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

Wyon, MA[1]; Wolman, R[2]; Martin, C[3]; Galloway, S[1]

[1] Research Centre for Sport, Exercise and Performance, Institute of Sport and Health Sciences, University of Wolverhampton, UK

[2] Department of Rheumatology and Sport and Exercise Medicine, Royal National Orthopaedic Hospital, Stanmore, UK

[3] Worcester Biomedical Science Research Group, Institute of Science and the Environment, St. John's Campus, University of Worcester, Henwick Grove, Worcester, WR2 6AJ, UK

Corresponding Author

Prof Matthew Wyon
Research Centre for Sport, Exercise and Performance,
University of Wolverhampton,
Gorway Rd, Walsall, UK
Email: m.wyon@wlv.c.uk

Author	Orchid ID	Contribution
Wyon	0000-0003-0942-2333	Methodological design, data collection, write up
Wolman	0000-0001-9378-9044	Methodological design, write up
Martin	0000-0002-5497-4594	Methodological design, write up
Galloway	0000-0002-2265-5732	Data analysis

Acknowledgements

SunVit supplied serum 25(OH)D_3 testing kits and vitamin D_3 supplementation (tablet and liquid)

Summary

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

Abstract

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

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

Methods: Using a randomised repeated measures double-blind placebo design (registered under ClinicalTrials.gov Identifier no. NCT03463642), changes in serum 25(OH)D over a 4-week period using a capillary spot method were monitored. 62 female participants blindly chose a number related to a supplementation delivery method: pill placebo, pill, oral liquid, oral liquid placebo, Skin oil application (SOA) placebo, SOA plus vitamin D_3 suspension, or SOA plus vitamin D_3 suspension with essential oil enhancer; active vitamin D supplements contained 100,000IU. Participants took their allocated supplements over a 24-hr period with serum 25(OH)D retested 4 weeks later. Liquid chromatography-tandem mass spectrometry method was applied to dried blood spot samples by an independent laboratory.

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

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

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

Introduction

Vitamin D_3 is mainly synthesized in the skin during exposure to ultraviolet light of the sun during the summer months [1,2], though food, specifically fatty fish, can also be a source [3]. A recent study has suggested that exposure to sunlight might only have a limited effect [4]; the Binkley et al. study indicated a variable response to sunlight exposure with some participants maintaining a low 25(OH)D3 level despite abundant sun exposure. A recent review of the effect of sunscreen on serum 25(OH)D concluded that sunscreen had little effect for healthy adults with recreational sunlight exposure[5]. The reviewed controlled studies, 3 showed no change and 4 showed a decrease in serum 25(OH)D, though a series of methodological limitations including a lack of personal UVR exposure (n=4) and no baseline measure of serum 25(OH)D (n=3) highlight areas of concern. One short-term study (1-week high UVI exposure) noted significant increases in serum 25(OH)D in the sunscreen group (SPF 15) suggesting that only very low levels of UVB were required for the biosynthesis of vitamin D_3[6]. These studies only used sunscreens with a factor of 15-17, whilst a number of organisations, such as the American Cancer Society and the British Association of Dermatology, promote the use of higher protection (SPF 30-50).

The current research indicates that to achieve optimal levels of 25(OH)D_3, supplementation is required [7]. Although there has been much research on supplementation dose levels there is still a lot of variation, this is possibly due to recommendations being targeted at specific clinical conditions, e.g. bone health. Ross et al [8] suggested 600 IU/day to maintain bone health, whilst others [9] have

suggested a higher daily dose (1500-2000 IU/day) is needed. Ekwaru et al [10] suggested that high doses had a diminishing effect with serum 25(OH)D_3 increasing by 12nmol/L per 1000IU for supplementation between 0-1000 IU/day and only 1.1nmol/L for supplementation between 15,000-20,000 IU/day and there was a need to account for body weight with obese patients requiring 2-3 times more vit D and those overweight, 1.5 times. Other studies have utilised 1-2 high dose bolus supplementation to beneficial effect [11-14].

There has been little research on different delivery methods for supplementation. Biancuzzo et al [15] compared liquid and pill oral supplementation and noted no difference between the delivery methods. Leventis and Kiely [14] reported no difference between a single high bolus deliver by either intramuscular injection or tablet. A number of transdermal delivery methods have been examined with varying success[16]. Pre-treatment of ex-vivo skin with ethanol increased penetration but would eventually lead to toxicity[17]; Ramezanli et al[18] used nanoparticles coated with hydrophillic and hydrophobic polymers to beneficial effect; whilst Devaux et al[19] concluded that vitamin D enhanced creams applied to the skin only penetrates deep enough to treatment of skin disorders, such as psoriasis. Three studies have looked at the effect of penetration enhancers in vitamin D enhanced creams. D' Angelo Costa et al[20] used various penetration enhancers in either a gel or cream formulation on *ex-vivo* human skin; gel formulation with cereal alcohol and propylene glycol noted vitamin D_3 penetration to stratum corneum (4 hours post application) and epidermis and dermis (24 hours post application) but no active vitamin D3 was found in receptor fluid, therefore skin penetration was not fully achieved. Sadat-Ali et al [21] used aloe vera as a delivery system for dermal delivery of vitamin D and reported significant changes in serum 25(OH)D over a 3 month period. Essential oils have been shown to enhance different drugs ability to penetration the lower skin layers through either the disintegration of intercellular lipid structure between corneocytes and the conformational modification of proteins[22]. Bubshait et al[23] used a proniosomal delivery system over a 4-month period with a similar beneficial effect on serum 25(OH)D. Therefore, topical delivery systems seem to be a safe and suitable delivery method of vitamin D.

The aim of the present study was to examine the efficacy of different delivery methods on serum 25(OH)D changes in healthy adult females. Various delivery methods of vitamin D supplementation are available to consumers but there have been no studies providing evidence of whether one delivery method is superior to others. We wanted to compare the delivery of 100,000IU vitamin D_3 by three methods. Two methods of oral supplementation (pill [prolonged release] and liquid [immediate release]), and delivery through the skin (with and without a penetrator enhancer).

Materials and Methods

Experimental design: The trial was a randomised double-blind placebo design and was registered with the US Clinical Trials (NCT03463642). An independent technician randomly assigned numbers (1-70) to the supplement samples: placebo pill, vitamin D pill, oral placebo liquid, oral vitamin D liquid, placebo skin oil application (SOA), SOA plus vitamin D_3 suspension, or SOA plus vitamin D_3 suspension with essential oil enhancer. Volunteers then randomly selected a number between 1-70. The data collectors and the statistician were blind to the participant's group (intervention or placebo) and only after the statistical analysis was completed were the group codes reviewed by the independent researcher.

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

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

Table 1: Participant descriptive data

Delivery method		N	Age (yrs)	Height (cm)	Body mass (kg)	BMI (kg/m^2)	Serum 25(OH)D ($nmol.L^{-1}$)
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

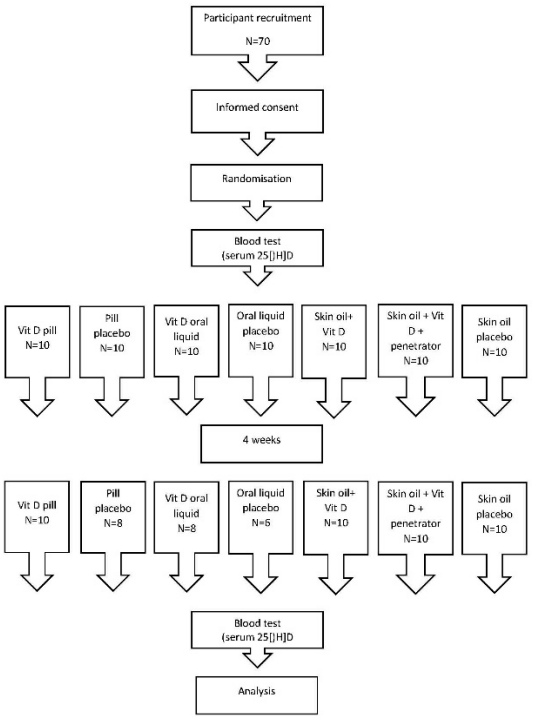

Figure 1: Participant flow chart

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

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

Table 2: Intervention Groups

	Active	Placebo
Pill	100 Vitamin D_3 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_3 suspension in orange syrup (1,000 IU per drop)	100 drops of orange syrup
Skin oil application	100,000 IU vitamin D_3 suspension in mineral oil (paraffinum liquidum, isopropyl palmitate, parfum) (100ml total) 100,000 IU vitamin D_3 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

Blood analysis:

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was applied to dried blood spot samples, utilising blood spot calibrators[27]. The method is standardised against conventional 25-hydroxyvitamin D3 and D2 LC-MS/MS service for serum (r^2=0.98; intra assay variation <10%; inter assay variation <11%). Blood spot results show good comparability to serum/plasma results with a 3.3% difference (95% CI: -6.3-12.1%; p=0.48)[28]. The City Assays laboratory participates in the DEQAS external quality assurance scheme.

Data analysis:

Group data were tested for homogeneity/sphericity prior to further analysis using Levene's test of equality of variance (SPSS v20). Analysis of covariance (ANCOVA) was conducted to detect changes in serum 25(OH)D; the dependent variable was post vitamin D_3; fixed factors were the different groups (pill placebo, pill, oral liquid, oral liquid placebo, skin oil application [SOA] placebo, SOA plus vitamin D_3 suspension, or SOA plus vitamin D_3 suspension with essential oil enhancer), and pre vitamin D_3 was the covariate. Bonferroni post hoc analyses were used where applicable. Analysis of variance within the delivery methods (pill, skin oil application, oral liquid) was carried on the absolute change in serum 25(OH)D with Bonferroni post hoc analyses. Significance for all analyses was set at $p \leq 0.05$.

Results

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

Table 3: Pre and post intervention serum 25(OH)D3 for the different delivery methods [mean, standard deviation (95%CI)]

Delivery method		Pre	Post	Change
		nmol.L^{-1}		
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)*[ǂ]	26.03 ±19.68 *(18.57, 33.49)*[‡]
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)*[ǂ]	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)*[ǂ]	14.92 ±10.80 *(6.86, 22.98)*[‡]

[ǂ] Significant changes over time ($p<0.05$); [‡]significantly greater change

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

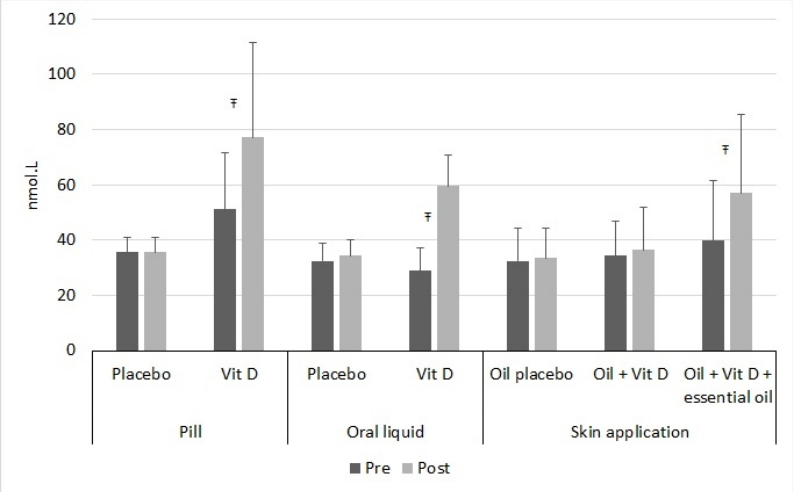

Figure 2: Serum 25(OH)D changes over time for the different delivery methods

Discussion

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

Biancuzzo et al [15] supplemented participants over a 11-week period with 1000IU/day and reported no significant difference between the two delivery methods (oral liquid and pill) though the liquid supplementation increase was approximately 70% whilst the pill supplementation was 42%. In our study participants took the equivalent of 100,000IU over a 24-hr period. The reduced efficacy of bolus oral liquid versus slower release pill may be due to rate limited hepatic hydroxylation of vit D to 25(OH)D following rapid intestinal absorption. The benefits of an essential oil as a dermalogical penetration enhancer is highlighted with the significantly greater absorption rates between the different skin application groups. Human skin has a multifunctional role but one of its primary functions is to act as a barrier against xenobiotic materials such as drugs [36]. The penetration enhancer interacts with the skin's stratum corneum, disrupting its lipid bilayers by modifying permeant diffusivity [37], thereby reducing the barrier properties. This may be due to the competitive hydrogen bonding of oxygen containing monoterpenes with ceramide head groups, thereby breaking the interlamellar hydrogen bonding network of lipid bilayer of stratum corneum and new polar pathways or channels are formed. This study highlights the efficacy of essential oils as a penetration enhancer in the delivery of vitamin D across the skin barrier. D'Angelo Costa et al[20] used the same amount of vitamin D_3 (100,000IU) on *ex-vivo* skin application but used different penetration enhancers (cereal alcohol, soybean lecithin, isopropyl palmitate, propylene glycerol and ethoxydiglycol) and although they noted vitamin D3 did reach the epidermis and dermis within 24

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

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

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

References

1. Heaney R. Vitamin D in Health and Disease. *Clin J Am Soc Nephrol.* 2008;3(5):1535-1541
2. Holick M, Chen T. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr.* 2008;87(suppl):1080S-1086S
3. Halliday T, Peterson N, Thomas J, Kleppinger K, Hollis B, Larson-Meyer D. Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Med Sci Sports Exerc.* 2011;43(2):335-343
4. Binkley N, Novotny R, Krueger D, Kawahara T, Daida Y, Lensmeyer G, Hollis B, Drezner M. Low vitamin D status despite abundant sun exposure. *J Clin Endocrinol Metab.* 2007;92(6):2130-2135 DoI:10.1210/jc.2006-2250.
5. Passeron T, Bouillon R, Callender V, Cestari T, Diepgen T, Green AC, van der Pols J, Bernard B, Ly F, Bernerd F. Sunscreen photoprotection and vitamin D status. *Br J Dermatol.* 2019;181(5):916-931
6. Young A, Narbutt J, Harrison G, Lawrence K, Bell M, O'Connor C, Olsen P, Grys K, Baczynska K, Rogowski-Tylman M. Optimal sunscreen use, during a sun holiday with a very high ultraviolet index, allows vitamin D synthesis without sunburn. *Br J Dermatol.* 2019;181(5):1052-1062
7. Wahl D, Cooper C, Ebeling P, Eggersdorfer M, Hilger J, Hoffman K, Josse R, Kanis J, Mithal A, Pierroz D, Stenmark J, Stocklin E, Dawson-Hughes B. A global representation of vitamin D status in healthy populations *Archives of Osteoporosis.* 2012 DoI:10.1007/s11657-012-0093-0.
8. Ross A, Manson J, Abrams S, Aloia J, Brannon P, Clinton S, Durazo-Arvizu R, Gallagher J, Gallo R, Jones G, Kovacs C, Mayne S, Rosen C, Shapses S. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know *J Clin Endocrinol Metab.* 2011;96(1):53-58
9. Pramyothin P, Holick M. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. *Curr Opin Gastroenterol.* 2012;28(2):139-150 DoI:10.1097/MOG.0b013e32835004dc.
10. Ekwaru J, Zwicker J, Holick M, Giovannucci E, Veugelers P. The importance of body weight for the dose response relationship of oral vitamin D supplementation and serum 25-hydroxyvitamin D in healthy volunteers. *PLoS One.* 2014;9(11):e111265 DoI:doi.org/10.1371/journal.pone.0111265
11. Stoll D, Dudler J, Lamy O, Hans D, Krieg M, Aubry-Rozier B. Can one or two high doses of oral vitamin D3 correct insufficiency in a non-supplemented rheumatologic population? *Osteoporos Int.* 2013;24(2):495-500
12. Wyon M, Nevill A, Cloak R, Metsios G, Gould D, Ingham A, Wolman R, Koutedakis Y. Acute effects of vitamin D3 supplementation on muscle strength in judoka athletes: a randomised placebo-controlled, double-blind trial. *Clin J Sport Med.* 2015 DoI:10.1097/JSM.0000000000000264.
13. Strickera H, Biandaa F, Guidicelli-Nicolosia S, Limonib C, Coluccic G. Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study. *Eur J Vasc Endovasc Surg.* 2012;44(3):307–312 DoI:doi.org/10.1016/j.ejvs.2012.06.023.

14. Leventis P, Kiely PDW. The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. *Scand J Rheumatol.* 2009;38(2):149-153 DoI:10.1080/03009740802419081.
15. Biancuzzo R, Young A, Bibuld D, Cai M, Winter M, Klein E, Ameri A, Reitz R, Salameh W, Chen T, Holick M. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr.* 2010;91(6):1621-1626 DoI:10.3945/ajcn.2009.27972
16. Sawarkar S, Ashtekar A. Transdermal vitamin D supplementation—A potential vitamin D deficiency treatment. *J Cosmet Dermatol.* 2020;19(1):28-32 DoI:https://doi.org/10.1111/jocd.13085.
17. Alsaqr A, Rasoully M, Musteata FM. Investigating Transdermal Delivery of Vitamin D 3. *AAPS PharmSciTech.* 2015;16(4):963-972
18. Ramezanli T, Kilfoyle BE, Zhang Z, Michniak-Kohn BB. Polymeric nanospheres for topical delivery of vitamin D3. *Int J Pharm.* 2017;516(1-2):196-203
19. Devaux S, Castela A, Archier E, Gallini A, Joly P, Misery L, Aractingi S, Aubin F, Bachelez H, Cribier B, Jullien D, Le Maître M, Richard M-A, Ortonne J-P, Paul C. Topical vitamin D analogues alone or in association with topical steroids for psoriasis: a systematic review. *J Eur Acad Dermatol Venereol.* 2012;26:52-60 DoI:doi:10.1111/j.1468-3083.2012.04524.x.
20. D'Angelo Costa GM, Sales de Oliveira Pinto CA, Rodrigues Leite-Silva V, Rolim Baby A, Robles Velasco MV. Is Vitamin D3 Transdermal Formulation Feasible? An Ex Vivo Skin Retention and Permeation. *AAPS PharmSciTech.* 2018;19(5):2418-2425 DoI:10.1208/s12249-018-1065-5.
21. Sadat-Ali M, Bubshait DA, Al-Turki HA, Al-Dakheel DA, Al-Olayani WS. Topical delivery of vitamin d3: a randomized controlled pilot study. *International journal of biomedical science: IJBS.* 2014;10(1):21
22. Herman A, Herman AP. Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. *J Pharm Pharmacol.* 2015;67(4):473-485
23. Bubshait DA, Al-Dakheel DA, Alanii FM. Topical vitamin D3: A randomized controlled trial (RCT). *Clinical nutrition ESPEN.* 2018;27:16-19
24. Hopkins W. Estimating Sample Size for Magnitude-Based Inferences. *Sportscience* 2006;10:63-70
25. Wyon MA, Wolman R, Nevill AM, Barber A, Edwards M, Bowd B, Clarke F, Bryant J, Cloak R. The influence of hormonal contraception on vitamin D supplementation on serum 25(OH)D 3 status in premenopausal women: A prospective double-blind placebo random controlled trial. *Journal of Endocrinology and Metabolism.* 2017;7(4):117-121
26. DEFRA. UV Radiation Data. *Reading University Atmospheric Observatory* 2020; https://uk-air.defra.gov.uk/data/uv-data?action=search. Accessed 28/04/2020, 2020.
27. Keevil BG. The analysis of dried blood spot samples using liquid chromatography tandem mass spectrometry. *Clin Biochem.* 2011;44(1):110-118
28. Larkin EK, Gebretsadik T, Koestner N, Newman MS, Liu Z, Carroll KN, Minton P, Woodward K, Hartert TV. Agreement of blood spot card measurements of vitamin D levels with serum, whole blood specimen types and a dietary recall instrument. *PLoS One.* 2011;6(1)
29. Mundasad S. Vitamin D supplements 'advised for everyone'. 2016; http://www.bbc.co.uk/news/health-36846894. Accessed 22 November 2017, 2017.
30. Holick M. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers and cardiovascular disease *Am J Clin Nutr.* 2004;80 1678-16788
31. Pearce S, Cheetham T. Diagnosis and management of vitamin D deficiency *Br Med J (Clin Res Ed).* 2010;340 142-147
32. Pfeifer M, Begerow B, Minne H, Suppan K, Fahrleitner-Pammer A, Dobnig H. Effects of long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos Int.* 2009;20:315-322
33. Poole C, Smith J, Davies J. Cost-effectiveness and budget impact of empirical vitamin D therapy on unintentional falls in older adults in the UK. *BMJ Open.* 2015;29(5) DoI:10.1136/bmjopen-2015-007910.
34. Wyon M, Koutedakis Y, Wolman R, Nevill A, Allen N. The influence of winter vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: A controlled study. *J Sci Med Sport.* 2014;17(1):8-12 DoI:10.1016/j.jsams.2013.03.007.
35. Osborn J, Germann A, St Anna L. Which regimen treats vitamin D deficiency most effectively? *J Fam Pract.* 2011;60(11):682-683
36. Brown M, Traynor M, Martin G, Akomeah F. Transdermal drug delivery systems: skin perturbation devices. In: KK J, ed. *Drug Delivery Systems. Methods in Molecular Biology.* Vol 437: Humana Press 2008:119-139.
37. Aggarwal S, Agarwal S, Jalhan S. Essential oils as novel human skin penetration enhancer for transdermal drug delivery: a review. *Int J Pharm Bio Sci.* 2013;4(1):857-868
38. Verbrugghe M, Verhaeghe S, Lauwaert K, Beeckman D, Van Hecke A. Determinants and associated factors influencing medication adherence and persistence to oral anticancer drugs: a systematic review. *Cancer Treat Rev.* 2013;39(6):610-621