BRITISH JOURNAL of NUTRITION

CAMBRIDGE UNIVERSITY PRESS

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

Journal:	*British Journal of Nutrition*
Manuscript ID	BJN-RA-16-0837.R1
Manuscript Type:	Research Article
Date Submitted by the Author:	25-Aug-2016
Complete List of Authors:	Todd, Joshua; University of Ulster, NICHE: Northern Ireland Centre for Food and Health McSorley, Emeir; University of Ulster, NICHE: Northern Ireland Centre for Food and Health Pourshahidi, Laura; University of Ulster, NICHE: Northern Ireland Centre for Food and Health Madigan, Sharon; Irish Inst. of Sport, Sports Campus Ireland Laird, Eamon; Trinity College Dublin , Inst. of Molecular Medicine Healy, Martin; St. James Hospital, Dept. of Medicine Magee, Pamela; University of Ulster, NICHE: Northern Ireland Centre for Food and Health
Keywords:	Oral spray, Capsules, vitamin D, Supplementation, Comparitive effectiveness
Subject Category:	Human and Clinical Nutrition

Title: Vitamin D_3 supplementation in healthy adults: a comparison of capsule and oral spray solution as a method of delivery in a wintertime randomised, open-label crossover study.

Author names: Joshua J Todd[1], Emeir M McSorley[1], L Kirsty Pourshahidi[1], Sharon M Madigan[2], Eamon Laird[3], Martin Healy[4] and Pamela J Magee[1].

Author affiliations and contributions:

[1] Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, United Kingdom

[2] Irish Institute of Sport, Sports Campus Ireland, Dublin 15, Ireland

[3] School of Biochemistry and Immunology, Trinity College, Dublin 2, Ireland

[4] Department of Biochemistry, Central Pathology Laboratory, St. James's Hospital, Dublin 8, Ireland

JJ Todd, EM McSorley, LK Pourshahidi, SM Madigan and PJ Magee designed the research. JJ Todd conducted the research, analysed data and wrote the paper. E Laird and M Healy conducted laboratory analysis. All authors read and approved the final manuscript and PJ Magee had responsibility for final content.

Corresponding author: Pamela J Magee, Northern Ireland Centre for Food and Health, University of Ulster Coleraine, Londonderry, United Kingdom, BT52 1SA; pj.magee@ulster.ac.uk; Tel. +44 28 70124360.

Key words:

Oral spray, capsules, vitamin D, supplementation, crossover, comparative effectiveness

Running head: Oral spray versus capsule vitamin D_3

Clinical Trial Registration: NCT02608164 www.clinicaltrials.gov

Abstract

Vitamin D is typically supplied in capsule form, both in trials and clinical practice. Yet little is known regarding the efficacy of vitamin D administered via oral spray; a method that primarily bypasses the gastrointestinal absorption route. This study aimed to compare the efficacy of vitamin D_3 liquid capsules and oral spray solution, at increasing wintertime total 25-hydroxyvitamin D [25(OH)D] concentrations. In this randomised, open-label crossover trial, healthy adults (n=22) received 3000IU (75µg) vitamin D_3 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 pre and post-supplementation, with samples analysed for total 25(OH)D, creatinine, intact parathyroid hormone and adjusted calcium concentrations. At baseline, vitamin D sufficiency [total 25(OH)D >50nmol/L], insufficiency (31-49nmol/L) and clinical deficiency (<30nmol/L) was evident in 59%, 23% and 18% of participants respectively. Overall, baseline mean ± SD total 25(OH)D concentration averaged 59.76±29.88nmol/L, representing clinical sufficiency. Analysis of covariance revealed no significant difference in the mean ± SD change from baseline in total 25(OH)D concentration between oral spray and capsule supplementation methods (26.15±17.85 versus 30.38±17.91nmol/L respectively (F=1.044, adjusted r^2=0.493, P=0.313)). Oral spray vitamin D_3 is an equally effective alternative to capsule supplementation in healthy adults.

Introduction

Epidemiological studies have revealed that vitamin D insufficiency and deficiency, defined as a total 25-hydroxyvitamin D [25(OH)D] concentration below 50 and 30nmol/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 [4, 7]. 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 to 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 triglyceride 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 [10]. This is achieved through dispersion across capillary beds in the oral submucosa [11]. Following entry into systemic circulation, vitamin D [including both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) compounds] is bound to vitamin D binding proteins and transported to the liver where it undergoes hydroxylation, catalysed by 25-hydroxylase. This process forms the biomarker of vitamin D status, 25(OH)D, that 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 body including sites such as the skeleton, prostate and immune system [13]. It is $1,25(OH)_2D$ that governs vitamin D-related mechanisms of action through binding to the vitamin D receptor which has been identified in an array of cell types

47 [14]. Indeed, researchers have compared the efficacy of vitamin D injections, tablets and capsules at
48 increasing total 25(OH)D concentration [15, 16]. Yet to our knowledge no study to date has directly
49 compared the total 25(OH)D response between oral spray and capsule vitamin D_3 supplementation
50 in a Western population residing at a northerly latitude. Therefore, the aim of this study was to
51 compare the efficacy of two forms of vitamin D_3 supplement; liquid capsules and oral spray
52 solution, at increasing total 25(OH)D concentrations during wintertime in healthy adults.

53 **Materials and methods**

54 Study overview

55 This randomised, open-label, two-period crossover study was conducted at the University of Ulster
56 Coleraine at a latitude of 55º N during wintertime when vitamin D synthesis is minimal at this
57 latitude (October 2015 to March 2016). The study was approved by the University of Ulster
58 Research Ethics Committee (REC/15/0083), registered at www.clinicaltrials.gov (NCT02608164)
59 and was conducted in accordance to the declaration of Helsinki. The protocol comprised two 4-
60 week interventions that were separated by a 10-week washout period, **Figure 1**. Washout length
61 was based upon the United States Food and Drug Administration (FDA) guidelines, which state
62 that a washout 5x the plasma half-life of the measured substance is required to achieve over 95%
63 elimination from the body, and evidence that the plasma half-life of total 25(OH)D is
64 approximately 2-weeks [17-19].

65 Subjects

66 A total of 22 healthy adults (males *n*=10 and females *n*=12) were recruited from the university and
67 local area through circular e-mails and online advertisements. Participants completed a screening
68 questionnaire and were provided with an information sheet prior to study enrollment. Inclusion

criteria consisted of being over 18 years of age 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 / calcitonin, 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 D_3 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 supplement at the same time each day (in the morning prior to breakfast). Those allocated to sequence allocation one received an oral spray solution containing 3000IU (75µg) vitamin D_3, per 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 1000IU (25µg) vitamin D_3 capsules per day with water for a period of 4 weeks. Following the washout period, participants completed a final 4-week supplementation phase on the opposite treatment. Capsules were provided in pill boxes to aid compliance. The vitamin D_3 content of a single oral spray bottle solution from the supplied batch and 50g of capsule matrix were confirmed by an independent laboratory using high-performance liquid chromatography. The oral spray solution tested contained 75±7.5µg vitamin D_3/spray and the capsules sample contained 25±5µg D_3/capsule. The 3000IU

(75µg) daily dose chosen fell below the 4000IU (100µg) 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 post-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 instructed to fast from 10pm the night prior to blood sampling and encouraged to drink water as usual. Blood samples were obtained from the antecubital vein by a trained phlebotomist. Samples were processed within 1 hour of collection. Following inversion, serum samples were allowed to clot for up to 60 minutes and plasma samples placed in refrigeration until centrifugation. Tubes were centrifuged at 2200rpm for 15 minutes at 4˚Celsius. Separated fractions of serum and plasma were then transferred into 0.5mL aliquots and stored at -80˚Celsius until further analysis.

Blood analysis

Total serum 25(OH)D concentrations [25(OH)D_2 plus 25(OH)D_3] were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a commercially available kit (API 4000; AB SCIEX; Chromsystems Instruments and Chemicals GmbH; MassChrom 25-OH-Vitamin D3/D2). Vitamin D analysis was conducted at the biochemistry department of St James' Hospital Dublin. This laboratory is fully accredited to ISO 15189 Standard and complies with the Vitamin D External Quality Assessment Scheme (DEQAS) and use of the National Institute of Standards and Technology 972 vitamin D standard reference material. The respective inter- and intra-assay

coefficients of variation were 6.5% and 7.5% respectively. Intact parathyroid hormone (PTH) concentrations were measured in duplicate using a commercially available enzyme-linked immunosorbent assay (MD Biosciences Inc., Minnesota, USA). Intra and inter-assay coefficients of variation were 4.52% and 6.18% respectively. Serum calcium, albumin and creatinine concentrations were quantified, in duplicate, using an ILab 650 clinical chemistry analyser (Instrumentation Laboratory, Massachusetts, United States). Intra-assay coefficients of variation were 1.11%, 0.80% and 1.19% respectively. The following equation was applied to total calcium and albumin concentrations to account for protein-bound calcium; $Adjusted\ calcium = 0.04 + total\ calcium \times (40 - albumin)$ [23] with adjusted calcium concentrations used in analyses thereafter. To confirm healthy renal function, the Modification of Diet in Renal Disease (MDRD) equation [24] was used in order to obtain estimated glomerular filtration rate (eGFR) from creatinine concentrations.

Dietary vitamin D intake

Participants completed a validated vitamin D food frequency questionnaire 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 22 participants were required to observe a significant 9.4nmol/L difference in the total 25(OH)D response 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 analysis was performed with the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, version 22.0, IBM Corp, Armonk, NY), 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 prior to further analysis. Missing data were subject to intention to treat (ITT) analysis in-line with the Consolidated Standards for Reporting Trials (CONSORT) 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 40 imputed datasets with minimum and maximum value constraints pre-specified using *per protocol* data. An overview of imputed data is provided in **Figure 1**. Comparisons between sequence allocations at baseline were made using and independent samples *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 versus capsule vitamin D_3 supplementation on total 25(OH)D concentration tested using analysis of covariance controlling for pre-intervention total 25(OH)D concentration. Magnitude of change in total 25(OH)D concentration was calculated as percentage change from baseline by dividing the change in total 25(OH)D concentration during intervention by baseline concentration and multiplying by a factor of 100.

Results

The participant flow is detailed in **Figure 1**. Overall, 4 participants did not complete the trial as a result of sun holidays (n=2), illness unrelated to intervention (n=1) or undisclosed reasons (n=1). In

participants that returned their oral spray bottle (*n*=16) and pill boxes (*n*=19), 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 was therefore included in ITT. At baseline, vitamin D sufficiency (>50nmol/L), insufficiency (31-49nmol/L) and clinical deficiency (<30nmol/L) was evident in 59%, 23% and 18% of participants respectively. Overall, baseline mean ± SD total 25(OH)D concentration averaged 59.76±29.88nmol/L, representing clinical sufficiency while dietary vitamin D intake averaged 6.25±6.24µg/day. Baseline characteristics of 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 ± SD total 25(OH)D concentration [59.76±29.88nmol/L (baseline) versus 59.90±19.86nmol/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±16.15nmol/L (sequence allocation 1) versus 23.82±18.62nmol/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 ± SD change from baseline in total 25(OH)D concentration between oral spray and capsule supplementation methods (26.15±17.85 versus 30.38±17.91nmol/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). 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 hypercalcemia (>2.2mmol/L) in response to intervention; highlighting the safety of the dose and duration provided.

Discussion

184 This randomised, open-label crossover study has revealed, for the first time in healthy Western
185 adults residing at a northerly latitude (55° N), that vitamin D_3 supplied in oral spray form is equally
186 effective at raising total 25(OH)D concentrations when compared to capsule supplementation. Our
187 findings therefore advocate use of oral spray vitamin D_3 as a suitable alternative, if desired, to
188 capsule supplementation in the general population. There is a lack of comparable studies however
189 a recent crossover trial that compared oral spray and capsule vitamin D_3 supplementation [1000IU
190 (25µg) daily for 4 weeks] in healthy Indian adults (assigned to oral spray, *n*=7; capsules, *n*=7;
191 control, *n*=6) and patients with gastrointestinal malabsorption (assigned to oral spray, *n*=7;
192 capsules, *n*=7; control, *n*=6) found that oral spray supplementation was superior to capsules in both
193 healthy and patient population groups, contrasting with the results of the current study [9]. Although
194 Satia and colleagues employed washout phase only 2x the plasma half-life of 25(OH)D and did not
195 account for sunlight exposure in statistical analyses, these factors are unlikely to account for the
196 abovementioned difference between studies as total 25(OH)D concentrations returned to baseline
197 concentrations following washout and remained stable in the control group throughout the study.
198 The magnitude of change in total 25(OH)D concentration (mean percentage increase from
199 baseline) was similar between the current study and the findings of Satia and colleagues for oral
200 spray supplementation (+44% versus +43% respectively) however this was not the case for capsule
201 supplementation (+51%, versus +22% respectively). The permeability and absorption potential of
202 the gastrointestinal tract is known to vary according to an individual's geographical location, with
203 Asians exhibiting lower absorption and membrane permeability than Europeans [29]. Although the
204 exact mechanism responsible for this disparity is yet to be elucidated it is possible that this
205 phenomenon may explain why Satia and colleagues found the oral spray to be more effective than
206 capsules at increasing total 25(OH)D concentrations and why their finding was not replicated in the
207 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 D_3 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 D_3 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 [8, 31]. It is important to recognise that, irrespective of the route of absorption, both oral spray and capsule-based vitamin D_3 must first undergo hepatic hydroxylation prior to 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 D_3 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 crossover 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 to numerous others conducted across Ireland and is a result of limited dietary sources that are not readily consumed [22, 33, 34]. The Scientific Advisory Committee on Nutrition (SACN) recently proposed a vitamin D recommended nutrient intake (RNI) of 10µg/day for the entire UK population [35]. However, 86% of 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 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 D_3 supplementation methods compare over longer-term interventions exceeding 4 weeks in duration. Future studies in this area should focus on comparing the effectiveness of oral spray vitamin D_3 supplementation against alternative methods in those with gastrointestinal malabsorption. If our findings are replicated or oral spray vitamin D_3 is indeed found to be advantageous over capsules in these individuals; oral spray supplementation may offer a non-invasive alternative to injections and therefore lower patient administration burden.

Acknowledgements

The authors would like to thank Ms Callan Dickey for her assistance in data collection and Mr Neil Dennison for his assistance in laboratory analyses.

Financial support

The work conducted in this study was supported by a PhD grant from the Department of Employment and Learning, Northern Ireland and seed funding from Translational Research Group: Diabetes, Endocrinology & Nutrition, HSC Research & Development Division, Public Health Agency, Belfast. Oral spray solutions were gifted by BetterYou Ltd. PJ Magee, LK Pourshahidi and EM McSorley received a grant from BetterYou Ltd for consultancy on previous unrelated work.

Conflict of Interest

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

Authorship

JJ Todd, EM McSorley, LK Pourshahidi, SM Madigan and PJ Magee designed the research. JJ Todd conducted the research, analysed data and wrote the paper. E Laird and M Healy conducted laboratory analysis. All authors read and approved the final manuscript and PJ Magee had responsibility for final content.

References

1. Holick M, Chen T. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87:1080S-6S.

2. Mithal A, Wahl DA, Bonjour J, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 2009;20:1807-20.

3. Kopec A, Solarz K, Majda F, Slowinska-Lisowska M, Medras M. An evaluation of the levels of vitamin d and bone turnover markers after the summer and winter periods in Polish professional soccer players. J Hum Kinet 2013;8:135-40.

4. Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K. Calcium and vitamin D supplementation decreases incidence of stress fractures in female navy recruits. J Bone Miner Res 2008;23:741-9.

5. He C, Handzlik M, Fraser WD, Muhamad A, Preston H, Richardson A, Gleeson M. Influence of vitamin D status on respiratory infection incidence and immune function during 4 months of winter training in endurance sport athletes. Exerc Immunol Rev 2013;19:86-101.

6. Todd JJ, Pourshahidi LK, McSorley EM, Madigan SM, Magee PJ. Vitamin D: Recent Advances and Implications for Athletes. Sports Med 2015;45:213-9.

7. Barnes MS, Horigan G, Cashman KD *et al.* (2011) Maintenance of wintertime vitamin D status with cholecalciferol supplementation is not associated with alterations in serum cytokine concentrations among apparently healthy younger or older adults. J Nutr 2008;141:476-81.

8. Narang N, Sharma J. Sublingual mucosa as a route for systemic drug delivery. Int J Pharm Pharm Sci 2011;3:18-22.

9. Satia MC, Mukim AG, Tibrewala KD, Bhavsar MS. A randomized two way cross over study for comparison of absorption of vitamin D3 buccal spray and soft gelatin capsule formulation in healthy subjects and in patients with intestinal malabsorption. Nutr J 2015;14:114.

10. Strickley RG. Solubilizing excipients in oral and injectable formulations. Pharm Res 2004;21:201-30.

11. Kalepu S, Manthina M, Padavala V. Oral lipid-based drug delivery systems – an overview. Acta Pharmaceutica Sinica B 2013;3:361-72.

12. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. Endocrinol Metab Clin North Am 2010;39:243-53.

13. Haussler M, Haussler C, Jurutka P, Thompson P, Hsieh J, Remus L, Selznick S, Whitfield G. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. J Endocrinol 1997;154:S57-73.

14. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009;94:26-34.

15. Grossmann RE, Tangpricha V. Evaluation of vehicle substances on vitamin D bioavailability: a systematic review. Mol Nutr Food Res 2010;54:1055-61.

16. Tellioglu A, Basarana S, Guzela R, Seydaoglub G. Efficacy and safety of high dose intramuscular or oral cholecalciferol in vitamin D deficient/insufficient elderly. Maturitas 2012;72:332-8.

17. Food and Drug Administration. Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA. Guidance for industry. Version current December 2013. Internet: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm377465.pdf (accessed June 2016).

18. Jones G. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 2008;88:582S-6S.

19. Food and Drug Administration. Bioequivalence testing. Guidance for industry. Version current 8th November 2006. Internet: http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052363.pdf (accessed June 2016).

20. Evans S, Royston P, Day S. Minim: allocation by minimisation in clinical trials. Version current 14th March 2013. Internet: https://www-users.york.ac.uk/~mb55/guide/minim.htm (accessed June 2015).

21. European Food Safety Authority (EFSA), Parma, Italy. Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal 2012;10:2813.

22. Todd JJ, McSorley EM, Pourshahidi LK, Madigan SM, Laird E, Healy M, Magee PJ. Vitamin D3 supplementation using an oral spray solution resolves deficiency but has no effect on VO2 max in Gaelic footballers: results from a randomised, double-blind, placebo-controlled trial. Eur J Nutr 2016; (Epub ahead of print).

23. Steele T, Kolamunnage-Dona R, Downey C, Toh CH, Welters I. Assessment and clinical course of hypocalcemia in critical illness. Crit Care 2013;17:R106.

24. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, Rahman M, Deysher AE, Zhang YL, Schmid CH, et al. Evaluation of the modification of diet in renal disease study equation in a large diverse population. J Am Soc Nephrol 2007;18:2749-57.

25. Spiro A, Buttriss J. Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull 2014;39:322-50.

26. Weir RR, Carson EL, Mulhern MS, Laird E, Healy M, Pourshahidi LK. Validation of a food frequency questionnaire to determine vitamin D intakes using the method of triads. J Hum Nutr Diet 2016;29:255-61.

27. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.

28. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 Explanation and Elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:C869.

29. Menzies IS, Zuckerman MJ, Nukajam WS, Somasundaram SG, Murphy B, Jenkins AP, Crane RS, Gregory GG. Geography of intestinal permeability and absorption. Gut 1999;44:483-89.

30. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene 2004;338:143-56.

31. Kotilea K, Quennery S, Decroës V, Hermans DA. Successful sublingual cobalamin treatment in a child with short-bowel syndrome. J Pediatr Pharmacol Ther 2014;19:60-3.

32. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21:319-29.

33. Hill TR, O'brien MM, Cashman KD, Flynn A, Kiely M. Vitamin D intakes in 18-64-y-old Irish adults. Eur J Clin Nutr 2004;58:1509-17.

34. McCarthy D, Collins A, O'Brien M, Lamberg-Allardt C, Jakobsen J, Charzewska J, Kiely M, Flynn A, Cashman KD. Vitamin D intake and status in Irish elderly women and adolescent girls. Ir J Med Sci 2006;175:14-20.

35. Scientific Advisory Committee on Nutrition. Vitamin D and Health report. Version

current 21st July 2016. Internet: www.gov.uk/government/publications/sacn-vitamin-d-and-health-report (accessed August 2016).

Tables and Figures

Table 1. Baseline participant characteristics by sequence allocation [a]

	Sequence allocation				
Measure	Capsules → oral spray (*n*=11)		Oral spray → capsules (*n*=11)		*P* [b]
Age, y	23.0	2.7	27.4	8.4	0.157
Height, cm	168.3	10.2	171.6	8.8	0.427
Weight, kg	67.4	17.8	76.4	10.8	0.166
BMI, kg/m^2	23.4	3.8	25.8	3.2	0.177
Total 25(OH)D, nmol/L	62.4	31.6	57.1	29.3	0.686
Adjusted calcium, mmol/L	2.3	0.1	2.2	0.1	0.114
PTH, pg/mL	43.5	15.5	53.2	29.1	0.647
eGFR, mL/min/1.73m^2	92.7	10.8	90.6	7.9	0.608

Abbreviations: Body mass index, BMI; 25-hydroxyvitamin D, 25(OH)D; parathyroid hormone, PTH, estimated glomerular filtration rate, eGFR

[a] All values are provided as mean ± SDs

[b] Difference between sequence allocation values at baseline compared using an independent *t* test

Table 2. Participant characteristics before and after supplementation with vitamin D_3 capsules or oral spray solution [a]

	Treatment and time point									
	Capsules (*n*=22)					Oral spray solution (*n*=22)				
Measure	Pre-intervention		Post-intervention		*P* [b]	Pre-intervention		Post-intervention		*P* [b]
Age, years	25.2	6.5	25.2	6.5	0.329	25.2	6.5	25.2	6.5	1.000
Weight, kg	71.5	15.1	71.0	15.1	0.578	70.9	14.9	70.8	15.0	0.747
BMI, kg/m^2	24.4	3.6	24.2	3.6	0.574	24.2	3.5	24.2	3.5	0.649
Total 25(OH)D, nmol/L	60.0	26.3	90.4	21.0	0.001 [c]	59.6	24.4	85.8	19.4	0.001 [c]
Adjusted calcium, mmol/L	2.2	0.1	2.2	0.1	0.783	2.2	0.1	2.2	0.1	0.666
PTH, pg/mL	50.3	25.5	52.2	19.3	0.373	52.1	26.0	48.2	27.3	0.475
eGFR, $mL/min/1.73m^2$	91.0	9.3	92.1	11.8	0.347	90.8	11.2	88.4	10.8	0.173

Abbreviations: Body mass index, BMI; 25-hydroxyvitamin D, 25(OH)D; parathyroid hormone, PTH, estimated glomerular filtration rate, eGFR

[a] All values are provided as mean ± SDs

[b] Difference between pre versus post-intervention values tested using a paired *t* test

[c] Significantly different from pre-intervention mean, $P<0.001$

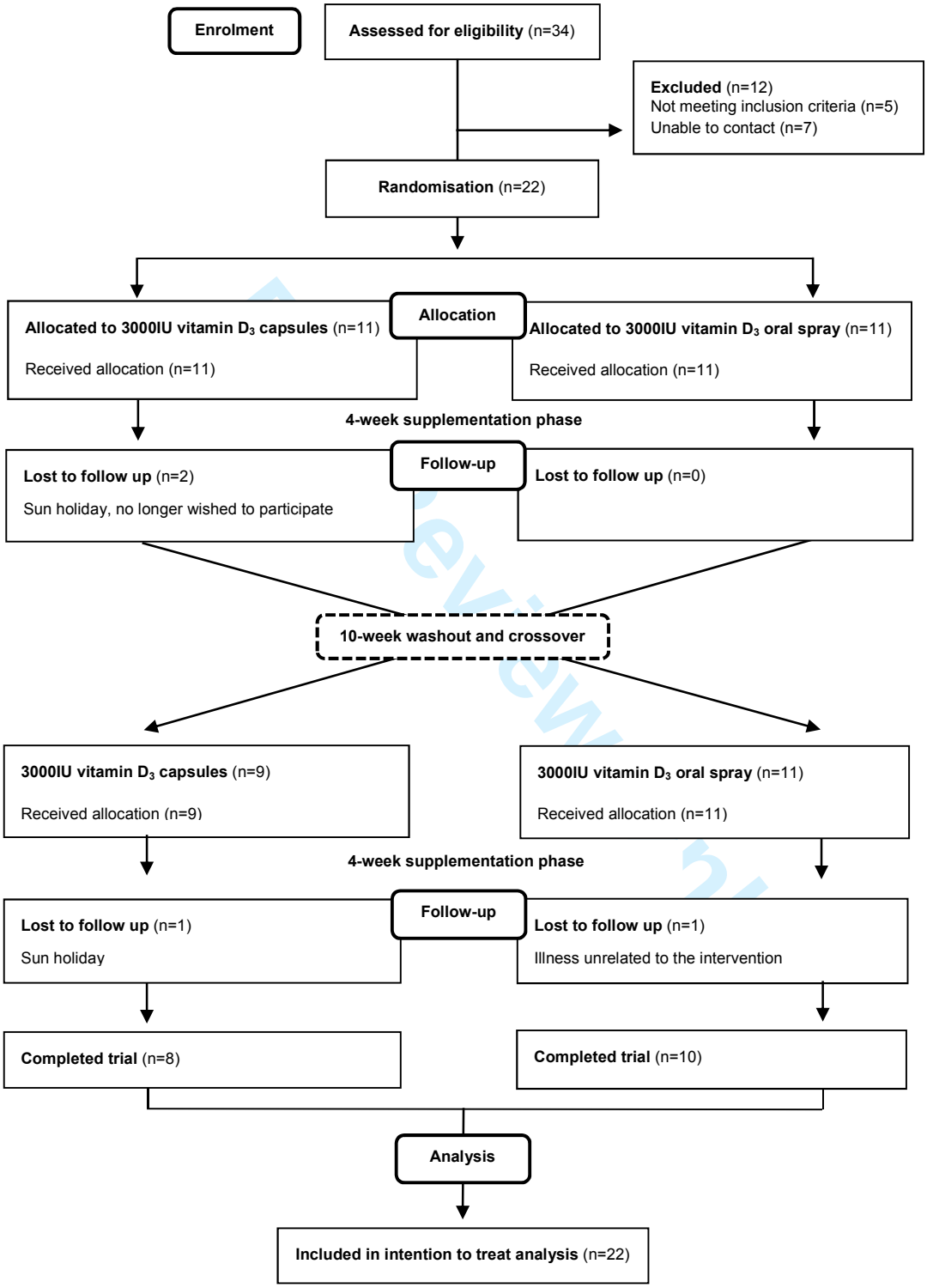
Enrolment
Assessed for eligibility (n=34)
Excluded (n=12)
Not meeting inclusion criteria (n=5)
Unable to contact (n=7)
Randomisation (n=22)
Allocation
Allocated to 3000IU vitamin D3 capsules (n=11)
Received allocation (n=11)
Allocated to 3000IU vitamin D3 oral spray (n=11)
Received allocation (n=11)
4-week supplementation phase
Follow-up
Lost to follow up (n=2)
Sun holiday, no longer wished to participate
Lost to follow up (n=0)
10-week washout and crossover
3000IU vitamin D3 capsules (n=9)
Received allocation (n=9)
3000IU vitamin D3 oral spray (n=11)
Received allocation (n=11)
4-week supplementation phase
Follow-up
Lost to follow up (n=1)
Sun holiday
Lost to follow up (n=1)
Illness unrelated to the intervention
Completed trial (n=8)
Completed trial (n=10)
Analysis
Included in intention to treat analysis (n=22)

Figure 1. CONSORT flow diagram. A total of 34 healthy adults expressed interest in the study and completed screening questionnaires. Overall, 12 individuals were excluded for either not meeting inclusion criteria (*n*=5) or were unable to contact (*n*=7). Twenty-two healthy adults satisfied inclusion criteria and were randomised to receive 3000IU (75µg) vitamin D_3 daily in either an oral spray (*n*=11) or capsules (*n*=11) for 4 weeks. Two participants were lost to follow-up during the first supplementation phase owing to sun holiday (*n*=1) or nor longer wishing to participate (*n*=1). Following a 10-week washout, participants crossed-over to the opposite treatment for a final 4 weeks. Two further participants were lost to follow-up in the second supplementation phase owing to sun holiday (*n*=1) or illness unrelated to the intervention (*n*=1). Overall, 18 participants completed the study *per protocol*. All participants randomised at baseline were included in the final analysis.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Title page
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 2 Lines 1-18
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Pages 3-4 Lines 23-51
	2b	Specific objectives or hypotheses	Page 4 Lines 50-52
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 4-5 lines 67- 73 and Page 5 lines 78-79
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	Pages 4-5 Lines 66-72
	4b	Settings and locations where the data were collected	Page 5 Lines 55-57
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Pages 5 Lines 78-90
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Page 4 lines 50-52 Pages 6-7 lines 98-114
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	Page 7 Lines 131-135
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A

Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Page 5 Lines 78-79
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Page 5 Lines 78-79
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Page 5 Lines 78-79
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Page 5 Lines 78-79
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	Page 5 Lines 87-90
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Page 8 Lines 149-154
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Pages 18-19 (Figure 1)
	13b	For each group, losses and exclusions after randomisation, together with reasons	Pages 18-19 (Figure 1) and Page 8 lines 156-157
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 4 Line 56-57
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Page 16 (Table 1)
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Pages 18-19 (Figure 1)
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Page 9 Lines 172-177

	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Page 9 Lines 177-179 (No harms observed)
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Pages 11 Lines 229-231
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Pages 11 Lines 229-231
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Pages 9-11 Lines 181-235
Other information			
Registration	23	Registration number and name of trial registry	Title Page
Protocol	24	Where the full trial protocol can be accessed, if available	Title Page
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Page 12

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.