

**BRITISH JOURNAL**  
*of* **NUTRITION**



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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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|-------------------------------|---|
| Journal:                      | <i>British Journal of Nutrition</i>   |
| 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<br>McSorley, Emeir; University of Ulster, NICHE: Northern Ireland Centre for Food and Health<br>Pourshahidi, Laura; University of Ulster, NICHE: Northern Ireland Centre for Food and Health<br>Madigan, Sharon; Irish Inst. of Sport, Sports Campus Ireland<br>Laird, Eamon; Trinity College Dublin, Inst. of Molecular Medicine<br>Healy, Martin; St. James Hospital, Dept. of Medicine<br>Magee, Pamela; University of Ulster, NICHE: Northern Ireland Centre for Food and Health |
| Keywords:                     | Oral spray, Capsules, vitamin D, Supplementation, Comparative effectiveness   |
| Subject Category:             | Human and Clinical Nutrition  |
|                               |   |

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**Title:** Vitamin D<sub>3</sub> 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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**Key words:**

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

**Running head:** Oral spray versus capsule vitamin D<sub>3</sub>

**Clinical Trial Registration:** NCT02608164 [www.clinicaltrials.gov](http://www.clinicaltrials.gov)

## 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 D<sub>3</sub> 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 $\mu$ g) vitamin D<sub>3</sub> 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 D<sub>3</sub> 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 <sup>(1, 2)</sup>. 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 <sup>(3-6)</sup>. **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 <sup>(4,</sup>  
30 <sup>7)</sup>. 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 <sup>(8)</sup>. 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 <sup>(9)</sup>. 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  $\alpha$ -tocopherol and oleic acid, which promote  
37 passive absorption of the micro-emulsified solution into systemic circulation <sup>(10)</sup>. This is achieved  
38 through dispersion across capillary beds in the oral submucosa <sup>(11)</sup>. Following entry into systemic  
39 circulation, vitamin D [including both ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>)  
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)<sub>2</sub>D] in the kidneys and by cells elsewhere that also express 1 $\alpha$ -  
44 hydroxylase <sup>(12)</sup>. Such cells are present throughout the body including sites such as the skeleton,  
45 prostate and immune system <sup>(13)</sup>. It is 1,25(OH)<sub>2</sub>D 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 <sup>(14)</sup>. Indeed, researchers have compared the efficacy of vitamin D injections, tablets and capsules at  
48 increasing total 25(OH)D concentration <sup>(15, 16)</sup>. 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<sub>3</sub> 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<sub>3</sub> 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](http://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 <sup>(17-19)</sup>.

#### 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 D<sub>3</sub> 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<sup>(20)</sup>.  
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 D<sub>3</sub>, 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 D<sub>3</sub>  
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 D<sub>3</sub> 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 D<sub>3</sub>/spray and the capsules sample contained 25±5µg D<sub>3</sub>/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 <sup>(21)</sup>. 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 <sup>(22)</sup>.

#### 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)D<sub>2</sub> plus 25(OH)D<sub>3</sub>] 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 <sup>(24)</sup>  
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 <sup>(25)</sup>. 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 <sup>(26)</sup>.

### 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 D<sub>3</sub> supplementation  
137 strategies (GPower version 3.1) <sup>(16, 27)</sup>. 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<sup>(28)</sup>. 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 D<sub>3</sub> 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 D<sub>3</sub> 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 D<sub>3</sub> 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 ( $26.15\pm 17.85$  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 D<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sup>(9)</sup>. 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<sup>(29)</sup>. 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 <sup>(30)</sup>.

210 Our findings demonstrate that oral spray vitamin D<sub>3</sub> 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 D<sub>3</sub> 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 <sup>(8, 31)</sup>. It is important to recognise that,  
215 irrespective of the route of absorption, both oral spray and capsule-based vitamin D<sub>3</sub> must first  
216 undergo hepatic hydroxylation prior to forming 25(OH)D which is detected by LC-MS/MS <sup>(32)</sup>. 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 D<sub>3</sub> 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 <sup>(22,</sup>  
226 <sup>33, 34)</sup>. 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 <sup>(35)</sup>. 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 D<sub>3</sub> 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 D<sub>3</sub> supplementation against alternative methods in those  
237 with gastrointestinal malabsorption. If our findings are replicated or oral spray vitamin D<sub>3</sub> 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.

#### 240 **Acknowledgements**

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

#### 243 **Financial support**

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

| Measure                         | Sequence allocation                   |      |                                       |      | <i>P</i> <sup>b</sup> |
|---------------------------------|---------------------------------------|------|---------------------------------------|------|-----------------------|
|                                 | 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 <sup>2</sup>          | 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 <sup>2</sup> | 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

<sup>a</sup> All values are provided as mean ± SDs

<sup>b</sup> Difference between sequence allocation values at baseline compared using an independent *t* test

**Table 2.** Participant characteristics before and after supplementation with vitamin D<sub>3</sub> capsules or oral spray solution<sup>a</sup>

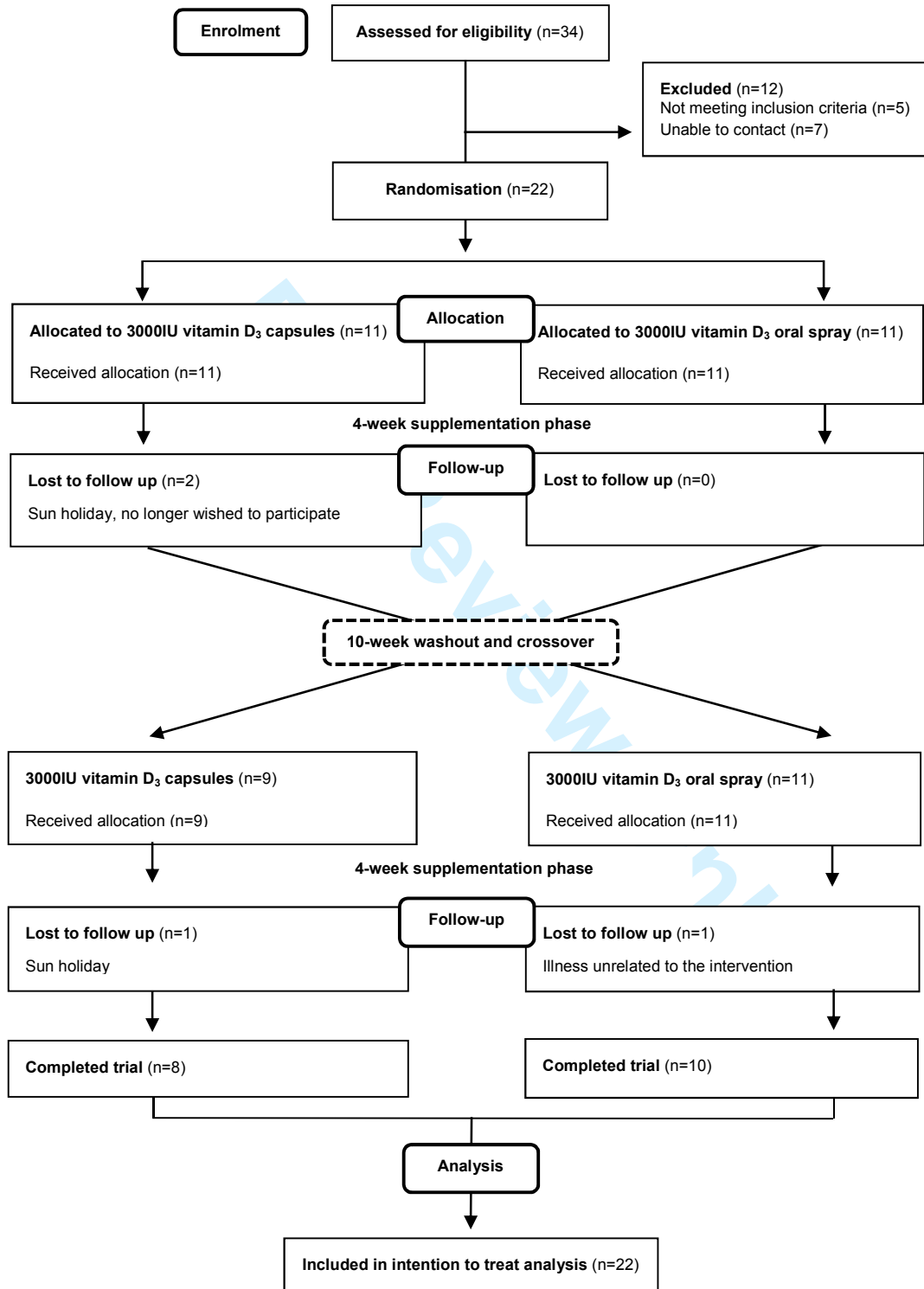
| Measure                         | Treatment and time point |      |                   |      |                       |                                     |      |                   |      |                       |
|---------------------------------|--------------------------|------|-------------------|------|-----------------------|-------------------------------------|------|-------------------|------|-----------------------|
|                                 | Capsules ( <i>n</i> =22) |      |                   |      |                       | Oral spray solution ( <i>n</i> =22) |      |                   |      |                       |
|                                 | Pre-intervention         |      | Post-intervention |      | <i>P</i> <sup>b</sup> | Pre-intervention                    |      | Post-intervention |      | <i>P</i> <sup>b</sup> |
| 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 <sup>2</sup>          | 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 <sup>c</sup>    | 59.6                                | 24.4 | 85.8              | 19.4 | 0.001 <sup>c</sup>    |
| 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 <sup>2</sup> | 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

<sup>a</sup> All values are provided as mean ± SDs

<sup>b</sup> Difference between pre versus post-intervention values tested using a paired *t* test

<sup>c</sup> 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 $\mu$ g) vitamin D<sub>3</sub> 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                                 |
|---------------------------|---------|---|---|
| <b>Title and abstract</b> |         |   |   |
|                           | 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<br>Lines 1-18                                |
| <b>Introduction</b>       |         |   |   |
| Background and objectives | 2a      | Scientific background and explanation of rationale  | Pages 3-4<br>Lines 23-51                            |
|                           | 2b      | Specific objectives or hypotheses   | Page 4<br>Lines 50-52                               |
| <b>Methods</b>            |         |   |   |
| Trial design              | 3a      | Description of trial design (such as parallel, factorial) including allocation ratio  | Page 4-5 lines<br>67- 73 and Page<br>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<br>Lines 66-72                            |
|                           | 4b      | Settings and locations where the data were collected  | Page 5<br>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<br>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-<br>52<br>Pages 6-7 lines<br>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<br>Lines 131-135                             |
|                           | 7b      | When applicable, explanation of any interim analyses and stopping guidelines  | N/A   |

|  |     |   |  |
|--|-----|---|--|
| Randomisation:<br>Sequence generation                | 8a  | Method used to generate the random allocation sequence  | Page 5<br>Lines 78-79                                    |
|  | 8b  | Type of randomisation; details of any restriction (such as blocking and block size)   | Page 5<br>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<br>Lines 78-79                                    |
| Implementation                                       | 10  | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions   | Page 5<br>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<br>Lines 87-90                                    |
| Statistical methods                                  | 12a | Statistical methods used to compare groups for primary and secondary outcomes   | Page 8<br>Lines 149-154                                  |
|  | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses  | N/A  |
| <b>Results</b>                                       |     |   |  |
| 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<br>(Figure 1)                                |
|  | 13b | For each group, losses and exclusions after randomisation, together with reasons  | Pages 18-19<br>(Figure 1) and<br>Page 8 lines<br>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<br>(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<br>(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<br>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<br>Lines 177-179<br>(No harms observed) |
| <b>Discussion</b>        |     |   |  |
| Limitations              | 20  | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses                          | Pages 11<br>Lines 229-231                      |
| Generalisability         | 21  | Generalisability (external validity, applicability) of the trial findings   | Pages 11<br>Lines 229-231                      |
| Interpretation           | 22  | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence                             | Pages 9-11<br>Lines 181-235                    |
| <b>Other information</b> |     |   |  |
| 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](http://www.consort-statement.org).