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

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ABSTRACT

Background: 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 achievable or typical rates of change. There has been considerable interest in the potential use of sublingual sprays for delivery of nutrient supplements, but data on efficacy remains sparse.

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

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

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

INTRODUCTION

Vitamin D is essential for the homeostasis of calcium and phosphate and well known for its role in the development and maintenance of bone health [1] . Once vitamin D has been ingested or synthesised via sunlight exposure it requires activation in the liver to form 25 hydroxyvitamin D (25(OH)D) and in the kidney to form 1,25 dihydroxyvitamin D (1,25 $(OH)_2D^2$. 25(OH)D is the most abundant circulating form in the human body and is used to determine vitamin D status. 25(OH)D levels can be defined as; sufficient (≥50nmol/L), insufficient (30-<5049 nmol/L) of deficient (<30 nmol/L)[3, 4] . There is limited research on rates of repletion; one paper reports amounts for maintenance of blood 25(OH)D at 50nmol/L requires around 11-weeks of dosing at 1000 IU vitamin D per day [5]. Hypovitaminosis is evident worldwide and is a major public health concern [6] leading to advocacy for supplementation in at-risk groups. Research has also shown African Americans may require a higher dose of vitamin D supplementation to reach optimal serum 25(OH)D concentrations compared to the Caucasian participants [7], perhaps as a result of lower baseline 25(OH)D levels in this population [8]. It is also known that serum 25(OH)D levels is inversely associated with body fat mass [9].

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

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

METHODS

Study design

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

Sample size and randomisation

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

Participants

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

Participant measures

The concentration of 25(OH)D in the blood was assessed by blood sample using a finger-prick blood spot kits at 0,3,7,14,21 and 42 days of supplementation. Blood spots were analysed by liquid chromatography tandem mass spectrometry (Waters TQD and Acquity UPLC) for total blood 25(OH)D (25(OH)D_2 and 25(OH)D_3). LC-MS was undertaken by City Assays, Department of Pathology, Birmingham Sandwell Hospitals NHS Trust. Previous work has shown that this method is comparable to other commercial assays with intra and interassay coefficients of <10% and <11% respectively [13-15]. Anthropometric measurements included; height, weight, BMI, and body fat percentage. Body fat and weight were measured using Tanita BC-543 [16]. Skin tone was assessed by the researcher using 1= Caucasian, 2 = Asian, 3 = Black.

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

"*Did you have a preference between the two preparations? If so which one?"*

Answers were categorised as; "yes, the spray", "yes, the capsule" and "no preference".

Intervention

The vitamin D_3 and corresponding placebos were manufactured by Cultech Ltd., Port Talbot, UK and provided by Better You Ltd, Barnsley, UK. Preparations of vitamin D_3 and corresponding placebos were provided as 15 ml sprays and capsule. Each capsule and spray contained 3000 IU (75 µg) of vitamin D_3 per dose. The content of the spray and the capsule from the manufacturer was prepared to 97.5 µg/dose in order to maintain shelf life and to guarantee dose. Volunteers were instructed to ingest one capsule per day with water and one spray orally per day for 6 weeks. Compliance was measured by weighing the spray bottles and counting the remaining capsules at the end of the study. 86% and 96.4% of participants reached 100% compliance with the spray and capsules respectively.

Adverse events

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

Statistical analyses

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

RESULTS

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

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

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

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

DISCUSSION

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

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

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

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

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

CONCLUSIONS

In summary, we have shown the capsule and sublingual spray are equally effective at delivery of a vitamin D supplement. There was an overwhelming preference (64%) for the spray over capsules for mode of supplement delivery. Rate of change, reported for the first time, exhibits a monotonic distribution in this population. This study saw a reduction in 25(OH)D levels as blood 25(OH)D concentrations increased over 21 days in both preparations. This suggests the oral spray has the same known mechanism as the capsule for slower conversions of vitamin D_3 when concentrations are higher [22] . These data illustrate the need for further studies to explore rate of change across mixed population groups, especially those identified as high risk.

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CONFLICT OF INTEREST

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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±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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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 (±SD)	22.9(±4.82)	22.4(±2.72)	21.7(±3.05)	22.4(±3.65)	0.504
BMI (kg/m^2)	23.6(±2.95)	22.7(±2.72)	23.8(±2.59)	23.4(±2.77)	0.294
Bodyfat (%)	23.4(±7.75)	19.1(±5.91)	23.7(±7.65)	22.1(±737)	0.043
Height (m)	171.3(±7.54)	173.5(±10.20)	170.0(±8.35)	171.6(±8.77)	0.357
Weight (kg)	69.6(±10.71)	68.6(±12.77)	69.0(±11.32)	69.1(±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(±19.73)	45.6(±21.30)	54.9(±27.84)	50.5(±23.24)	0.381
Mean serum 25(OH)D nmol/L (exit)	91.35(±19.78)	55.62(±34.40)	95.78(±28.03)	81.13(±33.02)	0.001

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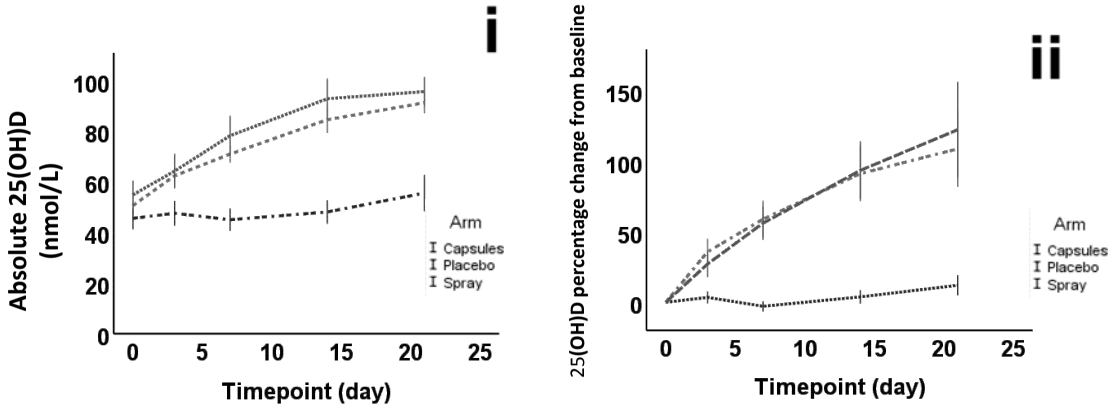
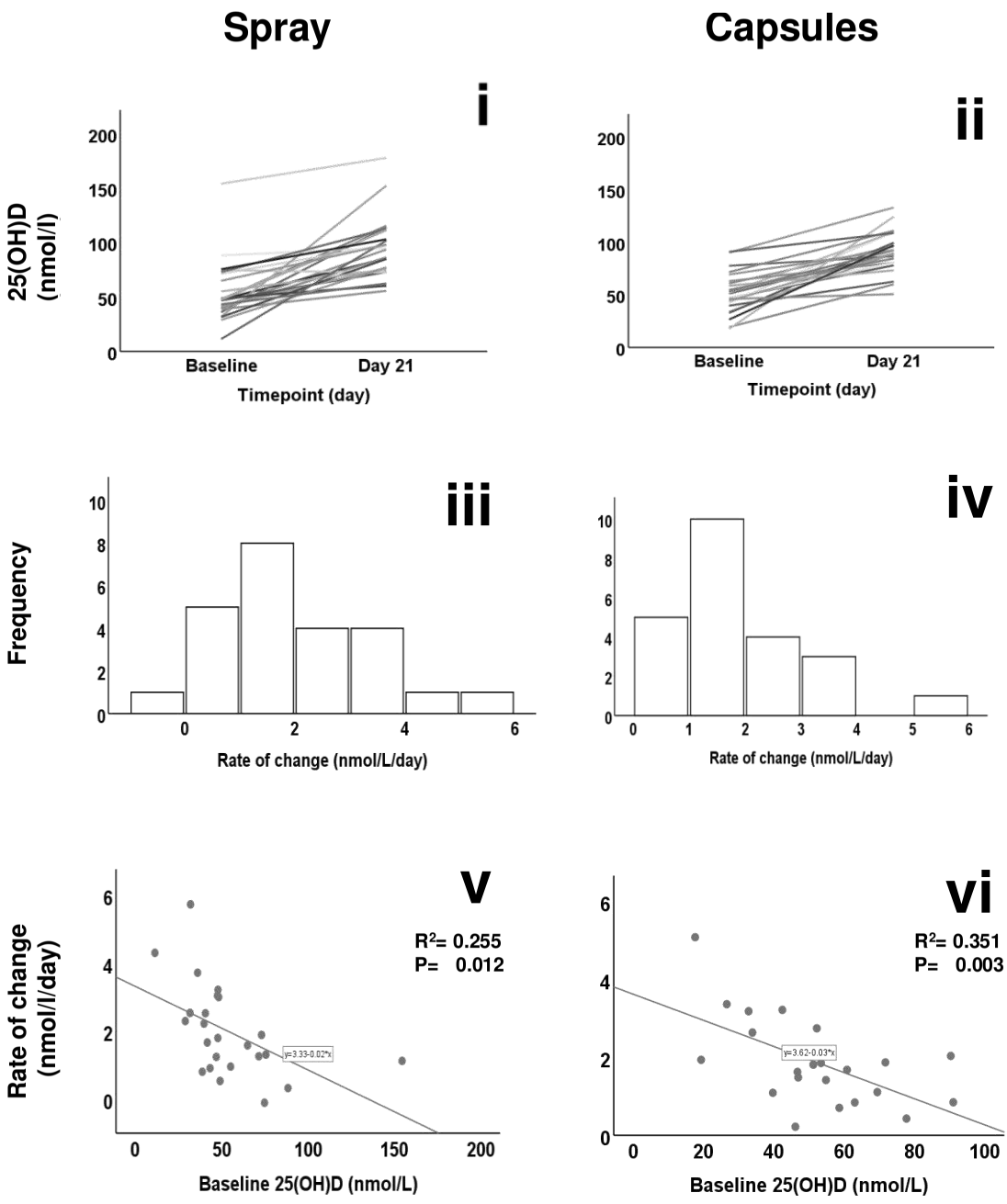

i
Absolute 25(OH)D (nmol/L)
Timepoint (day)
Arm
Capsules
Placebo
Spray
ii
25(OH)D percentage change from baseline
Timepoint (day)
Arm
Capsules
Placebo
Spray

B


Spray
Capsules
i
ii
25(OH)D (nmol/l)
Baseline
Day 21
Timepoint (day)
iii
iv
Frequency
Rate of change (nmol/L/day)
v
vi
Rate of change (nmol/l/day)
R²= 0.255
P= 0.012
R²= 0.351
P= 0.003
Baseline 25(OH)D (nmol/L)