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**The efficacy of different vitamin D supplementation delivery methods on serum 25(OH)D: a randomised double-blind placebo trial.**

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Galloway	0000-0002-2265-5732	Data analysis

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SunVit supplied serum 25(OH)D<sub>3</sub> testing kits and vitamin D<sub>3</sub> supplementation (tablet and liquid)

## 1 **Summary**

2 The aim of the study was to see which method of taking vitamin D supplements (pill, liquid, skin  
3 application) resulted in the greatest increase in blood vitamin D levels. The oral pill had the best  
4 increase then skin application with a penetrator agent.

## 5 **Abstract**

6 Background: The use of vitamin D supplementation has increased due to greater recognition of  
7 widespread deficiency.

8 Aims: There has been little research on the effectiveness of different delivery methods and therefore  
9 the aim of was to test the efficacy of different delivery methods on serum 25(OH)D.

10 Methods: Using a randomised repeated measures double-blind placebo design (registered under  
11 ClinicalTrials.gov Identifier no. NCT03463642), changes in serum 25(OH)D over a 4-week period using  
12 a capillary spot method were monitored. 62 female participants blindly chose a number related to a  
13 supplementation delivery method: pill placebo, pill, oral liquid, oral liquid placebo, Skin oil  
14 application (SOA) placebo, SOA plus vitamin D<sub>3</sub> suspension, or SOA plus vitamin D<sub>3</sub> suspension with  
15 essential oil enhancer; active vitamin D supplements contained 100,000IU. Participants took their  
16 allocated supplements over a 24-hr period with serum 25(OH)D retested 4 weeks later. Liquid  
17 chromatography-tandem mass spectrometry method was applied to dried blood spot samples by an  
18 independent laboratory.

19 Results: ANCOVA reported a significant difference between the groups ( $F_{1,6}=146.68$ ;  $p<0.001$ ,  $\eta^2 =$   
20  $.51$ ). Separate analysis within the delivery methods (pill, SOA, oral liquid) indicated significant  
21 differences between the active and placebo supplementation groups ( $p<0.01$ ). Post hoc analysis of  
22 absolute changes indicated vit D pill and SOA + vit D + essential oil had significant increases ( $p<0.05$ )  
23 in serum 25(OH)D compared to all other interventions with no significant difference between them

24 Conclusions: In human participants vitamin D oral pill has the greatest effect on serum 25(OH)D  
25 levels. Skin oil application delivery of vitamin D using a penetrator enhancer has also been shown to  
26 be an effective method of delivery.

27 **Keywords:** Skin penetrator enhancer, pill, oral liquid, human, vitamin D,

## 28 **Introduction**

29 Vitamin D<sub>3</sub> is mainly synthesized in the skin during exposure to ultraviolet light of the sun during the  
30 summer months<sup>1,2</sup>, though food, specifically fatty fish, can also be a source<sup>3</sup>. A recent study has  
31 suggested that exposure to sunlight might only have a limited effect<sup>4</sup>; the Binkley et al. study  
32 indicated a variable response to sunlight exposure with some participants maintaining a low  
33 25(OH)D<sub>3</sub> level despite abundant sun exposure. A recent review of the effect of sunscreen on serum  
34 25(OH)D concluded that sunscreen had little effect for healthy adults with recreational sunlight  
35 exposure<sup>5</sup>. The reviewed controlled studies, 3 showed no change and 4 showed a decrease in serum  
36 25(OH)D, though a series of methodological limitations including a lack of personal UVR exposure  
37 ( $n=4$ ) and no baseline measure of serum 25(OH)D ( $n=3$ ) highlight areas of concern. One short-term  
38 study (1-week high UVI exposure) noted significant increases in serum 25(OH)D in the sunscreen  
39 group (SPF 15) suggesting that only very low levels of UVB were required for the biosynthesis of  
40 vitamin D<sub>3</sub><sup>6</sup>. These studies only used sunscreens with a factor of 15-17, whilst a number of  
41 organisations, such as the American Cancer Society and the British Association of Dermatology,  
42 promote the use of higher protection (SPF 30-50).

43 The current research indicates that to achieve optimal levels of 25(OH)D<sub>3</sub>, supplementation is  
44 required<sup>7</sup>. Although there has been much research on supplementation dose levels there is still a lot  
45 of variation, this is possibly due to recommendations being targeted at specific clinical conditions,  
46 e.g. bone health. Ross et al<sup>8</sup> suggested 600 IU/day to maintain bone health, whilst others<sup>9</sup> have

47 suggested a higher daily dose (1500-2000 IU/day) is needed. Ekwaru et al<sup>10</sup> suggested that high  
48 doses had a diminishing effect with serum 25(OH)D<sub>3</sub> increasing by 12nmol/L per 1000IU for  
49 supplementation between 0-1000 IU/day and only 1.1nmol/L for supplementation between 15,000-  
50 20,000 IU/day and there was a need to account for body weight with obese patients requiring 2-3  
51 times more vit D and those overweight, 1.5 times. Other studies have utilised 1-2 high dose bolus  
52 supplementation to beneficial effect<sup>11-14</sup>.

53 There has been little research on different delivery methods for supplementation. Biancuzzo et al<sup>15</sup>  
54 compared liquid and pill oral supplementation and noted no difference between the delivery  
55 methods. Leventis and Kiely<sup>14</sup> reported no difference between a single high bolus deliver by either  
56 intramuscular injection or tablet. A number of transdermal delivery methods have been examined  
57 with varying success<sup>16</sup>. Pre-treatment of ex-vivo skin with ethanol increased penetration but would  
58 eventually lead to toxicity<sup>17</sup>; Ramezanli et al<sup>18</sup> used nanoparticles coated with hydrophilic and  
59 hydrophobic polymers to beneficial effect; whilst Devaux et al<sup>19</sup> concluded that vitamin D enhanced  
60 creams applied to the skin only penetrates deep enough to treatment of skin disorders, such as  
61 psoriasis. Three studies have looked at the effect of penetration enhancers in vitamin D enhanced  
62 creams. D' Angelo Costa et al<sup>20</sup> used various penetration enhancers in either a gel or cream  
63 formulation on ex-vivo human skin; gel formulation with cereal alcohol and propylene glycol noted  
64 vitamin D<sub>3</sub> penetration to stratum corneum (4 hours post application) and epidermis and dermis (24  
65 hours post application) but no active vitamin D<sub>3</sub> was found in receptor fluid, therefore skin  
66 penetration was not fully achieved. Sadat-Ali et al<sup>21</sup> used aloe vera as a delivery system for dermal  
67 delivery of vitamin D and reported significant changes in serum 25(OH)D over a 3 month period.  
68 Essential oils have been shown to enhance different drugs ability to penetration the lower skin  
69 layers through either the disintegration of intercellular lipid structure between corneocytes and the  
70 conformational modification of proteins<sup>22</sup>. Bubshait et al<sup>23</sup> used a proniosomal delivery system over  
71 a 4-month period with a similar beneficial effect on serum 25(OH)D. Therefore, topical delivery  
72 systems seem to be a safe and suitable delivery method of vitamin D.

73 The aim of the present study was to examine the efficacy of different delivery methods on serum  
74 25(OH)D changes in healthy adult females. Various delivery methods of vitamin D supplementation  
75 are available to consumers but there have been no studies providing evidence of whether one  
76 delivery method is superior to others. We wanted to compare the delivery of 100,000IU vitamin D<sub>3</sub>  
77 by three methods. Two methods of oral supplementation (pill [prolonged release] and liquid  
78 [immediate release]), and delivery through the skin (with and without a penetrator enhancer).

79

## 80 **Materials and Methods**

81 Experimental design: The trial was a randomised double-blind placebo design and was registered  
82 with the US Clinical Trials (NCT03463642). An independent technician randomly assigned numbers  
83 (1-70) to the supplement samples: placebo pill, vitamin D pill, oral placebo liquid, oral vitamin D  
84 liquid, placebo skin oil application (SOA), SOA plus vitamin D<sub>3</sub> suspension, or SOA plus vitamin D<sub>3</sub>  
85 suspension with essential oil enhancer. Volunteers then randomly selected a number between 1-70.  
86 The data collectors and the statistician were blind to the participant's group (intervention or  
87 placebo) and only after the statistical analysis was completed were the group codes reviewed by the  
88 independent researcher.

89 Participants: Advertisements were placed around campus and blast emails via the university  
90 intranet. Power analysis based upon effect size (0.8), alpha error probability (0.05), power 0.95, 7  
91 groups tested twice (repeated measures)<sup>24</sup>, estimated the required total sample size to be 40  
92 participants. To account for potential drop out 10 participants were recruited per group. Exclusion  
93 criteria included any participant that was taking vitamin supplementation, were non-Caucasian, had  
94 a skin condition that would prevent them from applying oil to their skin or were taking, had taken a

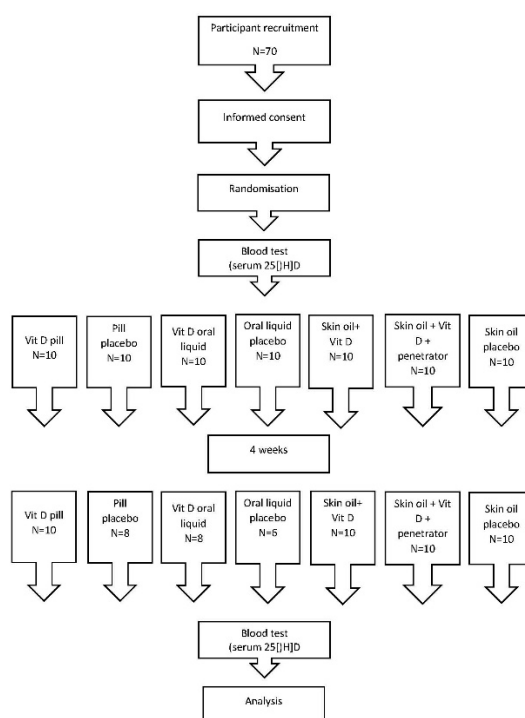
95 sunny holiday in the last 6-months, planned to take a sunny holiday during the study period, or had  
 96 been taking in the past 6-months, oestrogen-based contraception<sup>25</sup>. Seventy Caucasian volunteers  
 97 were recruited from a female university population that included students and academics (latitude  
 98 52.58° N) during the month of March. Sunlight hours during this month averaged 3.8 eight hours per  
 99 day with a mean UV total index of 0.86<sup>26</sup>. Eight participants dropped out over the intervention  
 100 period.

101 Table 1: Participant descriptive data

	Delivery method	N	Age (yrs)	Height (cm)	Body mass (kg)	BMI (kg/m <sup>2</sup> )	Serum 25(OH)D (nmol.L <sup>-1</sup> )
Pill	Placebo	8	28 ±10.24	166.1 ±7.39	69.5 ±7.29	20.9 ±1.66	32.79 ±5.39
	Vit D	10	29 ±14.61	161.5 ±6.41	64.3 ±6.63	19.9 ±1.39	40.03 ±24.18
Oral liquid	Placebo	6	21 ±4.68	172.2 ±9.95	68.3 ±4.72	19.9 ±1.52	29.58 ±6.54
	Vit D	8	31 ±8.62	170.1 ±9.93	69.1 ±7.64	20.3 ±1.97	26.15 ±8.34
Skin application	Oil placebo	10	24 ±5.99	165.6 ±9.03	67.0 ±9.23	20.8 ±1.92	32.87 ±12.6
	Oil + Vit D	10	22 ±4.56	169.2 ±6.32	70.6 ±12.33	20.8 ±2.97	31.54 ±12.43
	Oil + Vit D + essential oil	10	27 ±11.71	166.3 ±5.32	68.8 ±9.28	20.7 ±2.61	33.87 ±20.39

Pre intervention group differences: age  $p=0.243$ ; height  $p=0.197$ ; body mass  $p=0.824$ ; BMI  $p=0.936$ ; serum 25(OH)D  $p=0.632$

102



103

104 Figure 1: Participant flow chart

105 Protocol: Participants read and signed an informed consent form prior to data collection. Age  
 106 (years), height (centimetres with a Seca height measure) and body mass (kg with digital Seca scale)

107 were collected on all participants prior to a blood sample. Using a capillary blood spot sample  
 108 method, the tester used a single use lancet on the participant's selected finger and the first show of  
 109 blood was wiped away. Four blood spots were collected on the blood collection card (City Assays,  
 110 Birmingham UK) making sure the spots were of sufficient size and had soaked through the paper.  
 111 The card was then sealed before being sent to an independent laboratory for analysis (City Assays,  
 112 Pathology Department Sandwell and West Birmingham Hospital NHS Trust, UK). Each participant  
 113 was asked to select a number from a number grid 1-70. The relevant supplement sample was then  
 114 issued to the participant with the instructions to complete the supplementation within 24 hours  
 115 (table 2). The skin application group was asked to apply the oil twice in the 24-hour period on their  
 116 limbs and torso until it was fully absorbed. The active pill and oral supplementations were all  
 117 available commercially (Sunvit-D3 Ltd, UK), the active skin application used commercially available  
 118 hypoallergenic mineral oil (Johnson & Johnson, Inc) combined with the aforementioned oral  
 119 supplementation and the essential oil (Miaroma, France). The placebo supplements were either  
 120 manufactured by a university pharmacy department (pills), commercially available syrup (PureGusto,  
 121 UK) and hypoallergenic mineral oil (Johnson & Johnson, Inc). All participants confirmed completion  
 122 of their supplementation via email to the independent researcher. Four weeks later participants  
 123 were called in for their post-supplementation blood sample using the same methodology. Feedback  
 124 was provided to each participant on their second test serum 25(OH)D3 levels and appropriate advice  
 125 provided. The participants that had selected a placebo sample, were offered subsequent  
 126 supplementation.

127 Table 2: Intervention Groups

	Active	Placebo
Pill	100 Vitamin D <sub>3</sub> pills (1000IU, dicalcium phosphate, microcrystalline cellulose, silicium dioxide, magnesium stearate)	100 pills (dicalcium phosphate, microcrystalline cellulose, silicium dioxide, magnesium stearate)
Oral liquid	100 drops vitamin D <sub>3</sub> suspension in orange syrup (1,000 IU per drop)	100 drops of orange syrup
Skin oil application	100,000 IU vitamin D <sub>3</sub> suspension in mineral oil (paraffinum liquidum, isopropyl palmitate, parfum) (100ml total)  100,000 IU vitamin D <sub>3</sub> suspension in mineral oil (paraffinum liquidum, isopropyl palmitate, parfum) with 10ml tangerine essential oil (100ml total)	100ml of mineral oil (paraffinum liquidum, isopropyl palmitate, parfum) coloured with food colourant to match active oil sample

128

129 Blood analysis:

130 A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was applied to dried  
 131 blood spot samples, utilising blood spot calibrators<sup>27</sup>. The method is standardised against  
 132 conventional 25-hydroxyvitamin D<sub>3</sub> and D<sub>2</sub> LC-MS/MS service for serum ( $r^2=0.98$ ; intra assay  
 133 variation <10%; inter assay variation <11%). Blood spot results show good comparability to  
 134 serum/plasma results with a 3.3% difference (95% CI: -6.3-12.1%;  $p=0.48$ )<sup>28</sup>. The City Assays  
 135 laboratory participates in the DEQAS external quality assurance scheme.

136

137 Data analysis:

138 Group data were tested for homogeneity/sphericity prior to further analysis using Levene's test of  
 139 equality of variance (SPSS v20). Analysis of covariance (ANCOVA) was conducted to detect changes in  
 140 serum 25(OH)D; the dependent variable was post vitamin D<sub>3</sub>; fixed factors were the different groups  
 141 (pill placebo, pill, oral liquid, oral liquid placebo, skin oil application [SOA] placebo, SOA plus vitamin  
 142 D<sub>3</sub> suspension, or SOA plus vitamin D<sub>3</sub> suspension with essential oil enhancer), and pre vitamin D<sub>3</sub>  
 143 was the covariate. Bonferroni post hoc analyses were used where applicable. Analysis of variance  
 144 within the delivery methods (pill, skin oil application, oral liquid) was carried on the absolute change  
 145 in serum 25(OH)D with Bonferroni post hoc analyses. Significance for all analyses was set at  $p \leq 0.05$ .

146

## 147 Results

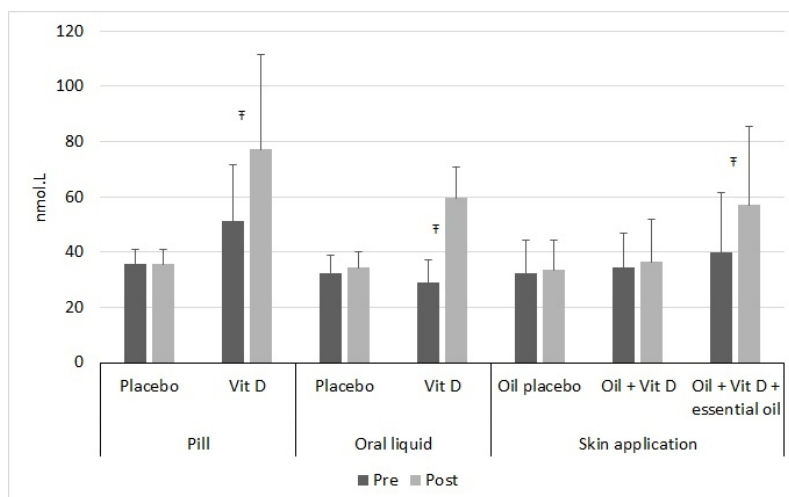
148 Pre-intervention there were no statistical differences between the groups for anthropometric  
 149 measurements or baseline serum 25(OH)D ( $p > 0.05$ ). Post-intervention ANCOVA reported a  
 150 significant difference in serum 25(OH)D between the groups ( $F_{1,6} = 146.68$ ;  $p < 0.001$ ,  $\eta^2 = .51$ ); post  
 151 hoc comparisons revealed that SOA placebo, SOA +vit D, oral liquid placebo and pill placebo groups  
 152 did not significantly increase ( $p > 0.05$ ). The vit D pill group had significantly higher serum 25(OH)D  
 153 than the following groups: SOA placebo ( $p < .01$ ), SOA +vit D ( $p < .05$ ), oral liquid placebo ( $p < .01$ )  
 154 and pill placebo ( $p < .01$ ). For the active supplementation groups, no significant difference was noted  
 155 between them with the exception of vit D pill group and SOA +vit D ( $p < 0.01$ ) (Table 3).

156 Table 3: Pre and post intervention serum 25(OH)D<sub>3</sub> for the different delivery methods  
 157 [mean, standard deviation (95%CI)]

Delivery method		Pre	Post	Change
		nmol.L <sup>-1</sup>		
Pill	Placebo	32.79 ±5.39 (28.28, 37.29)	32.89 ±5.23 (28.52, 37.27)	0.11 ±1.31 (-6.87, 7.09)
	Vit D	40.03 ±24.018 (21.45, 58.62)	74.39 ±34.26 (42.70, 106.07) <sup>‡</sup>	26.03 ±19.68 (18.57, 33.49) <sup>‡</sup>
Oral liquid	Placebo	29.58 ±6.54 (22.71, 36.44)	31.61 ±5.75 (25.57, 37.62)	2.04 ±2.90 (-6.02, 10.10)
	Vit D	26.15 ±8.34 (19.18, 33.12)	34.40 ± 6.47 (28.99, 39.81) <sup>‡</sup>	8.25 ±4.29 (1.27, 15.23)
Skin application	Oil placebo	32.87 ±12.6 (20.84, 44.90)	30.77 ±29.25 (22.45, 39.09)	7.81 ±10.59 (0.83, 14.79)
	Oil + Vit D	31.54 ±12.43 (21.15, 41.93)	33.73 ±15.38 (20.86, 46.59)	2.19 ±7.05 (-4.79, 9.17)
	Oil + Vit D + essential oil	33.87 ±20.39 (18.19, 49.54)	48.13 ±28.71 (18.00, 78.26) <sup>‡</sup>	14.92 ±10.80 (6.86, 22.98) <sup>‡</sup>

158 <sup>‡</sup> Significant changes over time ( $p < 0.05$ ); <sup>‡</sup>significantly greater change

159 Analysis of the actual change in serum 25(OH)D between the pre and post- tests indicated significant  
 160 differences ( $F_{6,49} = 5.016$ ,  $p < 0.001$ ;  $\eta^2 = .55$ ) between the supplementation methods. Within each  
 161 delivery method (pill, skin oil application, oral liquid) there were significant differences between the  
 162 active and placebo supplementation groups ( $p < 0.01$ ). Post hoc analysis indicated that vit D pill and  
 163 SOA + vit D + essential oil had significantly greater increases in serum 25(OH)D compared to all other  
 164 interventions ( $p < 0.05$ ). There was no significant difference in the amount of serum 25(OH)D change  
 165 between them. The skin oil application groups reported a significant difference between the SOA +  
 166 vit D + essential oil and both the SOA + vit D and SOA placebo groups (Fig 2), but not between the  
 167 SOA + vit D and the SOA placebo group.



168

169 Figure 2: Serum 25(OH)D changes over time for the different delivery methods

170

## 171 Discussion

172 Vitamin D insufficiency, within the general population, has been highlighted in both the academic  
 173 and popular press over the last decade<sup>2,7,29,30</sup> with the advice to take supplementation<sup>31-34</sup> especially  
 174 during the winter months.. Previous studies have examined the effects of different supplementation  
 175 doses on serum 25(OH)D<sup>8,9,35</sup>. Consumers have an array of different supplementation methods  
 176 available (pill, liquid, skin oil application, nasal spray, injection etc) without evidence of their efficacy.  
 177 Biancuzzo et al<sup>15</sup> compared liquid and oral vitamin D supplementation and the present study added  
 178 skin oil application to examine the efficacy of different delivery methods. With the exception of  
 179 vitamin D skin oil application, all the vitamin D active supplementation methods significantly  
 180 increased serum 25(OH)D compared with their equivalent placebo. The greatest change in serum  
 181 25(OH)D for an equal supplementation dose (100,000IU) was noted for pill supplementation (26.03  
 182  $\pm$ 19.68 nmol.L<sup>-1</sup>) followed by skin oil application with essential oil (14.92  $\pm$ 10.80 nmol.L<sup>-1</sup>) and finally  
 183 oral liquid (8.25  $\pm$ 4.29 nmol.L<sup>-1</sup>). Skin oil application without the addition of an essential oil reported  
 184 a similar change as the placebo groups but less than the other active interventions.

185 Biancuzzo et al<sup>15</sup> supplemented participants over a 11-week period with 1000IU/day and reported  
 186 no significant difference between the two delivery methods (oral liquid and pill) though the liquid  
 187 supplementation increase was approximately 70% whilst the pill supplementation was 42%. In our  
 188 study participants took the equivalent of 100,000IU over a 24-hr period. The reduced efficacy of  
 189 bolus oral liquid versus slower release pill may be due to rate limited hepatic hydroxylation of vit D  
 190 to 25(OH)D following rapid intestinal absorption. The benefits of an essential oil as a dermalogical  
 191 penetration enhancer is highlighted with the significantly greater absorption rates between the  
 192 different skin application groups. Human skin has a multifunctional role but one of its primary  
 193 functions is to act as a barrier against xenobiotic materials such as drugs<sup>36</sup>. The penetration  
 194 enhancer interacts with the skin's stratum corneum, disrupting its lipid bilayers by modifying  
 195 permeant diffusivity<sup>37</sup>, thereby reducing the barrier properties. This may be due to the competitive  
 196 hydrogen bonding of oxygen containing monoterpenes with ceramide head groups, thereby  
 197 breaking the interlamellar hydrogen bonding network of lipid bilayer of stratum corneum and new  
 198 polar pathways or channels are formed. This study highlights the efficacy of essential oils as a  
 199 penetration enhancer in the delivery of vitamin D across the skin barrier. D'Angelo Costa et al<sup>20</sup> used  
 200 the same amount of vitamin D<sub>3</sub> (100,000IU) on *ex-vivo* skin application but used different  
 201 penetration enhancers (cereal alcohol, soybean lecithin, isopropyl palmitate, propylene glycerol and  
 202 ethoxydiglycol) and although they noted vitamin D<sub>3</sub> did reach the epidermis and dermis within 24



203 hours, it was not detectable in the receptor fluid. A direct comparison to Sadat-Ali et al<sup>21</sup> study is  
 204 not possible as in that study the total amount of vitamin D delivered was not reported beyond the  
 205 concentration of the gel (5000IU/gram). The total usage of the gel, area of the body applied to and  
 206 frequency was not reported. The current study only recruited female participants within a  
 207 premenopausal age range to increase the compliance with skin application and reduce possible  
 208 confounding; further studies are required to examine whether there are sex or age effects.

209 A limitation of the current study could be participant compliance the administration of the  
 210 interventions. Although we asked for confirmation that the supplement had been taken/used within  
 211 the 24-hour time period direct observation of the administration might have strengthened the  
 212 methodology particularly in the oral pill and skin application conditions. The size of the bolus  
 213 (100,000IU) particularly the active oral liquid supplementation could have saturated the absorption  
 214 capabilities of the gut if taken all at once and a more measured ingestion of three intakes over the  
 215 24-hour period might have been more efficacious. The drop-out of participants within the study was  
 216 an issue, all participants were from an academic environment and the issue was scheduling post-  
 217 intervention tests before a vacation and placement periods, this asymmetrically effected the oral  
 218 liquid groups more than the other groups.

219 The present study has highlighted the effectiveness of different vitamin D supplementation delivery  
 220 methods. It has demonstrated that dermal delivery in the presence of a penetration enhancer is as  
 221 beneficial as oral supplementation. In patients that already take a number of oral medications there  
 222 is increased risk of non-compliance<sup>38</sup> and therefore an alternative to oral supplementation is  
 223 beneficial. The use of high dose oral pill bolus, to reduce the potential of non-compliance has been  
 224 reported previously<sup>12,34</sup> and the present study has underlined this outcome for oral pill and liquid  
 225 delivery and dermal delivery.

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