European Journal of Nutrition

Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations

--Manuscript Draft--

Manuscript Number:	
Full Title:	Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations
Article Type:	Short Communication
Keywords:	vitamin D; uptake rate; healthy subjects
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Funding Information:	University of Sheffield — Dr. Bernard Corfe
	BetterYou Ltd — Dr. Bernard Corfe
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 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. Methods: 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. Results: 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. Conclusions: 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.
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Efficacy and comparative uptake rates of sublingual and capsular vitamin D preparations

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Short title: Efficacy of sublingual vitamin D supplements

Keywords: vitamin D, oral spray, capsules, uptake rates, supplementation

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 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.

Methods: 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*.

Results: 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.

Conclusions: 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.

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 (3). Vitamin D levels can be defined as; sufficient (>50nmol/L), insufficient (31-49 nmol/L) of deficient (<30 nmol/L) (4). There is limited research on rates of repletion; one paper reports amounts for maintenance of serum 25(OH)D at 50nmol/L requires around 11-weeks of dosing at study requires 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 (7). 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, perhaps as a result of lower baseline vitamin D levels in this population (8).

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, of uptake and repletion rates, and of any potential interaction between vitamin D status and uptake.

METHODS

Study design

This was a 6-week double blind, placebo-controlled 3-arm parallel design study. The participants attended three visits to The Medical School at 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 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. All participants were 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.

Patient measures

Participant's serum 25(OH)D status was assessed by blood sample using at home 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 serum 25(OH)D (25(OH)D2 and 25(OH)D3). LC-MS was undertaken by City Assays,

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

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

Intervention

The vitamin D3 and corresponding placebos were manufactured by Cultech Ltd., Port Talbot, UK and provided by Better You Ltd, Barnsley, UK. Preparations of vitamin D3 and corresponding placebos were provided as 15 mL sprays and capsule. Each capsule and spray contained 3000 IU (75 ug) of vitamin D_3 per 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% of participants reached 100% compliance with the spray.

Adverse events

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

Statistical analyses

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. Spearman's correlations for rate of change in vitamin D per day was performed. Change in vitamin D over 6 time points were analysed by repeated measures ANOVA (there was a high failure rate in terms of assessment of vitamin D at day 42 leading to the exclusion of this timepoint's data from the main analysis). Comparisons between percentage change in 25(OH)D from baseline in deplete and replete participants were assessed by independent t tests. Two-tailed tests were used in all analyses with the significance value of <0.05.

RESULTS

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

Serum vitamin D levels analysed across the time course in all three trial arms by ANOVA showed a significant improvement in vitamin D status in those receiving vitamin D compared to placebo. *Post hoc* analyses revealed significant differences between each active and 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 uptake rate of ingested vitamin D, we assessed the inter-individual and inter-preparation difference as change in serum nmol/ml/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.

In order to investigate a potential homeostatic mechanism for vitamin D status, we investigated the relationship between serum status and uptake rate (Fig 1Bv-vi). We observed inverse relationships between baseline serum 25(OH)D and uptake rates over 21 days using Spearman's correlation for both the spray (r^2 0.26, P= 0.014) and capsule (r^2 0.35, 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 serum 25(OH)D concentrations with no significant difference between uptake rates 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 rates of vitamin D uptake and the spread of the uptake 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). The availability of reference values for rate will allow other populations to be compared to examine the effects of age, ethnicity, BMI, GI function upon rate.

These data also suggest that vitamin D status may influence uptake rate, as a correlation between baseline status and uptake rate exhibited a moderate inverse relationship, furthermore the circulating levels started to saturate towards the end of the intervention. 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.

CONCLUSIONS

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

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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198 **ACKNOWLEDGEMENTS**

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200 **FINANCIAL SUPPORT**

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

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203 **CONFLICT OF INTEREST**

204 BetterYou markets vitamin D supplements.

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218 **REFERENCES**

1. Ross AC, Manson JE, Abrams SA *et al.* (2011) The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D From the Institute of Medicine: What Clinicians Need to Know. *Obstetrical & Gynecological Survey* **66**, 356-357.

2. Calvo MS, Whiting SJ, Barton CN (2005) Vitamin D intake: A global perspective of current status, vol. 135, pp. 310-316.

3. Holick MF, Holick MF (2010) *Vitamin D [electronic resource] : physiology, molecular biology, and clinical applications*. 2nd ed. ed. Totowa, N.J.: Totowa, N.J. : Humana, 2010.

4. Palacios C, Gonzalez L (2014) Is vitamin D deficiency a major global public health problem? *Journal of Steroid Biochemistry and Molecular Biology* **144**, 138-145.

5. Quraishi SA, Camargo CA, Manson JE (2016) Low vitamin D status in Europe: moving from evidence to sound public health policies. *The American journal of clinical nutrition* **103**, 957.

6. Lips P (2006) Vitamin D physiology. *Progress in Biophysics and Molecular Biology* **92**, 4-8.

7. Dulai PS, Singh S, Marquez E *et al.* (2016) Chemoprevention of colorectal cancer in individuals with previous colorectal neoplasia: systematic review and network meta-analysis. *BMJ* **355**.

8. Zhang X, Giovannucci E (2011) Calcium, vitamin D and colorectal cancer chemoprevention. *Best Practice & Research Clinical Gastroenterology* **25**, 485-494.

9. Cai GH, Li MX, Lu L *et al.* (2015) The Current Role and Therapeutic Targets of Vitamin D in Gastrointestinal Inflammation and Cancer. *Current Pharmaceutical Design* **21**, 2917-2923.

10. Hiew HJ, Naghibi M, Wu J *et al.* (2013) Efficacy of high and low dose oral vitamin D replacement therapy in inflammatory bowel disease: Single centre cohort. *Gut* **62**, A2.

11. Souberbielle JC, Body JJ, Lappe JM *et al.* (2010) Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice.

12. Haroon M, FitzGerald O (2012) Vitamin D Deficiency: Subclinical and Clinical Consequences on Musculoskeletal Health. *Current Rheumatology Reports* **14**, 286-293.

13. Satia MC, Mukim AG, Tibrewala KD *et al.* (2015) A randomized two way cross over study for comparison of absorption of vitamin D3 buccal spray and soft gelatin capsule formulation in healthy subjects and in patients with intestinal malabsorption. *Nutrition journal* **14**, 114.

14. Todd JJ, McSorley EM, Pourshahidi LK *et al.* (2016) Vitamin D 3 supplementation in healthy adults: a comparison between capsule and oral spray solution as a method of delivery in a wintertime, randomised, open-label, cross-over study. **116**, 1402-1408.

15. SACN (2016) SACN vitamin D and health report.

16. Dou R, Ng K, Giovannucci EL *et al.* (2016) Vitamin D and colorectal cancer: molecular, epidemiological and clinical evidence. *The British journal of nutrition* **115**, 1643.

17. Tazzyman S, Richards N, Trueman AR *et al.* (2015
) Vitamin D associates with improved quality of life in participants with irritable bowel syndrome: outcomes from a pilot trial. *BMJ Open Gastroenterology* **2**, e000052.

18. Peterlik M, Cross HS (2009) Vitamin D and calcium insufficiency-related chronic diseases: molecular and cellular pathophysiology. *European Journal of Clinical Nutrition* **63**, 1377-1386.

FIGURE LEGENDS

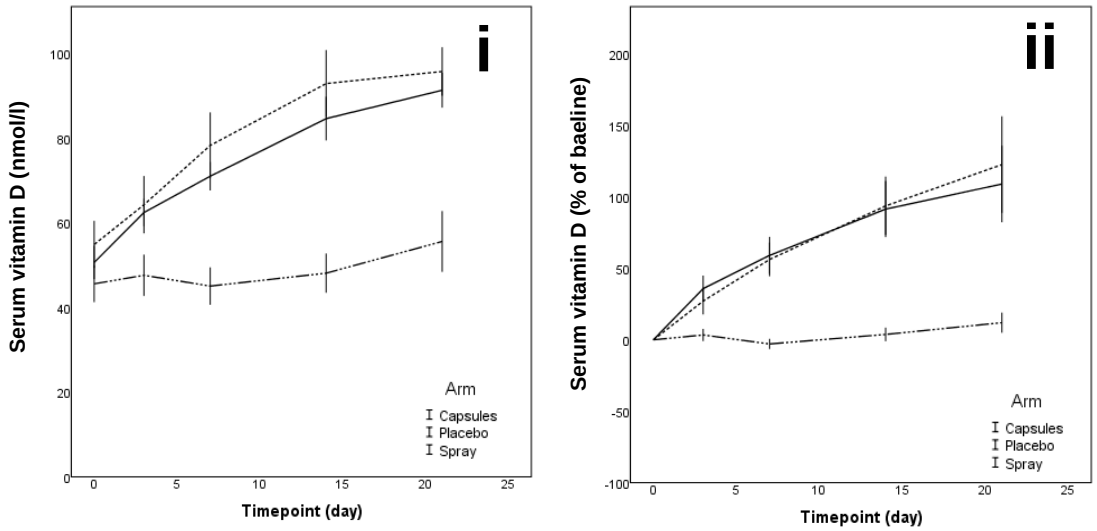
Figure 1. Efficacy and rates of vitamin D uptake with differing delivery platforms. Panel A shows change in vitamin D circulating levels over time in each of the three study arms, presented as absolute levels (panel Ai) or relative to baseline (Panel Aii). Panel B shows rates of uptake comparing spray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

Table 1 Baseline characteristics of participants

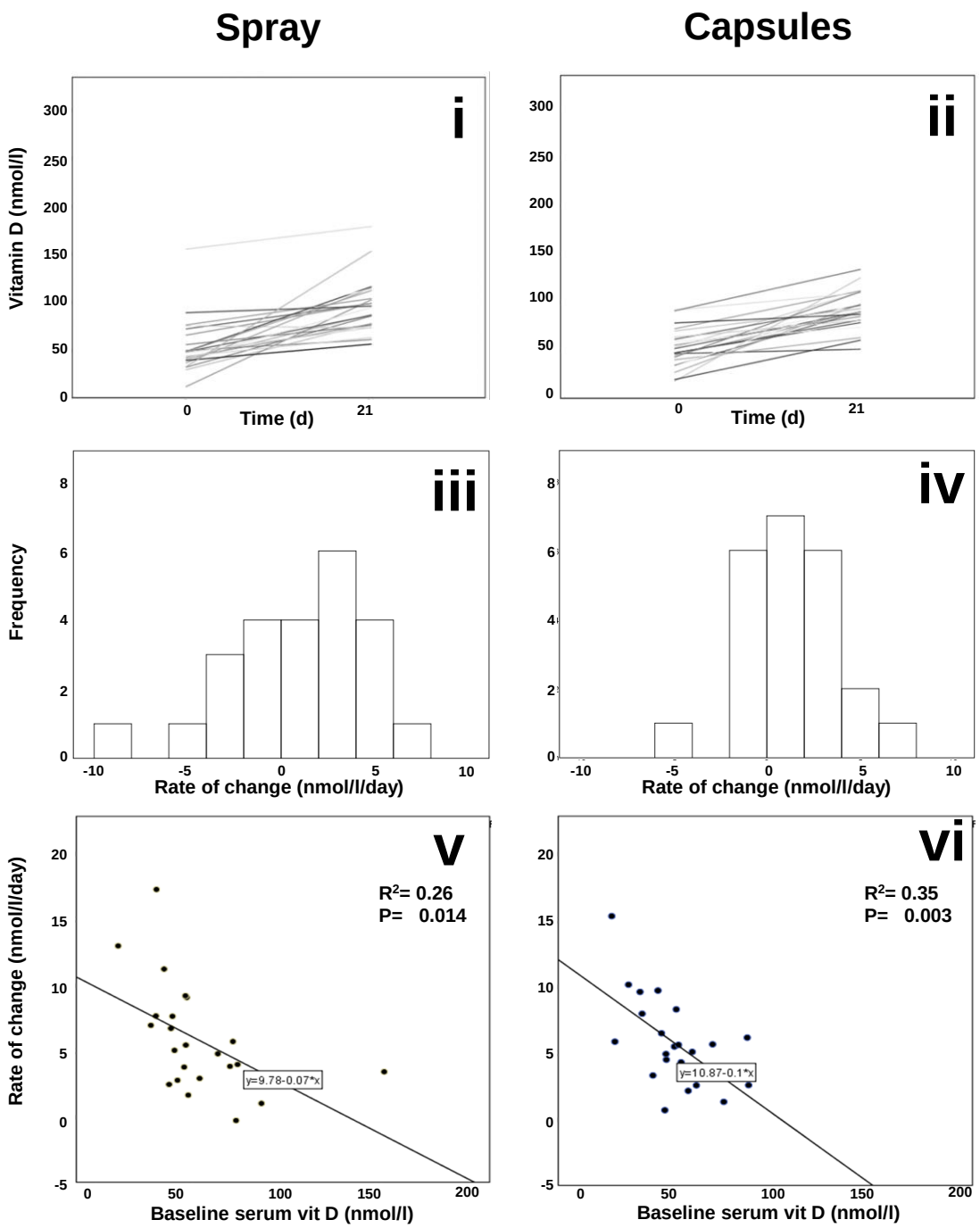
	Capsules	Placebo	Spray	All	P Value
Participants n	25	25	25	75	
Female n	14	10	15	39	0.326
Mean age (±*SD*)	22.9 (±4.62)	22.4 (±2.72)	21.7 (±3.05)	22.4 (±3.65)	0.504
Mean serum 25(OH)D nmol/L	50.7(±19.73)	45.6(±21.30)	54.2(±27.84)	50.5(±23.24)	0.38
BMI	23.7(±2.95)	22.7(±2.72)	23.8(±2.59)	23.4(±2.77)	0.294
Body fat	23.4(±7.75)	19.1(±5.91)	23.7(±7.65)	22.1(±7.37)	0.043
Height	171.3(±7.54)	173.5(±10.20)	170.0(±8.35)	171.6(±8.77)	0.357
Weight	69.6(±10.71)	68.6(±12.77)	69.0(±11.32)	69.1(±11.48)	0.958
Skin tone	22/2/1	24/0/1	25/0/0	71/2/2	0.268

Figure

A


i
Serum vitamin D (nmol/l)
Timepoint (day)
Arm
Capsules
Placebo
Spray
ii
Serum vitamin D (% of baeline)
Timepoint (day)
Arm
Capsules
Placebo
Spray

B


Spray
Capsules
i
ii
Vitamin D (nmol/l)
Time (d)
iii
iv
Frequency
Rate of change (nmol/l/day)
v
vi
Rate of change (nmol/l/day)
R2= 0.26
P= 0.014
y=9.78-0.07*x
R2= 0.35
P= 0.003
y=10.87-0.1*x
Baseline serum vit D (nmol/l)