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## Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations --Manuscript Draft--

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<b>Funding Information:</b>	<table border="1"> <tr> <td>University of Sheffield</td> <td>Dr. Bernard Corfe</td> </tr> <tr> <td>BetterYou Ltd</td> <td>Dr. Bernard Corfe</td> </tr> </table>	University of Sheffield	Dr. Bernard Corfe	BetterYou Ltd	Dr. Bernard Corfe
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<b>Abstract:</b>	<p><b>Background:</b> Vitamin D is critical for skeletal health and is increasingly associated with other pathologies encompassing gastrointestinal, immunological, psychological effects. A significant proportion of the population exhibit suboptimal levels of vitamin D, particularly in Northern latitudes in winter. Supplementation is advocated, but few data are available on relative efficacy of preparations, or rates of uptake, or whether serum status may influence uptake. There has been considerable interest in the potential use of sublingual sprays for delivery of nutrient supplements, but data on efficacy remains sparse.</p> <p><b>Methods:</b> A randomised, placebo-controlled, 3-arm parallel design study was conducted in healthy volunteers (n=75) to compare uptake rates of vitamin D supplementation in capsule and sublingual spray preparations over a six week period between January and April 2017. Serum 25(OH)D concentrations were measured after day 0, 3, 7, 14, 21 and 42 days of supplementation with 3000IU per diem.</p> <p><b>Results:</b> Baseline measurements show 25(OH)D deficiency, insufficiency and sufficiency in 14.9%, 44.6% and 40.5% of the participants respectively. There was a significant elevation in serum concentrations of 25(OH)D in the treatment arms (capsule p=0.003, spray p=0.001) compared to control. The capsule and spray were equally efficacious with average change in serum vitamin D of 2 nmol/ml/day. The data suggest that uptake rates are higher in individuals with lower serum vitamin D. 71% of the participants preferred the oral spray preparation to the capsule.</p> <p><b>Conclusions:</b> A sublingual vitamin D spray is an effective and preferential mode of delivery for supplementation in a healthy population. Achievable rates of vitamin D increment are suggested to be around 2 nmol/ml/day.</p>				
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## Short Communication: Efficacy of sublingual vitamin D supplements

# 1 Efficacy and comparative uptake rates of sublingual and 2 capsular vitamin D preparations

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32 **ABSTRACT**

33 **Background:** Vitamin D is critical for skeletal health and is increasingly associated with other  
34 pathologies encompassing gastrointestinal, immunological, psychological effects. A  
35 significant proportion of the population exhibit suboptimal levels of vitamin D, particularly in  
36 Northern latitudes in winter. Supplementation is advocated, but few data are available on  
37 relative efficacy of preparations, or rates of uptake, or whether serum status may influence  
38 uptake. There has been considerable interest in the potential use of sublingual sprays for  
39 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 uptake rates of vitamin D supplementation in capsule  
42 and sublingual spray preparations over a six week period between January and April 2017.  
43 Serum 25(OH)D concentrations were measured after day 0, 3, 7, 14, 21 and 42 days of  
44 supplementation with 3000IU *per diem*.

45 **Results:** Baseline measurements show 25(OH)D deficiency, insufficiency and sufficiency in  
46 14.9%, 44.6% and 40.5% of the participants respectively. There was a significant elevation in  
47 serum concentrations of 25(OH)D in the treatment arms (capsule p=0.003, spray p=0.001)  
48 compared to control. The capsule and spray were equally efficacious with average change in  
49 serum vitamin D of 2 nmol/ml/day. The data suggest that uptake rates are higher in individuals  
50 with lower serum vitamin D. 71% of the participants preferred the oral spray preparation to  
51 the capsule.

52 **Conclusions:** A sublingual vitamin D spray is an effective and preferential mode of delivery  
53 for supplementation in a healthy population. Achievable rates of vitamin D increment are  
54 suggested to be around 2 nmol/ml/day.

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61 **INTRODUCTION**

62 Vitamin D is essential for the homeostasis of calcium and phosphate and well known for its  
63 role in the development and maintenance of bone health. (1). Once vitamin D has been ingested  
64 or synthesised via sunlight exposure it requires activation in the liver to form 25  
65 hydroxyvitamin D (25(OH)D) and in the kidney to form 1,25 dihydroxyvitamin D (1,25  
66 (OH)<sub>2</sub>D (2). 25(OH)D is the most abundant circulating form in the human body and is used to  
67 determine vitamin D status (3). Vitamin D levels can be defined as; sufficient (>50nmol/L),  
68 insufficient (31-49 nmol/L) or deficient (<30 nmol/L) (4). There is limited research on rates  
69 of repletion; one paper reports amounts for maintenance of serum 25(OH)D at 50nmol/L  
70 requires around 11-weeks of dosing at study requires 1000 IU vitamin D per day (5).  
71 Hypovitaminosis is evident worldwide and is a major public health concern (6) leading to  
72 advocacy for supplementation in at-risk groups (7). Research has also shown African  
73 Americans may require a higher dose of vitamin D supplementation to reach optimal serum  
74 25(OH)D concentrations compared to the Caucasian participants, perhaps as a result of lower  
75 baseline vitamin D levels in this population (8).

76 Supplementation has classically been with capsule preparations, but sublingual sprays are  
77 increasingly available. There are few data available on the relative efficacy of each type of  
78 preparation, of uptake and repletion rates, and of any potential interaction between vitamin D  
79 status and uptake.

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83 **METHODS**

84 *Study design*

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

92 *Sample size and randomisation*

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

100 *Participants*

101 The University of Sheffield Research Ethics Committee granted ethical approval for this study  
102 (Ref: 011865). Participants were recruited via poster advertisements at the University of  
103 Sheffield and through a student volunteer email list. All participants were fit and healthy and  
104 aged between 18-50 years. Participants who reported any micronutrient supplement use  
105 (vitamin D, multi-vitamin, fish oils), recent or upcoming sunny holiday, pregnant or lactating,  
106 history of gastrointestinal disease, BMI >30, diabetes, >50 years of age were excluded.

107 *Patient measures*

108 Participant's serum 25(OH)D status was assessed by blood sample using at home finger-prick  
109 blood spot kits at 0,3,7,14,21 and 42 days of supplementation. Blood spots were analysed by  
110 liquid chromatography tandem mass spectrometry (Waters TQD and Acquity UPLC) for total  
111 serum 25(OH)D (25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>). LC-MS was undertaken by City Assays,

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112 Department of Pathology, Birmingham Sandwell Hospital. Anthropometric measurements  
113 included; height, weight, BMI, and body fat percentage.

114 Qualitative opinion of capsules and sprays were assessed via exit questionnaire and focus  
115 groups.

### 116 *Intervention*

117 The vitamin D<sub>3</sub> and corresponding placebos were manufactured by Cultech Ltd., Port Talbot,  
118 UK and provided by Better You Ltd, Barnsley, UK. Preparations of vitamin D<sub>3</sub> and  
119 corresponding placebos were provided as 15 mL sprays and capsule. Each capsule and spray  
120 contained 3000 IU (75 ug) of vitamin D<sub>3</sub> per dose. Volunteers were instructed to ingest one  
121 capsule per day with water and one spray orally per day for 6 weeks. Compliance was  
122 measured by weighing the spray bottles and counting the remaining capsules at the end of the  
123 study. 86% of participants reached 100% compliance with the spray.

### 124 *Adverse events*

125 Two participants reported that small blisters formed on cheek and tongue after use of the spray  
126 began. One participant stopped using the preparation for the duration of the study. The second  
127 participant continued to use the spray throughout the intervention despite discomfort.

### 128 *Statistical analyses*

129 Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS)  
130 (IBM SPSS Statistics for Windows, V.23; IBM Corp.). Percentage change in 25(OH)D from  
131 baseline was determined by analysis of variance (ANOVA) with Bonferroni correction.  
132 Spearman's correlations for rate of change in vitamin D per day was performed. Change in  
133 vitamin D over 6 time points were analysed by repeated measures ANOVA (there was a high  
134 failure rate in terms of assessment of vitamin D at day 42 leading to the exclusion of this  
135 timepoint's data from the main analysis). Comparisons between percentage change in  
136 25(OH)D from baseline in deplete and replete participants were assessed by independent t tests.  
137 Two-tailed tests were used in all analyses with the significance value of <0.05.

138

139 **RESULTS**

140 Baseline demographics are shown in Table 1. The three arms were similar in numbers, age,  
141 BMI, body fat, height, weight, skin tone, sex and baseline serum 25(OH)D concentrations.  
142 Baseline serum 25(OH)D levels showed 59% of participants had insufficient/deficient levels  
143 (<50nmol/L).

144 Serum vitamin D levels analysed across the time course in all three trial arms by ANOVA  
145 showed a significant improvement in vitamin D status in those receiving vitamin D compared  
146 to placebo. *Post hoc* analyses revealed significant differences between each active and placebo  
147 (capsules  $p= 0.003$ , spray  $p= 0.001$ ), but no difference between the active preparations at any  
148 time point (Fig 1A). As there are few available data on uptake rate of ingested vitamin D, we  
149 assessed the inter-individual and inter-preparation difference as change in serum nmol/ml/d  
150 (Fig1Bi-ii). Whilst there was a range of rates in each dataset, assessment of the distribution of  
151 rate showed a monotonic normal distribution for both preparations with similar peak rates (Fig  
152 1Biii-iv). Independent t-test was performed and found no significant difference between mean  
153 rates of change for capsule and spray.

154 In order to investigate a potential homeostatic mechanism for vitamin D status, we investigated  
155 the relationship between serum status and uptake rate (Fig 1Bv-vi). We observed inverse  
156 relationships between baseline serum 25(OH)D and uptake rates over 21 days using  
157 Spearman's correlation for both the spray ( $r^2$  0.26,  $P= 0.014$ ) and capsule ( $r^2$  0.35,  $P=0.003$ ).

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

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161



162 **DISCUSSION**

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

169 Our findings show that a sublingual spray is equally effective at raising serum 25(OH)D  
170 concentrations with no significant difference between uptake rates compared to capsules in this  
171 study population. The study participants reported a preference for the sublingual spray, and  
172 this study demonstrates that this delivery platform is of comparable efficacy. Sublingual sprays  
173 may be particularly advantageous in people with pre-existing malabsorption conditions or  
174 swallowing problems. Our analysis shows for the first time the likely rates of vitamin D uptake  
175 and the spread of the uptake rates, albeit in a relatively small, healthy sample. The monotonicity  
176 of our rate distribution suggests a limited spread of rates with no suggestions of outliers or  
177 subpopulations, however the relatively homogenous profile of the study population, whilst an  
178 advantage for this pilot exploration, is a limitation in terms of the prediction of rates in other  
179 groups (older adults, different ethnicities). The availability of reference values for rate will  
180 allow other populations to be compared to examine the effects of age, ethnicity, BMI, GI  
181 function upon rate.

182 These data also suggest that vitamin D status may influence uptake rate, as a correlation  
183 between baseline status and uptake rate exhibited a moderate inverse relationship, furthermore  
184 the circulating levels started to saturate towards the end of the intervention. The mechanistic  
185 basis of this is unclear, and it is notable that both delivery platforms exhibit this effect, implying  
186 control in both enteric and transbuccal absorption. Future work may address the strength of this  
187 inferred relationship more thoroughly and identify implied control mechanisms.

188 **CONCLUSIONS**

189 In summary, we have shown the capsule and sublingual spray are equally effective at delivery  
190 of vitamin D supplement. There was an overwhelming preference (64%) for the spray over  
191 capsules for mode of supplement delivery. Absorption rates, reported for the first time, exhibit  
192 a monotonic distribution in this population. This study saw a reduction in uptake of vitamin D3  
193 as serum 25(OH)D levels increased over 21 days which suggests vitamin D absorption may be

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194 influenced by vitamin D status. This data illustrates the need for further studies to explore  
195 uptake rates across mixed population groups, especially those identified as high risk.

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

202

### 203 **CONFLICT OF INTEREST**

204 BetterYou markets vitamin D supplements.

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261 **FIGURE LEGENDS**

262 **Figure 1. Efficacy and rates of vitamin D uptake with differing delivery platforms.** Panel  
263 A shows change in vitamin D circulating levels over time in each of the three study arms,  
264 presented as absolute levels (panel Ai) or relative to baseline (Panel Aii). Panel B shows rates  
265 of uptake comparing spray (left column) with capsules (right column). Panels Bi and Bii show  
266 ladder plots for individuals in each arm of the trial plotting difference in vitamin D between  
267 day 0 and day 21 (the abscissa for uptake, based on Panel A). Rates were derived as  
268 nmol/ml/day and binned into 5nmol bins (Panels Biii and Biv). KS tests showed the data were  
269 normally distributed (capsules  $p=0.200$ , spray  $p=0.200$ ). Finally, the rates for each individual  
270 were correlated with the baseline serum concentration for that individual (Panels Bv and Bvi).  
271 The  $r^2$  and  $p$  values for correlations are indicated.

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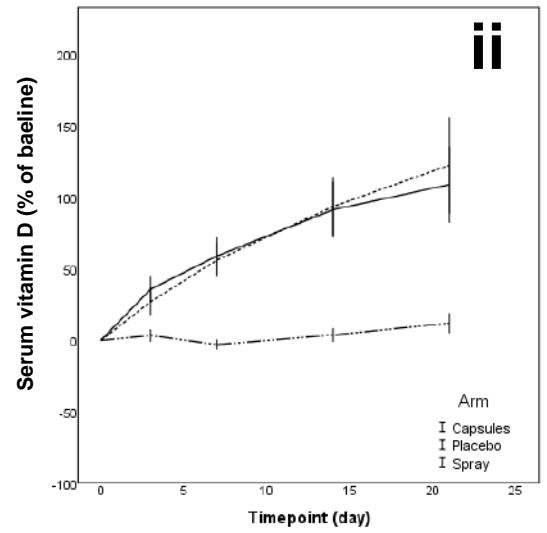
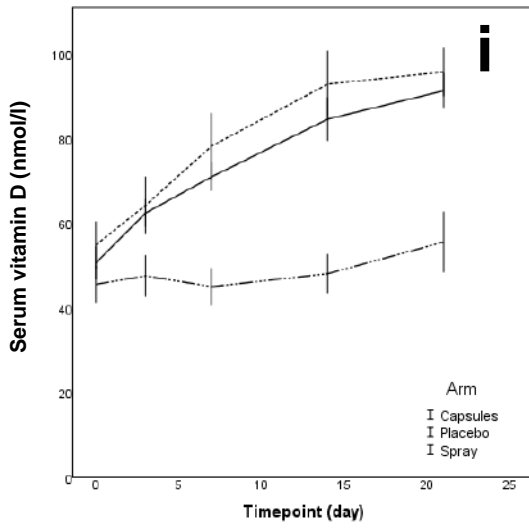
Table 1 Baseline characteristics of participants

	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 ( $\pm$ 4.62)	22.4 ( $\pm$ 2.72)	21.7 ( $\pm$ 3.05)	22.4 ( $\pm$ 3.65)	0.504
Mean serum 25(OH)D nmol/L	50.7 ( $\pm$ 19.73)	45.6 ( $\pm$ 21.30)	54.2 ( $\pm$ 27.84)	50.5 ( $\pm$ 23.24)	0.38
BMI	23.7 ( $\pm$ 2.95)	22.7 ( $\pm$ 2.72)	23.8 ( $\pm$ 2.59)	23.4 ( $\pm$ 2.77)	0.294
Body fat	23.4 ( $\pm$ 7.75)	19.1 ( $\pm$ 5.91)	23.7 ( $\pm$ 7.65)	22.1 ( $\pm$ 7.37)	0.043
Height	171.3 ( $\pm$ 7.54)	173.5 ( $\pm$ 10.20)	170.0 ( $\pm$ 8.35)	171.6 ( $\pm$ 8.77)	0.357
Weight	69.6 ( $\pm$ 10.71)	68.6 ( $\pm$ 12.77)	69.0 ( $\pm$ 11.32)	69.1 ( $\pm$ 11.48)	0.958
Skin tone	22/2/1	24/0/1	25/0/0	71/2/2	0.268

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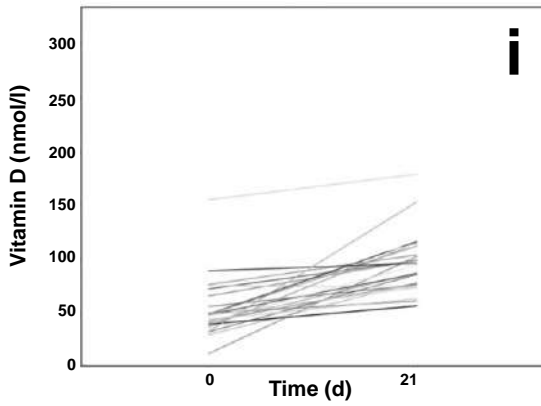
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# A



# B

## Spray



## Capsules

