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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

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Title: 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.

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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.

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1 Abstract

2 Vitamin D is typically supplied in capsule form, both in trials and clinical practice. Yet little is
3 known regarding the efficacy of vitamin D administered via oral spray; a method that primarily
4 bypasses the gastrointestinal absorption route. This study aimed to compare the efficacy of vitamin
5 D₃ liquid capsules and oral spray solution, at increasing wintertime total 25-hydroxyvitamin D
6 [25(OH)D] concentrations. In this randomised, open-label crossover trial, healthy adults ($n=22$)
7 received 3000IU (75 μ g) vitamin D₃ daily for 4 weeks in either capsule or oral spray form.
8 Following a 10-week washout phase, participants received the opposite treatment for a final 4
9 weeks. Anthropometrics and fasted blood samples were obtained pre and post-supplementation,
10 with samples analysed for total 25(OH)D, creatinine, intact parathyroid hormone and adjusted
11 calcium concentrations. At baseline, vitamin D sufficiency [total 25(OH)D >50nmol/L],
12 insufficiency (31-49nmol/L) and clinical deficiency (<30nmol/L) was evident in 59%, 23% and
13 18% of participants respectively. Overall, baseline mean \pm SD total 25(OH)D concentration
14 averaged 59.76 \pm 29.88nmol/L, representing clinical sufficiency. Analysis of covariance revealed no
15 significant difference in the mean \pm SD change from baseline in total 25(OH)D concentration
16 between oral spray and capsule supplementation methods (26.15 \pm 17.85 versus 30.38 \pm 17.91nmol/L
17 respectively ($F=1.044$, adjusted $r^2=0.493$, $P=0.313$)). Oral spray vitamin D₃ is an equally effective
18 alternative to capsule supplementation in healthy adults.

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23 Introduction

24 Epidemiological studies have revealed that vitamin D insufficiency and deficiency, defined as a
25 total 25-hydroxyvitamin D [25(OH)D] concentration below 50 and 30nmol/L respectively, are
26 endemic worldwide ^(1, 2). Such findings have led to significant investment in vitamin D research
27 with many exploring the impact of vitamin D supplementation on skeletal health as well as
28 potential extra-skeletal outcomes ⁽³⁻⁶⁾. **Scientists investigating the pleotropic role of vitamin D in
29 randomised controlled trials often use capsules or tablets as a peroral method of nutrient delivery ^{(4,}
30 ⁷⁾. However, despite being commercially available, little is known regarding the efficacy of oral
31 spray vitamin D which is primarily absorbed at the buccal, sublingual and palatal membranes in the
32 oral cavity rather than the gastrointestinal tract ⁽⁸⁾. Emerging evidence also suggests that oral spray
33 vitamin D may provide an accelerated route of absorption compared to capsules and may be
34 advantageous in those with gastrointestinal malabsorption ⁽⁹⁾. Owing to the lipophilic nature of
35 vitamin D, oral sprays containing this micronutrient typically contain a triglyceride carrier
36 substance as well as solubilising excipients, such as α -tocopherol and oleic acid, which promote
37 passive absorption of the micro-emulsified solution into systemic circulation ⁽¹⁰⁾. This is achieved
38 through dispersion across capillary beds in the oral submucosa ⁽¹¹⁾. Following entry into systemic
39 circulation, vitamin D [including both ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃)
40 compounds] is bound to vitamin D binding proteins and transported to the liver where it undergoes
41 hydroxylation, catalysed by 25-hydroxylase. **This process forms the biomarker of vitamin D status,
42 25(OH)D, that is subsequently hydroxylated into the biologically active vitamin D metabolite 1,25-
43 dihydroxyvitamin D [1,25(OH)₂D] in the kidneys and by cells elsewhere that also express 1 α -
44 hydroxylase ⁽¹²⁾. Such cells are present throughout the body including sites such as the skeleton,
45 prostate and immune system ⁽¹³⁾. **It is 1,25(OH)₂D that governs vitamin D-related mechanisms of
46 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 D₃ 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 D₃ 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 55° 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

69 criteria consisted of being over 18 years of age and apparently healthy. Exclusion criteria were as
70 follows; intending to consume a supplement containing vitamin D at any point during the study;
71 currently taking medication(s) known to influence vitamin D metabolism [calcium-channel
72 blockers, anticonvulsants, cardiac glycosides, thiazide diuretics, isoniazid, statins, active vitamin D
73 metabolites / calcitonin, laxatives (regular/continued use)]; those following a vegan diet, sun bed
74 users and those planning a sun holiday at any point during the study. Informed consent was
75 obtained at the first appointment. All appointments took place at either the Human Intervention
76 Studies Unit at the University of Ulster, Coleraine or the Northern Ireland Clinical Research
77 Facility in Belfast City Hospital.

78 Supplements and compliance

79 The order in which vitamin D₃ oral sprays or capsules were provided, was determined by the
80 clinical trials manager using MINIM randomisation software with an allocation ratio of 1:1⁽²⁰⁾.
81 Participants were asked to consume their respective supplement at the same time each day (in the
82 morning prior to breakfast). Those allocated to sequence allocation one received an oral spray
83 solution containing 3000IU (75µg) vitamin D₃, per spray, and were instructed to self-administer a
84 single spray targeting the buccal membrane on a daily basis for a period of 4 weeks. Those
85 allocated to sequence allocation two were instructed to consume three 1000IU (25µg) vitamin D₃
86 capsules per day with water for a period of 4 weeks. Following the washout period, participants
87 completed a final 4-week supplementation phase on the opposite treatment. Capsules were
88 provided in pill boxes to aid compliance. The vitamin D₃ content of a single oral spray bottle
89 solution from the supplied batch and 50g of capsule matrix were confirmed by an independent
90 laboratory using high-performance liquid chromatography. The oral spray solution tested contained
91 75±7.5µg vitamin D₃/spray and the capsules sample contained 25±5µg D₃/capsule. The 3000IU

92 (75µg) daily dose chosen fell below the 4000IU (100µg) daily tolerable upper limit for vitamin D
93 specified by the European Food Safety Authority ⁽²¹⁾. Participants were asked to return pill boxes
94 and oral spray bottles at the end of each supplementation phase, to enable estimation of
95 compliance. Percentage compliance to capsule supplementation was determined by capsule
96 counting post-intervention and by dividing the actual number of days on intervention by the
97 expected number of days and multiplying by a factor of 100. The method used to calculate
98 percentage compliance to oral spray supplementation is described elsewhere ⁽²²⁾.

99 Blood collection and processing

100 Participants were instructed to fast from 10pm the night prior to blood sampling and encouraged to
101 drink water as usual. Blood samples were obtained from the antecubital vein by a trained
102 phlebotomist. Samples were processed within 1 hour of collection. Following inversion, serum
103 samples were allowed to clot for up to 60 minutes and plasma samples placed in refrigeration until
104 centrifugation. Tubes were centrifuged at 2200rpm for 15 minutes at 4°Celsius. Separated fractions
105 of serum and plasma were then transferred into 0.5mL aliquots and stored at -80°Celsius until
106 further analysis.

107 Blood analysis

108 Total serum 25(OH)D concentrations [25(OH)D₂ plus 25(OH)D₃] were measured by liquid
109 chromatography-tandem mass spectrometry (LC-MS/MS) using a commercially available kit (API
110 4000; AB SCIEX; Chromsystems Instruments and Chemicals GmbH; MassChrom 25-OH-Vitamin
111 D₃/D₂). Vitamin D analysis was conducted at the biochemistry department of St James' Hospital
112 Dublin. This laboratory is fully accredited to ISO 15189 Standard and complies with the Vitamin D
113 External Quality Assessment Scheme (DEQAS) and use of the National Institute of Standards and
114 Technology 972 vitamin D standard reference material. The respective inter- and intra-assay

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

127 Dietary vitamin D intake

128 Participants completed a validated vitamin D food frequency questionnaire to estimate habitual
129 dietary vitamin D intake on one occasion, owing to the minimal contribution of dietary vitamin D
130 to overall vitamin D status in the Western diet⁽²⁵⁾. Researchers asked participants a series of
131 questions regarding their consumption of foods containing vitamin D and a food atlas was used to
132 estimate portion sizes⁽²⁶⁾.

133 Statistical analysis

134 An *a priori* power calculation with a two-sided significance level of 5% and power at 80%
135 concluded that a total of 22 participants were required to observe a significant 9.4nmol/L
136 difference in the total 25(OH)D response between two different vitamin D₃ supplementation
137 strategies (GPower version 3.1)^(16, 27). This figure was inclusive of an estimated 40% dropout rate.

138 All further statistical analysis was performed with the Statistical Package for the Social Sciences
139 (SPSS) (IBM SPSS Statistics for Windows, version 22.0, IBM Corp, Armonk, NY), with
140 significance set at $P < 0.05$. Normality of data was assessed using the Shapiro-Wilk test. Age and
141 PTH concentrations were skewed and therefore transformed using the logarithmic function to
142 achieve a more normal distribution prior to further analysis. Missing data were subject to intention
143 to treat (ITT) analysis in-line with the Consolidated Standards for Reporting Trials (CONSORT)
144 guidelines⁽²⁸⁾. As such, statistical analyses included all participants randomised at baseline ($n=22$).
145 As data were deemed to be missing completely at random, ITT consisted of 40 imputed datasets
146 with minimum and maximum value constraints pre-specified using *per protocol* data. An overview
147 of imputed data is provided in **Figure 1**. Comparisons between sequence allocations at baseline
148 were made using an independent samples t test. Potential carryover effects were ruled out using a
149 paired t test that compared total 25(OH)D concentration at baseline and at the beginning of the
150 second supplementation phase. **Following this, a time by treatment interaction was ruled-out using**
151 **an independent t test that compared overall change in total 25(OH)D concentration according to**
152 **sequence allocation.** Data from both sequence allocations were then pooled into a single database
153 and the effect of oral spray versus capsule vitamin D₃ supplementation on total 25(OH)D
154 concentration tested using analysis of covariance controlling for pre-intervention total 25(OH)D
155 concentration. **Magnitude of change in total 25(OH)D concentration was calculated as percentage**
156 **change from baseline by dividing the change in total 25(OH)D concentration during intervention**
157 **by baseline concentration and multiplying by a factor of 100.**

158 **Results**

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

161 participants that returned their oral spray bottle ($n=16$) and pill boxes ($n=19$), average compliance
162 to both interventions exceeded 80%. Nevertheless, two participants did not respond to oral spray
163 vitamin D supplementation, despite $>80\%$ compliance, and were considered outliers. Oral spray
164 supplementation phase data for these participants was therefore included in ITT. At baseline,
165 vitamin D sufficiency ($>50\text{nmol/L}$), insufficiency ($31\text{--}49\text{nmol/L}$) and clinical deficiency
166 ($<30\text{nmol/L}$) was evident in 59%, 23% and 18% of participants respectively. Overall, baseline
167 mean \pm SD total 25(OH)D concentration averaged $59.76\pm 29.88\text{nmol/L}$, representing clinical
168 sufficiency while dietary vitamin D intake averaged $6.25\pm 6.24\mu\text{g/day}$. Baseline characteristics of
169 participants in each sequence allocation are provided in **Table 1**. There was no evidence of a
170 carryover effect from the first supplementation phase with respect to mean \pm SD total 25(OH)D
171 concentration [$59.76\pm 29.88\text{nmol/L}$ (baseline) versus $59.90\pm 19.86\text{nmol/L}$ (end of washout),
172 $P=0.977$]. There was also no difference in the response to vitamin D₃ supplementation according to
173 sequence allocation, [$32.70\pm 16.15\text{nmol/L}$ (sequence allocation 1) versus $23.82\pm 18.62\text{nmol/L}$
174 (sequence allocation 2), $P=0.098$]. Participant characteristics before and after supplementation with
175 vitamin D₃ capsules or oral spray solution are presented in **Table 2**. ANCOVA revealed no
176 significant difference in the mean \pm SD change from baseline in total 25(OH)D concentration
177 between oral spray and capsule supplementation methods (26.15 ± 17.85 versus $30.38\pm 17.91\text{nmol/L}$
178 respectively ($F=1.044$, adjusted $r^2=0.493$, $P=0.313$). Use of ITT did not change the study outcome
179 when compared with *per protocol* analysis ($F=-4.709$; $r^2=0.476$, $P=0.329$). **Percentage change**
180 **from baseline in total 25(OH)D concentration for oral spray and capsule interventions was +44%**
181 **and +51% respectively**. There was no evidence of hypercalcemia ($>2.2\text{mmol/L}$) in response to
182 intervention; highlighting the safety of the dose and duration provided.

183 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 D₃ 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 D₃ 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 D₃ 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

208 in study outcomes as there is growing evidence of ethnic differences in the frequency of VDR
209 polymorphisms known to impact vitamin D metabolism ⁽³⁰⁾.

210 Our findings demonstrate that oral spray vitamin D₃ is just as effective as capsule supplementation
211 at increasing total 25(OH)D concentrations in the healthy adult population. Nevertheless, the
212 ability of oral spray vitamin D₃ to bypass the intestinal absorption route may well prove superior
213 for those with gastrointestinal malabsorption syndromes and for individuals with difficulty
214 swallowing such as the elderly, young children and babies ^(8, 31). It is important to recognise that,
215 irrespective of the route of absorption, both oral spray and capsule-based vitamin D₃ must first
216 undergo hepatic hydroxylation prior to forming 25(OH)D which is detected by LC-MS/MS ⁽³²⁾. As
217 such, in those with malabsorption syndromes, any potential long-term benefit of oral spray
218 supplementation over capsules on total 25(OH)D concentrations would likely be derived from
219 enhanced absorption rather than as a result of faster entry of vitamin D₃ into systemic circulation.
220 This concept is supported by the similar extent to which both oral spray and capsule
221 supplementation methods raised total 25(OH)D concentrations in the current study. Additional
222 well-designed crossover trials are required in order to elucidate the potential benefits of oral spray
223 vitamin D in patients with gastrointestinal malabsorption.

224 The low dietary vitamin D intake reported in this study is comparable to numerous others
225 conducted across Ireland and is a result of limited dietary sources that are not readily consumed ^{(22,}
226 ^{33, 34)}. The Scientific Advisory Committee on Nutrition (SACN) recently proposed a vitamin D
227 recommended nutrient intake (RNI) of 10µg/day for the entire UK population ⁽³⁵⁾. However, 86%
228 of participants in this study failed to meet this recommendation thus reinforcing the important role
229 of safe summertime UVB exposure and effective wintertime supplementation strategies in
230 optimising vitamin D status.

231 Strengths of this study include use of an adequate washout phase, independent vitamin D content
232 verification of supplements, inclusion of male and female participants and rigorous statistical
233 analysis that accounted for baseline total 25(OH)D concentrations. However, it remains unknown
234 how oral spray and capsule vitamin D₃ supplementation methods compare over longer-term
235 interventions exceeding 4 weeks in duration. Future studies in this area should focus on comparing
236 the effectiveness of oral spray vitamin D₃ supplementation against alternative methods in those
237 with gastrointestinal malabsorption. If our findings are replicated or oral spray vitamin D₃ is indeed
238 found to be advantageous over capsules in these individuals; oral spray supplementation may offer
239 a non-invasive alternative to injections and therefore lower patient administration burden.

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249 work.

250 **Conflict of Interest**

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

252 **Authorship**

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

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For Review Only

Tables and Figures

Table 1. Baseline participant characteristics by sequence allocation ^a

Measure	Sequence allocation				<i>P</i> ^b
	Capsules → oral spray (<i>n</i> =11)	Oral spray → capsules (<i>n</i> =11)	Capsules → oral spray (<i>n</i> =11)	Oral spray → capsules (<i>n</i> =11)	
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 D₃ capsules or oral spray solution^a

Measure	Treatment and time point									
	Capsules (<i>n</i> =22)					Oral spray solution (<i>n</i> =22)				
	Pre-intervention		Post-intervention		<i>P</i> ^b	Pre-intervention		Post-intervention		<i>P</i> ^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

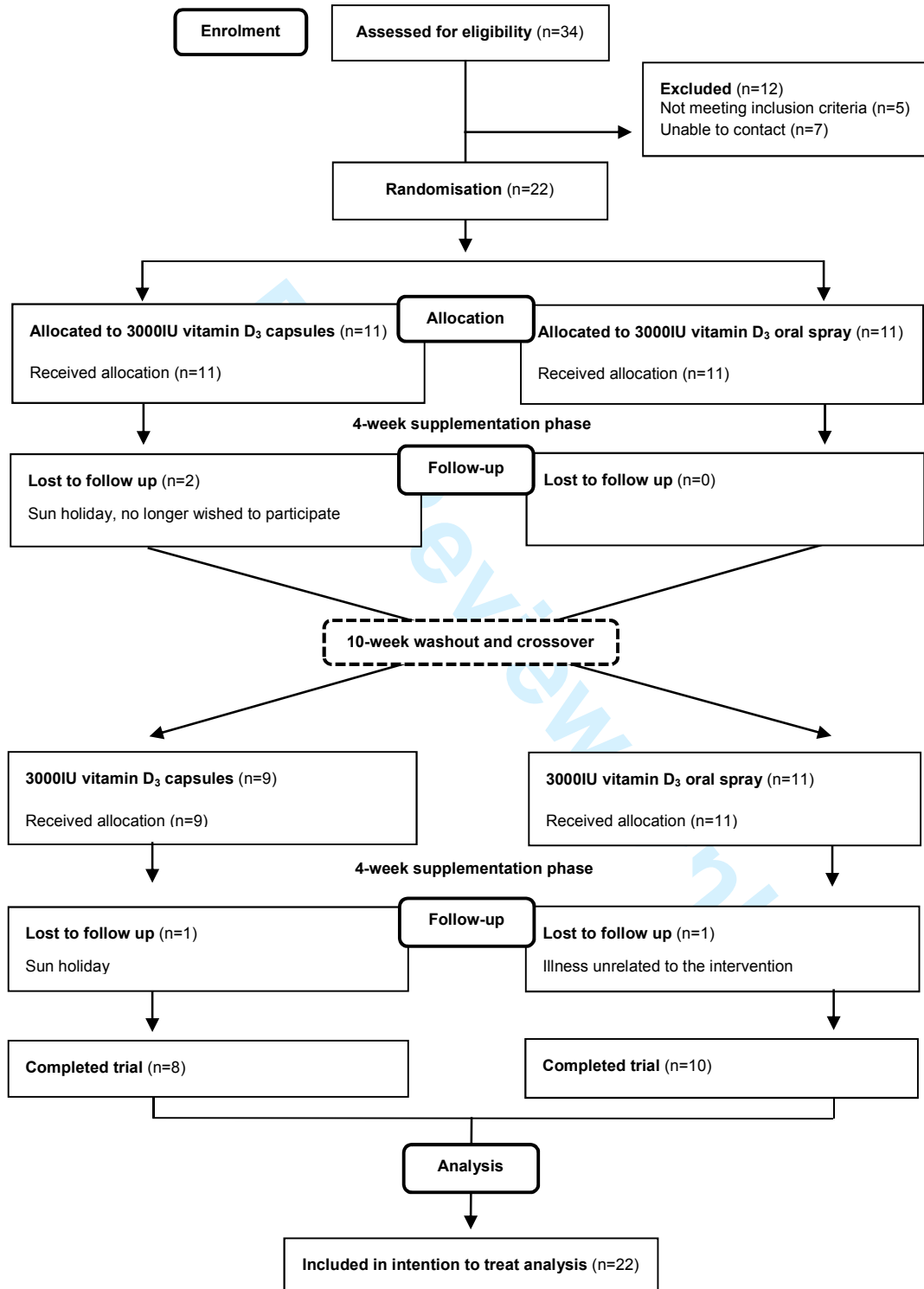


Figure 1. CONSORT flow diagram. A total of 34 healthy adults expressed interest in the study and completed screening questionnaires. Overall, 12 individuals were excluded for either not meeting inclusion criteria ($n=5$) or were unable to contact ($n=7$). Twenty-two healthy adults satisfied inclusion criteria and were randomised to receive 3000IU (75 μ g) vitamin D₃ 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.