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1           **Rate of change of circulating 25-hydroxyvitamin D**  
2           **following sublingual and capsular vitamin D preparations**

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5           Claire E. Williams<sup>1</sup>, Elizabeth A. Williams<sup>2</sup> & Bernard M. Corfe<sup>1,3,4</sup>  
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8           1. Molecular Gastroenterology Research Group, Academic Unit of Surgical Oncology,  
9           Department of Oncology & Metabolism, University of Sheffield, Beech Hill Road, Sheffield,  
10           S10 2RX

11           2. Department of Oncology & Metabolism, University of Sheffield, Beech Hill Road,  
12           Sheffield, S10 2RX

13           3. Insigneo Institute for *In Silico* Medicine, The University of Sheffield  
14

15           4. Author to whom correspondence should be addressed: Dr B.M. Corfe, Molecular  
16           Gastroenterology Research Group, Academic Unit of Surgical Oncology, Department of  
17           Oncology & Metabolism, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX

18           Tel: 0114 215 9044

19           Fax: 0114 2713314

20           Email: [b.m.corfe@sheffield.ac.uk](mailto:b.m.corfe@sheffield.ac.uk)  
21

22           Short title: Efficacy of sublingual vitamin D supplements  
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33 **ABSTRACT**

34 **Background:** Vitamin D is critical for skeletal health and is increasingly associated with  
35 other pathologies encompassing gastrointestinal, immunological, psychological effects. A  
36 significant proportion of the population exhibit suboptimal levels of vitamin D, particularly in  
37 Northern latitudes in winter. Supplementation is advocated, but few data are available on  
38 achievable or typical rates of change. There has been considerable interest in the potential use  
39 of sublingual sprays for delivery of nutrient supplements, but data on efficacy remains sparse.

40 **Methods:** A randomised, placebo-controlled, 3-arm parallel design study was conducted in  
41 healthy volunteers (n=75) to compare the rate of change of vitamin D status in response to  
42 vitamin D3 (3000IU/day) supplementation in capsule and sublingual spray preparations over  
43 a six-week period between January and April 2017. Blood 25(OH)D concentrations were  
44 measured after day 0, 3, 7, 14, 21 and 42 days of supplementation with 3000IU *per diem*.

45 **Results:** Baseline measurements show 25(OH)D deficiency (<30nmol/l), insufficiency (31-  
46 46nmol/l) and sufficiency (>50nmol/l) in 14.9%, 44.6% and 40.5% of the participants  
47 respectively. There was a significant elevation in blood concentrations of 25(OH)D in both of  
48 the treatment arms (capsule p=0.003, spray p=0.001) compared to control. The capsule and  
49 spray were equally efficacious. The rate of change ranged from 0.69-3.93 (capsule) and 0.64-  
50 3.34 (spray) nmol/L day with average change in blood 25(OH)D levels of 2 nmol/l/day. Rates  
51 followed a simple normal distribution in the study population (ks= 0.94 and 0.82 for capsule  
52 and spray respectively). The data suggest that rates of change are higher in individuals with  
53 lower levels of 25(OH)D.

54 **Conclusions:** A sublingual vitamin D spray is an effective mode of delivery for  
55 supplementation in a healthy population. The data provide reference values and ranges for  
56 the rate of change of 25(OH)D for nutrkinetic analyses.

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63 **INTRODUCTION**

64 Vitamin D is essential for the homeostasis of calcium and phosphate and well known for its  
65 role in the development and maintenance of bone health <sup>1</sup>. Once vitamin D has been ingested  
66 or synthesised via sunlight exposure it requires activation in the liver to form 25  
67 hydroxyvitamin D (25(OH)D) and in the kidney to form 1,25 dihydroxyvitamin D (1,25  
68 (OH)<sub>2</sub>D<sup>2</sup>. 25(OH)D is the most abundant circulating form in the human body and is used to  
69 determine vitamin D status. 25(OH)D levels can be defined as; sufficient (≥50nmol/L),  
70 insufficient (30-<5049 nmol/L) or deficient (<30 nmol/L)<sup>3, 4</sup>. There is limited research on  
71 rates of repletion; one paper reports amounts for maintenance of blood 25(OH)D at 50nmol/L  
72 requires around 11-weeks of dosing at 1000 IU vitamin D per day <sup>5</sup>. Hypovitaminosis is  
73 evident worldwide and is a major public health concern <sup>6</sup> leading to advocacy for  
74 supplementation in at-risk groups. Research has also shown African Americans may require a  
75 higher dose of vitamin D supplementation to reach optimal serum 25(OH)D concentrations  
76 compared to the Caucasian participants <sup>7</sup>, perhaps as a result of lower baseline 25(OH)D  
77 levels in this population <sup>8</sup>. It is also known that serum 25(OH)D levels is inversely associated  
78 with body fat mass <sup>9</sup>.

79 Supplementation has classically been with capsule preparations, but sublingual sprays are  
80 increasingly available. There are few data available on the relative efficacy of each type of  
81 preparation on rate of change in circulating levels. Dose response studies using capsular  
82 delivery of vitamin D supplementation <sup>10-12</sup> have shown evidence of efficiency in increasing  
83 serum 25(OH)D levels which plateau and begin to decrease.

84 This study aimed to measure and compare the rate of change of circulating vitamin D in  
85 response to capsular or sub-lingual delivery of a daily vitamin D supplement.

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91 **METHODS**

92 *Study design*

93 This was a 6-week double blind, placebo-controlled 3-arm parallel design study. The  
94 participants attended three visits at The Medical School of The University of Sheffield. The  
95 initial visit included anthropometrics, issue of first batch of blood test kits and completion of  
96 a first self-test blood sample. The second visit occurred approximately two weeks after the  
97 initial visit for issue of further test kits and to support participant retention in the trial. The  
98 final visit required participants to return their preparation bottles and answer five questions  
99 regarding the study.

100 *Sample size and randomisation*

101 There were no data upon which to base a power calculation. 75 healthy male and female  
102 participants were recruited between January 2017 and February 2017 and were randomly  
103 assigned to one of three arms: (i) active capsules and placebo spray (n= 25); (ii) active spray  
104 and placebo capsules (n= 25); (iii) double placebo (n= 25). Participants were randomised  
105 according to a computer generated random sequence using block randomisation with a block  
106 size of 9, with randomisation undertaken by an independent outside source. The allocation  
107 sequence was not available to any member of the team until databases had been completed  
108 and locked.

109 *Participants*

110 The University of Sheffield Research Ethics Committee granted ethical approval for this  
111 study (Ref: 011865). Participants were recruited via poster advertisements at the University  
112 of Sheffield and through a student volunteer email list. Inclusion criteria required  
113 participants to be fit and healthy and aged between 18-50 years. Participants who reported  
114 any micronutrient supplement use (vitamin D, multi-vitamin, fish oils), recent or upcoming  
115 sunny holiday, pregnant or lactating, history of gastrointestinal disease, BMI >30, diabetes,  
116 >50 years of age were excluded. A total of 124 potential participants were approached, of  
117 which 49 were excluded:28 did not meet inclusion criteria and 21 had no further contact after  
118 initial consultation.

119 *Participant measures*

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120 The concentration of 25(OH)D in the blood was assessed by blood sample using a finger-  
121 prick blood spot kits at 0,3,7,14,21 and 42 days of supplementation. Blood spots were  
122 analysed by liquid chromatography tandem mass spectrometry (Waters TQD and Acquity  
123 UPLC) for total blood 25(OH)D (25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>). LC-MS was undertaken by City  
124 Assays, Department of Pathology, Birmingham Sandwell Hospitals NHS Trust. Previous  
125 work has shown that this method is comparable to other commercial assays with intra and  
126 interassay coefficients of <10% and <11% respectively<sup>13-15</sup>. Anthropometric measurements  
127 included; height, weight, BMI, and body fat percentage. Body fat and weight were measured  
128 using Tanita BC-543<sup>16</sup>. Skin tone was assessed by the researcher using 1= Caucasian, 2 =  
129 Asian, 3 = Black.

130 Qualitative opinion of capsules and sprays were assessed via exit questionnaire. Participants  
131 were asked if they had a preference between preparations

132 *“Did you have a preference between the two preparations? If so which one?”*

133 Answers were categorised as; “yes, the spray”, “yes, the capsule” and “no preference”.

### 134 *Intervention*

135 The vitamin D<sub>3</sub> and corresponding placebos were manufactured by Cultech Ltd., Port Talbot,  
136 UK and provided by Better You Ltd, Barnsley, UK. Preparations of vitamin D<sub>3</sub> and  
137 corresponding placebos were provided as 15 ml sprays and capsule. Each capsule and spray  
138 contained 3000 IU (75 µg) of vitamin D<sub>3</sub> per dose. The content of the spray and the capsule  
139 from the manufacturer was prepared to 97.5 µg/dose in order to maintain shelf life and to  
140 guarantee dose. Volunteers were instructed to ingest one capsule per day with water and one  
141 spray orally per day for 6 weeks. Compliance was measured by weighing the spray bottles  
142 and counting the remaining capsules at the end of the study. 86% and 96.4% of participants  
143 reached 100% compliance with the spray and capsules respectively.

### 144 *Adverse events*

145 Two participants reported that small blisters formed on cheek and tongue after the study  
146 began. One participant stopped using the preparations for the duration of the study. The  
147 second participant continued to use the preparations throughout the intervention.

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## Short Communication: Efficacy of sublingual vitamin D supplements

### 150 *Statistical analyses*

151 The data on vitamin D status was held by a third party until all other data entry was complete,  
152 spreadsheets were then merged and analysis was undertaken at a group level with blinding to  
153 group identity. Statistical analysis was performed using the Statistical Package for the Social  
154 Sciences (SPSS) (IBM SPSS Statistics for Windows, V.23; IBM Corp.). Percentage change  
155 in 25(OH)D from baseline was determined by analysis of variance (ANOVA) with  
156 Bonferroni correction. Pearson's correlations for rate of change in 25(OH)D per day was  
157 performed. Change in 25(OH)D over 6 time points were analysed by repeated measures  
158 ANOVA (there was a high failure rate in assessments of 25(OH)D at day 42, leading to the  
159 exclusion of this time point's data from the main analysis). Comparisons between percentage  
160 change in 25(OH)D from baseline in deplete and replete participants were assessed by Mann-  
161 Whitney U Test. Two-tailed tests were used in all analyses with the significance value of  
162 <0.05.

163

164 **RESULTS**

165 Baseline demographics are shown in Table 1 and a CONSORT is supplied in the online  
166 supplement. The three arms were similar in numbers, age, BMI, body fat, height, weight,  
167 skin tone, sex and baseline blood 25(OH)D concentrations. Baseline blood 25(OH)D  
168 concentration showed 59% of participants had insufficient/deficient vitamin D status  
169 (<50nmol/L).

170 Intention-to-treat analysis was used to evaluate the 5 time points up to day 21. Kolmogorov–  
171 Smirnov test (ks) indicates that the rate of change of 25(OH)D for both treatment arms follow  
172 a normal distribution ( $p = 0.200$ ). Raw data are available in the online supplementary bundle.  
173 Blood 25(OH)D concentration analysed across the time course in all three trial arms by  
174 ANOVA showed a significant improvement in 25(OH)D status in those receiving vitamin D  
175 compared to placebo. *Post hoc* analyses revealed significant differences between each of the  
176 active treatments and the placebo (capsules  $p = 0.003$ , spray  $p = 0.001$ ), but no difference  
177 between the active preparations at any time point (Fig 1A). As there are few available data on  
178 the rates of change of ingested vitamin D, we assessed the inter-individual and inter-  
179 preparation difference as change in whole blood nmol/L/d (Fig1Bi-ii). Whilst there was a  
180 range of rates in each dataset, assessment of the distribution of rate showed a monotonic  
181 normal distribution for both preparations with similar peak rates (Fig 1Biii-iv). Independent t-  
182 test was performed and found no significant difference between mean rates of change for  
183 capsule and spray. A Mann Whitney U Test was used to compare differences between  
184 deplete and replete participants within the treatment arms (replete data was not normally  
185 distributed with a KS score of  $p = 0.001$ ). There was a significant difference ( $p = 0.001$ ) in the  
186 percentage change of 25(OH)D between the replete and deplete from baseline to day 21.

187 In order to investigate a potential homeostatic mechanism for 25(OH)D status, we  
188 investigated the relationship between 25(OH)D status and rate of change (Fig 1Bv-vi). We  
189 observed inverse relationships between baseline whole blood 25(OH)D and rates of change  
190 over 21 days using Pearson's correlation for both the spray ( $r^2 = 0.255$ ,  $p = 0.012$ ) and capsule  
191 ( $r^2 = 0.351$ ,  $p = 0.003$ ).

192 In an exit interview about preference for either the spray or capsule for delivery, 60%  
193 preferred spray, 24% capsules and 16% did not express a preference.

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196 **DISCUSSION**

197 Advocacy for vitamin D supplementation for some subpopulations, interest in its use,  
198 availability of over-the-counter preparations, and lack of information on the factors  
199 predisposing to development of excessive levels collectively identify a need for research on  
200 comparative efficacy of preparations and the saturability of uptake. This study used two  
201 commonly available vitamin D preparations; the widely used capsules and a more novel  
202 sublingual spray to investigate these factors.

203 Our findings show that a sublingual spray is equally effective at raising blood 25(OH)D  
204 concentrations with no significant difference between rate of change compared to capsules in  
205 this study population. The study participants reported a preference for the sublingual spray,  
206 and this study demonstrates that this delivery platform is of comparable efficacy. Sublingual  
207 sprays may be particularly advantageous in people with pre-existing malabsorption  
208 conditions or swallowing problems. Our analysis shows for the first time the likely rate of  
209 change in 25(OH)D and the range of these rates, albeit in a relatively small, healthy sample.  
210 The monotonicity of our rate distribution suggests a limited spread of rates with no  
211 suggestions of outliers or subpopulations, however the relatively homogenous profile of the  
212 study population, whilst an advantage for this pilot exploration, is a limitation in terms of the  
213 prediction of rates in other groups (older adults, different ethnicities). A recent review <sup>17</sup>  
214 does offer suggested optimal supplementation rates to achieve adequate serum 25(OH)D  
215 levels (75 nmol/L) in regional, population and age-specific groups.

216 These data also suggest that baseline 25(OH)D status may influence the rate of change, as a  
217 correlation between baseline status and change exhibited a moderate inverse relationship,  
218 furthermore the circulating 25(OH)D concentrations started to level off towards the end of  
219 the intervention. This is in agreement with previous research by Lips *et al.* 2001 who  
220 reported that change in serum 25OHD in response to 6 months vitamin D supplementation  
221 was dependent on baseline vitamin D status, with the greatest change observed in people with  
222 the lowest baseline vitamin D <sup>18</sup>. Our research complements the previous work by  
223 undertaking an intervention over a shorter timeframe with sampling along the timecourse,  
224 demonstrating a baseline status-dependent response to the intervention and the possibility of a  
225 plateau effect. The mechanistic basis of this is unclear, and it is notable that both delivery  
226 platforms exhibit this effect, implying control in both enteric and transbuccal absorption.  
227 Future work may address the strength of this inferred relationship more thoroughly and

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228 identify implied control mechanisms. This study had no data from which a power  
229 calculation could be determined, however the data presented herein may prove useful for the  
230 design of prospective intervention studies.

231 A limitation to this study is that we cannot show definitive absorption of the sublingual  
232 supplement. However, sublingual routes of drug delivery are established in pharmacokinetic  
233 studies<sup>19, 20</sup>. Recent research presented by Satia and colleagues found superior sublingual  
234 absorption compared to capsules in patients with malabsorption issues<sup>21</sup>. Participants were  
235 given clear guidelines on how to use the spray. Further studies should assess, 25(OH)D, and  
236 1,25(OH)D levels in localized tissues with the use of labelled D3.

### 237 CONCLUSIONS

238 In summary, we have shown the capsule and sublingual spray are equally effective at  
239 delivery of a vitamin D supplement. There was an overwhelming preference (64%) for the  
240 spray over capsules for mode of supplement delivery. Rate of change, reported for the first  
241 time, exhibits a monotonic distribution in this population. This study saw a reduction in  
242 25(OH)D levels as blood 25(OH)D concentrations increased over 21 days in both  
243 preparations. This suggests the oral spray has the same known mechanism as the capsule for  
244 slower conversions of vitamin D<sub>3</sub> when concentrations are higher<sup>22</sup>. These data illustrate the  
245 need for further studies to explore rate of change across mixed population groups, especially  
246 those identified as high risk.

### 247 ACKNOWLEDGEMENTS

248

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250 This work was jointly supported by BetterYou Ltd and The University of Sheffield

251

### 252 CONFLICT OF INTEREST

253 BetterYou co-funded this PhD and provided the supplements and placebos. This sponsor  
254 was not involved in the study design, delivery or interpretation of the data, which was  
255 undertaken entirely by The University of Sheffield.

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342 **FIGURE LEGENDS**

343 **Figure 1. Efficacy and rates of vitamin D uptake with differing delivery platforms.**

344 Panel A shows change in vitamin D circulating levels over time in each of the three study  
345 arms, presented as absolute levels (panel Ai) or relative to baseline (Panel Aii). Panel B  
346 shows rates of uptake comparing spray (left column) with capsules (right column). Panels Bi  
347 and Bii show ladder plots for individuals in each arm of the trial plotting difference in  
348 vitamin D between day 0 and day 21 (the abscissa for uptake, based on Panel A). Rates were  
349 derived as nmol/L/day and binned into 5nmol bins (Panels Biii and Biv). KS tests showed the  
350 data were normally distributed (capsules  $p=0.200$ , spray  $p=0.200$ ). Finally, the rates for each  
351 individual were correlated with the baseline serum concentration for that individual (Panels  
352 Bv and Bvi). The  $r^2$  and  $p$  values for correlations are indicated.

353 **Table 1. Demographic characteristics and mean serum vitamin D at baseline and exit.**

354 The data are presented in means $\pm$ SD. Baseline characteristics are given along with exit  
355 serum 25(OH)D. Significant values are  $p > 0.005$ . A one-way ANOVA was used to compare  
356 means at baseline and exit for serum 25(OH)D.

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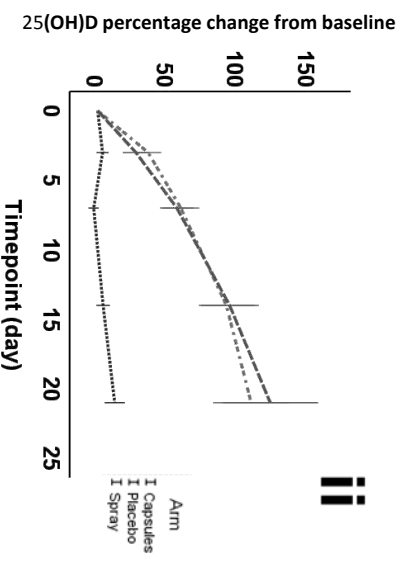
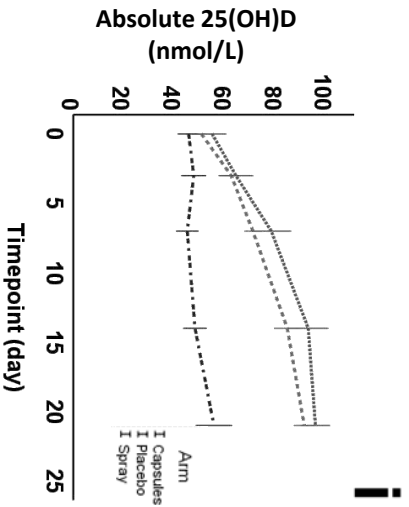
374

375 Table 1. Characteristics and mean serum vitamin D at baseline and exit

	Capsules	Placebo	Spray	All	P Value
Participants n	25	25	25	75	
Female n	14	10	15	39	0.326
Mean age ( $\pm$ SD)	22.9( $\pm$ 4.82)	22.4( $\pm$ 2.72)	21.7( $\pm$ 3.05)	22.4( $\pm$ 3.65)	0.504
BMI ( $\text{kg}/\text{m}^2$ )	23.6( $\pm$ 2.95)	22.7( $\pm$ 2.72)	23.8( $\pm$ 2.59)	23.4( $\pm$ 2.77)	0.294
Bodyfat (%)	23.4( $\pm$ 7.75)	19.1( $\pm$ 5.91)	23.7( $\pm$ 7.65)	22.1( $\pm$ 7.37)	0.043
Height (m)	171.3( $\pm$ 7.54)	173.5( $\pm$ 10.20)	170.0( $\pm$ 8.35)	171.6( $\pm$ 8.77)	0.357
Weight (kg)	69.6( $\pm$ 10.71)	68.6( $\pm$ 12.77)	69.0( $\pm$ 11.32)	69.1( $\pm$ 11.48)	0.958
Skintone	22/2/1	24/0/1	25/0/0	71/2/2	0.268
Mean serum 25(OH)D nmol/L (baseline)	50.7( $\pm$ 19.73)	45.6( $\pm$ 21.30)	54.9( $\pm$ 27.84)	50.5( $\pm$ 23.24)	0.381
Mean serum 25(OH)D nmol/L (exit)	91.35( $\pm$ 19.78)	55.62( $\pm$ 34.40)	95.78( $\pm$ 28.03)	81.13( $\pm$ 33.02)	0.001

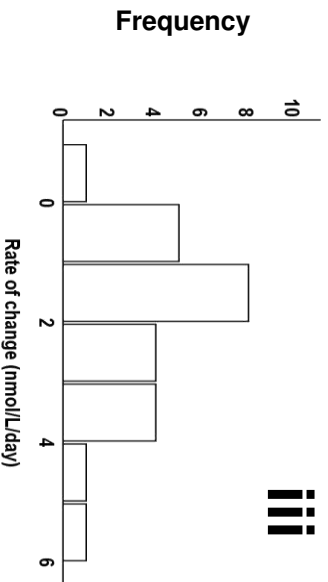
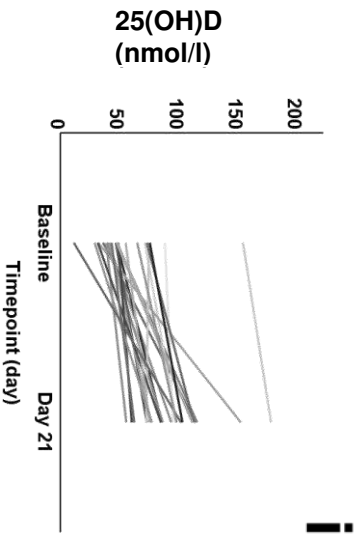
376

# A



# B

## Spray



## Capsules

