end of washout), *P* = 0·977). There was also no difference in the response to vitamin D\(_3\) supplementation according to sequence allocation (32·70 (SD 16·15) nmol/l (sequence allocation 1) *v.* 23·82 (SD 18·62) nmol/l (sequence allocation 2), *P* = 0·098). Participant characteristics before and after supplementation with vitamin D\(_3\) capsules or oral spray solution are presented in Table 2. ANCOVA revealed no significant difference in the mean change from baseline in total 25(OH)D concentrations between oral spray and capsule supplementation methods (26·15 (SD 17·85) *v.* 30·38 (SD 17·91) nmol/l, respectively (*F* = 1·044, adjusted *r*\(^2\) 0·493, *P* = 0·313). Use of ITT did not change the study outcome when compared with per protocol analysis (*F* = 4·709; *r*\(^2\) 0·476, *P* = 0·329). The percentage change from baseline in total 25(OH)D concentration for oral spray and capsule interventions was +44 and +51%, respectively. There was no evidence of hypercalcaemia (>2·2 mmol/l) in response to intervention, highlighting the safety of the dose and duration provided.

**Discussion**

This randomised, open-label, cross-over study has revealed, for the first time in healthy Western adults residing at a northerly latitude (55°N), that vitamin D\(_3\) supplied in oral spray form is equally effective at raising total 25(OH)D concentrations when compared with capsule supplementation. Our findings therefore advocate the use of oral spray vitamin D\(_3\) as a suitable alternative, if desired, to capsule supplementation in the general population. There is a lack of comparable studies; however, a recent cross-over trial that compared oral spray and capsule
vitamin D3 supplementation (1000 IU (25 µg) daily for 4 weeks) in healthy Indian adults (assigned to oral spray, n 7; capsules, n 7; control, n 6) and patients with gastrointestinal malabsorption (assigned to oral spray, n 7; capsules, n 7; control, n 6) found that oral spray supplementation was superior to capsules in both healthy and patient population groups, contrasting with the results of the current study. Although Satia et al. employed a washout phase with only 2X the plasma half-life of 25(OH)D and did not account for sunlight exposure in their statistical analyses, these factors were found to be unlikely to account for the above-mentioned difference between studies, as total 25(OH)D concentrations returned to baseline concentrations.
following washout and remained stable in the control group throughout the study. The magnitude of change in total 25(OH)D concentration (mean percentage increase from baseline) was similar between the current study and the findings of Satia et al. for oral spray supplementation (+44 v. +43%, respectively), but this was not the case for capsule supplementation (+51, v. +22%, respectively). The permeability and absorption potential of the gastrointestinal tract are known to vary according to an individual’s geographical location, with Asians exhibiting lower absorption and membrane permeability than Europeans(29). Although the exact mechanism responsible for this disparity is yet to be elucidated, it is possible that this phenomenon may explain why Satia et al. found the oral spray to be more effective than capsules at increasing total 25(OH)D concentrations and why their finding was not replicated in the current study. Furthermore, genetic variation between cohorts may have contributed to differences in study outcomes, as there is growing evidence of ethnic differences in the frequency of VDR polymorphisms known to impact vitamin D metabolism(30).

Our findings demonstrate that oral spray vitamin D₃ is just as effective as capsule supplementation at increasing total 25(OH)D concentrations in the healthy, adult population. Nevertheless, the ability of oral spray vitamin D₃ to bypass the intestinal absorption route may well prove superior for those with gastrointestinal malabsorption syndromes and for individuals with difficulty swallowing such as the elderly, young children and babies(25-31). It is important to recognise that, irrespective of the route of absorption, both oral spray and capsule-based vitamin D₃ must first undergo hepatic hydroxylation before forming 25(OH)D, which is detected by LC-MS/MS(32). As such, in those with malabsorption syndromes, any potential long-term benefit of oral spray supplementation over capsules on total 25(OH)D concentrations would likely be derived from enhanced absorption rather than as a result of faster entry of vitamin D₃ into systemic circulation. This concept is supported by the similar extent to which both oral spray and capsule supplementation methods raised total 25(OH)D concentrations in the current study. Additional well-designed, cross-over trials are required in order to elucidate the potential benefits of oral spray vitamin D in patients with gastrointestinal malabsorption.

The low dietary vitamin D intake reported in this study is comparable with numerous other studies conducted across Ireland and is a result of limited dietary sources that are not readily consumed(22-24). The Scientific Advisory Committee on Nutrition(33) recently proposed a vitamin D recommended nutrient intake of 10 µg/d for the entire UK population. However, 86% of the participants in this study failed to meet this recommendation, thus reinforcing the important role of safe summertime UVB exposure and effective wintertime supplementation strategies in optimising vitamin D status.

Strengths of this study include the use of an adequate washout phase, independent vitamin D content verification of supplements, inclusion of male and female participants and rigorous statistical analysis that accounted for baseline total 25(OH)D concentrations. However, it remains unknown how oral spray and capsule vitamin D₃ supplementation methods compare over longer-term interventions exceeding 4 weeks in

Table 1. Baseline participant characteristics by sequence allocation (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Measures</th>
<th>Sequence allocation</th>
<th>Capsules (n 22)</th>
<th>Oral spray (n 22)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>23.0</td>
<td>23.0</td>
<td>0.157</td>
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<tr>
<td>Height (cm)</td>
<td>Mean</td>
<td>168.3</td>
<td>168.3</td>
<td>0.427</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean</td>
<td>67.4</td>
<td>67.4</td>
<td>0.166</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean</td>
<td>23.4</td>
<td>23.4</td>
<td>0.177</td>
</tr>
<tr>
<td>Total 25(OH)D (nmol/l)</td>
<td>Mean</td>
<td>62.4</td>
<td>62.4</td>
<td>0.666</td>
</tr>
<tr>
<td>Adjusted Ca (mmol/l)</td>
<td>Mean</td>
<td>2.3</td>
<td>2.3</td>
<td>0.114</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>Mean</td>
<td>43.5</td>
<td>43.5</td>
<td>0.647</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>Mean</td>
<td>92.7</td>
<td>92.7</td>
<td>0.608</td>
</tr>
</tbody>
</table>

25(OH)D: 25-hydroxyvitamin D; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate.
* Difference between sequence allocation values at baseline compared using an independent t test.

Table 2. Participant characteristics before and after supplementation with vitamin D₃ capsules or oral spray solution (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Measures</th>
<th>Capsules (n 22)</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>25.2</td>
<td>25.2</td>
<td>0.329</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean</td>
<td>71.5</td>
<td>71.0</td>
<td>0.578</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>Mean</td>
<td>24.4</td>
<td>24.2</td>
<td>0.574</td>
</tr>
<tr>
<td>Total 25(OH)D (nmol/l)</td>
<td>Mean</td>
<td>60.0</td>
<td>90.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>Adjusted Ca (mmol/l)</td>
<td>Mean</td>
<td>2.2</td>
<td>2.2</td>
<td>0.783</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>Mean</td>
<td>50.3</td>
<td>52.2</td>
<td>0.373</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>Mean</td>
<td>91.0</td>
<td>92.1</td>
<td>0.347</td>
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<table>
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<tr>
<th>Measures</th>
<th>Oral spray solution (n 22)</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>P†</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>25.2</td>
<td>25.2</td>
<td>1.000</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean</td>
<td>70.9</td>
<td>70.8</td>
<td>0.747</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>Mean</td>
<td>24.2</td>
<td>24.2</td>
<td>0.649</td>
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<tr>
<td>Total 25(OH)D (nmol/l)</td>
<td>Mean</td>
<td>59.6</td>
<td>58.5</td>
<td>0.001*</td>
</tr>
<tr>
<td>Adjusted Ca (mmol/l)</td>
<td>Mean</td>
<td>2.2</td>
<td>2.2</td>
<td>0.666</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>Mean</td>
<td>52.1</td>
<td>48.2</td>
<td>0.475</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>Mean</td>
<td>90.8</td>
<td>88.4</td>
<td>0.173</td>
</tr>
</tbody>
</table>

25(OH)D: 25-hydroxyvitamin D; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate.
* Significantly different from the pre-intervention mean, P < 0.001.
† Difference between pre-intervention v. post-intervention values tested using a paired t test.
Vitamin D3 is indeed found to be advantageous over capsules in malabsorption. If our results were confirmed, this novel non-invasive alternative to injections, and therefore lower patient administration burden.

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J. J. T., E. M. M., L. K. P., S. M. M. and P. J. M. designed the study. J. J. T. conducted the study, analysed the data and wrote the paper. E. L. and M. H. conducted the laboratory analysis. All the authors read and approved the final manuscript, and P. J. M. had responsibility for the final content.

The authors have no further potential conflicts of interest to declare in relation to this article.

References