Trials

STUDY PROTOCOL

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A multistage double-blind placebo-controlled study to assess the safety and efficacy of transdermal vitamin D phosphate supplementation (TransVitD)

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Abstract

Background Lifestyle changes have meant that it is problematic for many people across the globe to maintain adequate vitamin D concentrations. As UV-catalysed production in the skin, which uses vitamin D-binding protein to facilitate systemic absorption, is the primary source of vitamin D, it is questionable if oral supplementation of this vitamin is the optimal means to replace it. However, supplementing an oil-soluble vitamin via the skin is problematic as it gets stuck in the stratum corneum after topical application. This clinical study will test if a new vitamin D ester, vitamin D phosphate, which is more water-soluble compared to vitamin D, administered via a transdermal patch, can be used to improve vitamin D status.

Method This is a two-part study comprising a dose-escalation with the vitamin D phosphate transdermal patch followed by a randomised, double-blind, placebo-controlled, multiarmed, multistage clinical trial. It is a single-centred, 12-week study that will enrol a maximum of 100 participants. The dose escalation study will monitor safety and tolerability using serum calcium and 25(OH)D_3 levels. The blinded, randomised trial will test different dose frequencies for 4 weeks compared to a placebo. Then, after an interim analysis, the best dosing frequency will be assessed against a placebo. The primary outcome for the multistage clinical study will be the percentage change in 25(OH)D_3 concentration in the serum (ng/mL) at weeks 4 and 8 compared to baseline. The secondary outcome measures include percentage change in serum vitamin D-binding protein levels, skin interstitial fluid biomarker concentrations, and nail appearance after 4 and 8 weeks compared to baseline.

Discussion This study will determine if a vitamin D phosphate transdermal patch can improve vitamin D status. In addition, it could provide a better understanding of how vitamin D is absorbed directly into the skin after application by measuring the serum vitamin D-binding protein and skin biomarker responses to transdermal supplementation.

Trial registration Clinical Trials.gov NCT06098846, registered on 23rd October 2023.

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Keywords Vitamin D phosphate, Vitamin D, Vitamin D-binding protein, Transdermal, Clinical trial, Interstitial fluid, 25(OH)D3

Introduction

Background and rationale

Vitamin D deficiency is common worldwide [1, 2]. The prevalence of severe vitamin D deficiency (< 30 nmol/L or 12 ng/mL) is 13% among Europeans, 7.4% among Canadians and 5.9% in the USA. In comparison, serum levels below the recommended 50 nmol/L (20 ng/mL) have been reported in 40% of Europeans, 37% of Canadians, and 4% of Americans [3]. Vitamin D supplementation is recommended to prevent vitamin D deficiency from impacting health [4]. For example, the UK government advises individuals to take daily vitamin D supplements (400 IU, 10 µg) to ensure adequate blood levels and to protect musculoskeletal health [5]. Despite national guidelines recommending vitamin D supplementation for both adults and children, the health benefits of vitamin D supplementation, especially the non-skeletal benefits, are not currently strongly supported by robust clinical trial data. Therefore, there remains a need to understand better the contribution of vitamin D to health [2].

Vitamin D is available in two forms, vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), which differ in chemical structure. Both forms are extensively metabolised in the liver to form 25(OH)VD_2 and 25(OH)VD_3, as well as subsequent active metabolites, 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2D_2$ (subsequently referred to as relevant vitamin D metabolites) [6]. Vitamin D_3 can be produced endogenously by UV-B light-mediated cutaneous synthesis and it can be absorbed from exogenous sources by consuming animal products that contain trace amounts. In contrast, vitamin D_2 can only be obtained in relatively small amounts through dietary sources [7]. Individuals with low vitamin D levels, due to insufficient dietary intake or limited exposure to direct sunlight that is often a result of geographical location or lifestyle, can improve serum levels of 25(OH)VD_3 (subsequently referred to as vitamin D status) through supplementation [8].

Vitamin D_3 is currently the most effective means of supplementing vitamin D and, as such, is provided in most supplement formulations on the market [9, 10]. Although their use is widespread, these oral formulations are subject to limited oral bioavailability, which is influenced by genetics and food in the gastrointestinal tract during supplement ingestion [11]. Even under controlled conditions, such as those in nutrition studies, the 25(OH)D_3 plasma levels can vary by > 50% in individual participants after each vitamin D supplement dose [12]. Furthermore, orally administered vitamin D has been shown to have lower biological activity than vitamin D produced in the skin [13]. This disparity is attributed to differences in absorption and transport to the liver. Orally ingested vitamin D is absorbed through the small intestine and transported in chylomicrons, whereas skin-synthesised vitamin D is collected and transported directly by the vitamin D-binding protein (VDBP) [14]. As a result of reduced biliary excretion of VDBP-bound vitamin D, the skin-synthesised form exhibits increased potency and duration of effect compared to orally administered vitamin D [14].

This work hypothesises that delivering vitamin D through the skin may overcome the disadvantages of oral administration and lead to an efficacious means of supplementation. The greater efficiency of transdermal supplementation was predicted to be due to the ready access to the vitamin D-binding protein (VDBP) in the skin, which then distributes the vitamin around the body and avoides clearance [15]. However, vitamin D does not readily pass through the outer lipidic barrier in the skin, the stratum corneum; therefore, a novel administration approach is needed for supplementation via the skin is to be realised [16]. One potential strategy is the generation of a pro-vitamin that can modify the physicochemical properties and facilitate improved skin absorption. Several vitamin D derivatives have previously been reported to modify the physicochemical properties compared to the parent molecule (e.g., acetate, propionate, stearate, butyrate, and laurate esters), but they do not make the compound more water-soluble, which is a requirement to enhance the transdermal penetration of vitamin D [17]. A new phosphate ester of vitamin D differs from these other pro-vitamins as it increases the compound's water solubility, which allows it to effectively pass through the stratum corneum of skin (vitamin D phosphate, VDP, World International Publication Number: WO2022084669A1, 2022). Unpublished cell culture and human skin metabolism studies have demonstrated that the VDP ester is metabolised into vitamin D in the skin prior to systemic absorption. However, there is a need to assess, in clinical studies, if this new compound can bind to the VDBP, and then be absorbed into the blood to act as an effective supplement [18, 19]. Vitamin D phosphate has been developed into a active-in-adhesive transdermal patch formulation, and it has been shown to increase vitamin D skin absorption in ex vivo studies. This new study will assess the safety and efficacy of the vitamin D phosphate transdermal patch in healthy human volunteers.

Hypothesis

Vitamin D phosphate administered as a transdermal patch will enhance human vitamin D status. An increase in serum 25(OH)D_3 levels of 2 ng/mL or greater will be considered clinically significant.

Objectives

The objectives of the study are:

- To investigate the extent of systemic exposure of vitamin D after administering a vitamin D phosphate transdermal patch in healthy individuals using 25(OH)D_3 as a biomarker.
- To determine how transdermal administration of vitamin D phosphate influences VDBP levels in the serum.
- To investigate how interstitial skin biomarkers in the local tissue are changed after the transdermal administration of vitamin D phosphate.
- To understand if the visual appearance of the human nail plate changes as a consequence of transdermal vitamin D supplementation.

Trial design

This two-part study comprises a dose-finding and a randomised, placebo-controlled, double-blind clinical trial using an adaptive design (Figs. 1 and 2). This study aims to evaluate the safety and efficacy of a vitamin phosphate transdermal supplement patch compared to a placebo.

Methods: participants, interventions and outcomes

Study setting

The study will take place in the Institute of Pharmaceutical Science, King's College London, London, UK. Recruitment of participants will be primarily from London and the surrounding areas; thus they will represent the multicultural and multiethnic population of the city. The study will be conducted over 9 months from October to June. This timeframe will coincide with the primary academic semesters, thereby maximising access to a diverse cohort of staff and students while avoiding major holiday periods that could potentially impact participant availability. In addition, this period covers the seasons associated with a higher risk of vitamin D deficiency, particularly from late autumn through early spring [6].

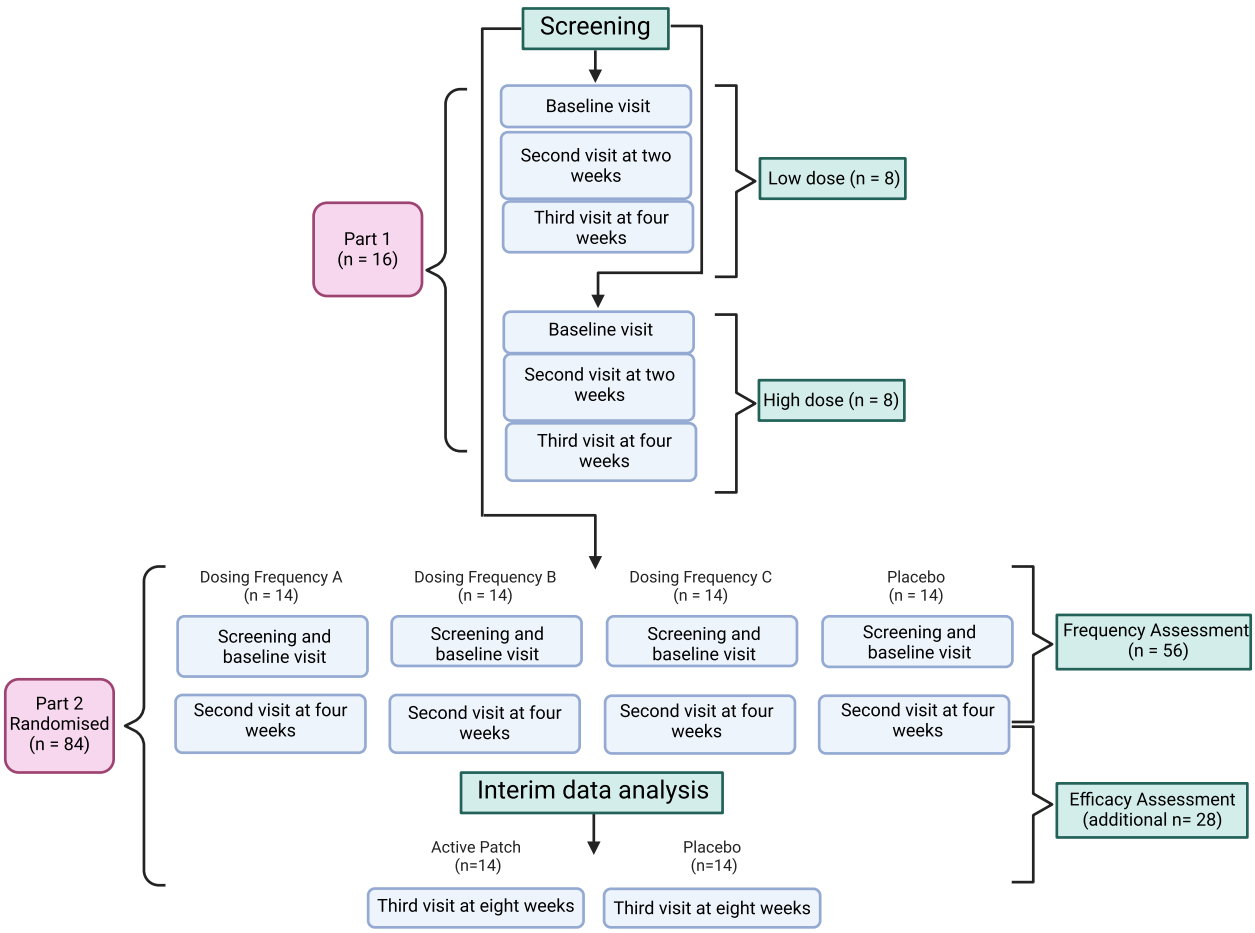

Fig. 1 Participant flow diagram

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					
TIMEPOINT	$-t_1$	0	*Part 1a 2 Week*	*Part 1a 4 Week*	*Part 1b 2 Week*	*Part 1b 4 Week*	*Part 2 4 Week*	*Part 2 8 Week**
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Demographics	X							
Allocation		X						
INTERVENTIONS:								
Part 1a low dose intervention		X	♦—	—♦				
Part 1b high dose intervention		X			♦—	—♦		
Part 2 Frequency A		X					♦—	—♦
Part 2 Frequency B		X					♦—	—♦
Part 2 Frequency C		X					♦—	—♦
Part 2 Placebo		X					♦—	—♦
ASSESSMENTS:								
Baseline Health Assessment	X							
Serum Vitamin D		X	X	X	X	X	X	X
Serum Calcium		X	X	X	X	X	X	X
Serum and ISF VDBP		X	X	X	X	X	X	X
TEWL		X	X	X	X	X	X	X
Fingernail Imaging		X	X	X	X	X	X	X
[$]Parathyroid Hormone		X					X	X
[$]Genetic Testing		X						

Fig. 2 SPIRIT diagram detailing enrollment, interventions, and assessments. *Note: Part 2, 8 Week time point only for one dose frequency and placebo following Part 2, week 4 interim analysis. [$]Only tested in Part 2. ISF = interstitial fluid, VDBP = vitamin D-binding protein, TEWL = transepidermal water loss

Eligibility criteria

Interested participants will be provided with a participant information sheet and a member of the trial staff will explain the study. Volunteers who cannot read or comprehend the information given will be considered not eligible. Initial screening and subsequent recruitment will be based on participant suitability as per the inclusion and exclusion criteria (Table 1) and a successful patch adhesive sensitivity test using a placebo patch.

Screening will occur at least 24 h before the baseline visit to allow time for the participant to understand the information provided. Suspected vitamin D deficiency will be established using the Deschasaux questionnaire, and no samples will be taken prior to the baseline visit. To ensure the study participants are "healthy" we will not allow participants who have been diagnosed with vitamin D deficiency by a GP in the prior 6 months to be included in the trial. Study staff will request that the participants sign a statement of informed consent, and if eligible, they will be invited to participate in three study visits. A maximum of 100 participants will be enrolled in the trial. A set of patch application instructions will be provided to

Table 1 Participant inclusion and exclusion criteria

Inclusion criteria
1. Healthy adults between 18 and 65 years of age
2. Suspected low vitamin D levels (defined as moderate or high risk using the Deschasaux questionnaire [20])
3. Written informed consent for study participation
4. Willingness to comply with the study requirements
5. Competent use of English language
Exclusion criteria
1. Patients unable to give informed consent
2. The use of vitamin D supplements 4 weeks prior to the commencement of the study (and unwilling to washout)
3. Pregnancy
4. Those with parathyroid, thyroid, or calcium disorders, sarcoidosis, taking calcium channel blocking medication, type I diabetes, and concurrent active malignancies
5. Those who have been diagnosed with vitamin D deficiency by a GP in the last 3 months using a blood test
6. Those who have been diagnosed with vitamin D deficiency by a GP in the last 6 months using a blood test and have not taken any vitamin D supplements

the participants to aid the daily application of the patches and to reinforce the instructions after the participants have left the clinical site.

Explanation for the choice of comparators

Although other patches containing vitamin D are available on the market, no clinical studies have demonstrated their efficacy in improving vitamin D status, presumably due to the limited transdermal bioavailability of vitamin D [19, 20]. Consequently, we will compare the innovative transdermal vitamin D supplementation approach using vitamin D phosphate to a placebo patch.

Intervention

The intervention will be the application of daily transdermal patches. They will be active in adhesive patches manufactured at King's College London comprising food-grade vitamin D phosphate (1–5 mg) and cosmetic/food-grade excipients. Placebo patch components will be the same as the active but adjusted in proportions to accommodate the absence of vitamin D phosphate and the antioxidant. The patches underwent ISO standard toxicology testing for medical devices before clinical use (cytotoxicity, irritation, and sensitisation). Patches will be packaged in aluminium sealable pouches (primary packaging) and then a cardboard box (secondary packaging). The patches are stable for at least 6 months after production.

Criteria for discontinuing or modifying allocated intervention

A mechanism will be in place to report any adverse events to the study team through a dedicated study email address. If any of the adverse events are classified as serious the study team will advise the participant to exit the study and they will be directed to receive appropriate medical attention. Participants can exit the study at any time.

Participant timeline

Baseline assessment

Volunteers will provide demographic data, relevant medical history, allergy status, Fitzpatrick skin type, and lifestyle data, including diet, to estimate the differences in vitamin D intake, and the levels of regular sun exposure, to estimate differences in the background levels of vitamin D. We will use the participant location to calculate UV exposure during the trials. Baseline measurements will be taken, including trans-epidermal water loss (TEWL), a blood sample to measure serum vitamin D (25(OH)VD_3) and calcium levels, skin interstitial fluid (ISF) sample, and fingernail photographs.

Part 1

After receiving informed consent and completing the baseline assessment, two cohorts of 8 participants will take part in a non-blinded dose escalation pilot study. These cohorts will be supplemented via transdermal patch application, with one cohort completing the 4-week study, the data being analysed, and the choice of second dose made, before dosing the second cohort. The primary outcome measure will be safety and tolerability. This will be assessed using calcium and 25(OH)VD_3 levels at 2 weeks and 4 weeks for systemic effects and visual observation for local tolerability. If analysis at either time point shows blood plasma levels with a serum calcium measure of more than 12 mg/dL or a 25(OH)VD_3 level > 60 ng/ml, this will indicate a risk of toxicity. The dosing will be terminated in this eventuality, and a

serious adverse event will be recorded. If there are serious adverse events in 2 or more participants, the dose will be classified as unsafe. The secondary outcome will be efficacy using the 25(OH)VD_3 blood level. A blood concentration change of < 2 ng/ml in < 75% of participants will indicate that the dose is ineffective after 4 weeks of treatment. The primary and secondary outcome measures for the "lowest dose" cohort will direct the dosing of the second cohort in Part 1. Participants partaking in Part 1 of the study will not be eligible for inclusion in Part 2.

Part 2

This part of the trial will be multistage. Four cohorts of 14 participants will be enrolled in a double-blinded, randomised, placebo-controlled study in the first stage. The randomisation sequence will be designed by a statistician independent of the trial team. Participants will be assigned according to the randomisation schedule by a local researcher independent of the study team into one of three treatment arms or one placebo arm. The treatment arms will measure different dosing frequencies of the same strength vitamin D patch. The participants in Part 2 will have their parathyroid hormone levels monitored and be subject to genetic analysis to help understand any changes in vitamin D-binding protein, and these are additional tests compared to Part 1. The first stage of the study will last 4 weeks, after which there will be an interim data analysis to select the treatment arm for the second stage. In the second stage, two cohorts of 14 new participants will be blinded and randomised into the final two study arms consisting of one treatment arm and one placebo arm (28 participants in each arm). The second stage will run for 8 weeks, with measurements in week 4 and a final measurement in week 8. The aim of Part 2 is to establish whether vitamin D phosphate supplementation via the skin increases the concentration of vitamin D in the blood and which dosing interval is best suited to achieve this.

Strategies to improve adherence to interventions

A specific set of written instructions regarding the patch application will be used to reinforce the verbal directions provided by the study team. Dosing reminders will be sent to participants on their mobile phones each day. At each visit, the participants will be asked to bring any unused transdermal batches back for inspection by the study team, who will count any unused patches to determine the percentage compliance over the study duration.

Relevant concomitant care permitted or prohibited during the trial

The trial will monitor the participant's UV exposure, vitamin D intake, medical history and medication intake during the trial. However, the trial will not change diet, sun exposure or medical treatment.

Ancillary and post-trial care

It is not anticipated that participants will require post-trial care; however, participants will be instructed to contact the study research team via the designated study email address if symptoms relating to the application of study patches are experienced following the completion of the trial. In such an event, participants will be assessed and referred for further medical attention if required.

Outcomes

Primary outcomes

Percentage change in the concentration of 25(OH)D_3 in the serum compared to baseline.

Secondary outcome

(1) Percentage change in the concentration of VDBP levels in the serum compared to baseline.
(2) Percentage change in the concentration of chemical biomarker levels in the skin interstitial fluid compared to baseline.
(3) Quantification of image feature change in nail photographs (unit pixels).

The outcomes may be normalised for confounding factors such as BMI, percentage body fat, age, ethnicity and initial vitamin D serum levels.

Sample size calculation

For Part 1 the sample size is based on clinical considerations. With 6 patients per cohort, there is greater than 80% probability of detecting an adverse event with a population frequency of 25% using the simple probabilistic calculations (Table 2). Considering a 20% dropout rate, 8 patients are required for each cohort in Part 1 of the study.

Table 2 Part 1 probability of detecting adverse event calculations

p	n	$n(1-p)$	np
0.01	6	0.94148	0.05852
0.2	6	0.262144	0.737856
0.25	6	0.177979	0.822021
Definitions			
Sample size		n	
Pr of observing an event		p	
Pr not observing event in a participant		$1-p$	
Pr not observing an event in n participants		$n(1-p)$	
Pr of observing an event in n participants		np	

For Part 2, the sample size calculation is based on a 4-arm 2-stage design. The minimum change in 25(OH)VD_3 from baseline to week 8 is 4 ng/ml based on previous studies with oral supplements [21]. The typical value of 25(OH)VD_3 in placebo arm was estimated at 15.14 ng/ml with standard deviation (SD) of 9.1 nmol/L. With 1000 simulation runs, considering 5% significance level 2-sided and Dunnet test for multiple comparisons to select the best treatment, 12 participants are need for stage 1 in each arm. Assuming a 15% dropout rate, 56 participants in total will be recruited in stage 1. For stage 2, 12 additional participants will be enrolled in each of the selected and placebo arms. Considering the same 15% dropout rate, 28 participants in each group are required for stage 2. The overall sample size for Part 2 of the study is 84. The overall sample size for two parts of the study is 84 + 16 = 100. A per-protocol analysis will be conducted using data collected at the study endpoint. This analysis will include only participants who adhered to their initially assigned treatment regimen throughout the study period (> 80% compliance).

Recruitment strategy

Participants will be primarily recruited from staff at King's College London, students, and members of the public from the local area who voluntarily respond to trial advertising. Participants expressing interest in the trial will receive an information leaflet and consent form and will be invited to screening and baseline assessments. To encourage participant retention, all study participants will be offered financial compensation to reimburse them for the inconvenience of clinical visits. As an additional incentive for retention, participant 25(OH)VD_3 blood results will be given as a report at the end of the trial stating, "results are *guideline only* and should not be used for *clinical diagnosis*". Participation in the trial is voluntary, and participants may withdraw at any time for any reason.

Assignment of interventions

Allocation, concealment, implementation

Part 2 of the trial will be randomised. A code generated by an independent statistician will ensure equal participant recruitment into each of the trial cohorts by a block randomisation process. Group allocation will be performed by an independent researcher using the randomisation code. The blinding will remain in place until the trial and the final data analysis have been completed. In stage 1 of Part 2, 56 participants will be randomised and allocated into 4 groups. Following the interim data analysis, 28 additional participants will be randomised and allocated into the two final groups for Part 2, stage two.

Blinding

Participants and researchers will be blinded to the treatment allocation. The statistician will be blinded to the treatment allocation but will be unblinded to the groups during the interim analysis and thus we consider this a double-blind study. Treatment and placebo patches will be visually identical and labelled by a pharmacist independent of the trial, who will package them in the same way and assign each box with a code according to the independent statistician's list of codes. The independent researcher responsible for participant allocations will check the codes against the statistician's randomisation list to ensure accuracy. Where the frequency of treatment patch application is assessed, daily patch application will still be used with a mixture of treatment and placebo patches to maintain participant and researcher blinding. Any interim analysis that needs to be completed will be performed by an independent statistician and data review committee so as not to break the blinding code. In the event of a major adverse event, the team will contact the independent researcher responsible for the group allocation and ask them to unblind the participant affected by the event to ensure they receive the appropriate medical attention.

Data collection, management, analysis

Data collection methods

Screening questionnaire

Participants will be identified as having a moderate or high risk of vitamin D deficiency via the validated Deschasaux questionnaire [20]. This simple and effective screening tool is designed to assess an individual's risk of vitamin D insufficiency in adults. It makes use of a scoring system based on key factors known to influence vitamin D status, including age, skin phototype, body mass index (BMI), physical activity level, sun exposure habits, and dietary vitamin D intake. The questionnaire will be given during the initial screening visit, and based on the responses, a total score will be calculated. Higher scores indicate a greater risk of vitamin D insufficiency. A score between 7 and 9 indicates a moderate risk of vitamin D insufficiency, while a score greater than 9 indicates a high risk of vitamin D insufficiency [7]. A score equal to or greater than 7 will satisfy the eligibility criteria.

Demographic, diet and exercise questionnaire

The participants will be asked a series of questions to gather demographic information. This will generate data for potential covariates. The most important information will include participant BMI, sex, age, body fat, dietary vitamin D intake, UV exposure levels (monitored through location information), skin type, ethnicity, and exercise habits. Although these factors will not

determine to which group participants will be assigned, the randomisation process is expected to inform the final demographic analysis. Further analysis may also be performed if confounding factors such as BMI, percentage body fat, age, ethnicity, and initial vitamin D serum levels significantly impact the study outcome.

Blood sampling

A trained phlebotomist will collect venous blood (max. 3 × 10 mL) from a peripheral vein. Serum vitamin D levels 25(OH)VD_3 and relevant vitamin D metabolite concentrations in blood serum will be determined using ELISA and/or gas/liquid mass spectrometry analysis. Plasma calcium concentration will be determined in triplicate based on colourimetric methods. Parathyroid hormone and vitamin D-binding protein levels will also be determined using ELISA assays. Once collected serum and plasma samples will be logged and stored at − 80 °C until analysis.

Skin interstitial fluid (ISF) extraction

ISF samples (max. 3 × 150 µL) will be collected from the inner side of the forearm for the determination of VDBP levels at the skin sites where the patches have been applied. ISF samples will be collected under hypobaric pressure (510 mBar) using an in-house designed extraction chamber and hand pump fitted with manometer. VDBP level will be determined using a commercial ELISA testing kit. Samples will be logged and stored at − 80 °C until analysis.

Transepidermal water loss

TEWL will be measured in triplicate at each study visit using a AquaFlux AF200 condensing chamber probe (Biox Systems Ltd., UK) as a means of measuring skin barrier dysfunction and skin irritation.

Fingernail imaging

Images of fingernails from the whole hand will be taken using a digital camera as a means of monitoring visual changes.

Genetic variation profiling

We will collect biological samples to generate profiles of genetic variation in participants to investigate the relationship between genetic variation and specific measured traits. Participants will be asked to use an Oragene saliva sample tube. These will be stored at − 20 °C until analysis will be performed. If this fails for any reason, a similar analysis will be performed on blood samples that have already been collected for the other analytical measurement. DNA extraction from these samples will be performed using standard protocols appropriate to the source of the sample. Following DNA extraction, genotyping will be conducted using microarrays, a reliable and widely accepted technique for simultaneously identifying genetic variants across the genome. Once the genotypic data is obtained, association testing will be carried out. This process will evaluate the correlation between the genetic variants identified and the traits measured in the study, allowing us to ascertain potential genetic contributions to these traits.

Data management

All study participants will be allocated a unique study identification number so that researchers will not have access to subject-identifiable information. Study data, patient contact details, and patient medical information will be entered onto a secure password-protected research database (provided by: REDCap®). Copies of protocols, case report forms, physiological test results, participant correspondence, informed consent documents, and other files relevant to the study will be kept securely by the Principal Investigator for at least 15 years following the completion or discontinuation of the research study. All data recording and retention will comply with King's College London policies for sensitive data.

Confidentiality

All study staff will endeavour to protect the privacy and consent rights of the participants and will adhere to the Data Protection act, 2018. Access to study information will be limited to study staff, investigators, and trial conduct auditors.

Statistical methods for primary and secondary outcomes

Descriptive statistics on participants' demographics and baseline clinical characteristics will be provided using frequency and percentage or mean or median and range in both parts of the study. The first part of the study focuses on safety endpoints. Frequency and percentage of occurrence of adverse events and calcium > 12 mg/dL or 25(OH)VD_3 > 60 ng/ml will be reported in the first cohort of 6. If there are serious adverse events in 2 or more of participants, the dose will not be safe. The secondary outcome is efficacy, the change in 25(OH)VD_3. Participants with a 25(OH)VD_3 change less than 2 ng/ml will be reported as frequency percentage. If the 2 ng/ml change is observed in less than 75% of participants, the dose will be considered ineffective after 4 weeks. The decision for second cohort dosing will be made according to these results. The safety and efficacy of second cohort will be reported similar to the first cohort.

For the second part of the study, an interim analysis is planned when 50% of the participants have completed

4 weeks. The mean change in 25(OH)VD_3 in each treatment arm will be tested against the placebo arm using a z-test. Two treatment arms will be dropped and the treatment arm with the highest observed response as compared to placebo will be selected for testing at the second stage. As multiple doses are compared to the shared control, multiplicity adjustment will be considered in the first stage, and familywise error rate (FWER) will be controlled at 2.5%. Therefore, the probability of incorrectly rejecting the null hypothesis for at least one experimental arm is 0.025. If the z-value of the test is greater than 2.797 in stage 1, the treatment arm will be considered efficacious. At the final stage, the endpoints of the treatment arms against placebo will be tested using an independent sample t-test. For secondary outcomes such as VDBP levels in the blood and ISF, similar analysis will be performed. The outcomes may be normalised for confounding factors such as BMI, age, ethnicity, and initial vitamin D serum levels. Descriptive statistics will be reported for the number of vitamin D patches used in each arm at weeks 2, 4, and 8. All statistical analysis will be conducted using RStudio.

Data monitoring

Data monitoring: formal committee and interim analysis

A data monitoring committee consisting of a chair, clinician, and statistician independent of the researchers will monitor the running of the trial. The committee will ensure consent, data collection, and recording of results are accurate to ensure the reliability of the trial. The monitoring committee will undertake interim data analysis between Part 1a and 1b and halfway through Part 2 to decide on the trial dosing and review side effects, trial withdrawals, and trial conduct. In addition, the committee has the power to terminate the trial in case of participant safety incidents as detailed below.

Adverse event reporting and harms

The safety and tolerability of the vitamin D phosphate patch will be monitored throughout the study. Patients will be asked to report any adverse events or changes to their health status during the study visits and encouraged to contact the research team via the designated study email address outside of these times if required.

Possible adverse events occurring due to this study are expected to be the same as those reported following the administration of vitamin D by other routes. According to vitamin D "Summary of Product Characteristics" possible adverse events of vitamin D supplementation include:

- Metabolic: hypercalemia, hypercalciuria (uncommon, 0.1–1%)
- Dermatologic: pruritis, rash, urticaria (rare, < 0.1%)
- Gastrointestinal: nausea, vomiting (frequency not reported)
- Hypersensitivity: angioedema, laryngeal oedema (frequency not reported)

This information will be included in the study information leaflet to aid participants in identifying adverse events.

Adverse events will be recorded using the standardised MEDDRA (https://www.meddra.org/how-to-use/basics/hierarchy) protocol and will include the time and date of the event, duration, symptoms, and severity. The MEDDRA classification guides the team on assessing the action required, from no medical attention being required, non-urgent medical attention being required, or urgent medical attention being required. All MDDRA Class 2 adverse drug reactions (ADRs) and above will be reported to the principal investigator of the study. The team will use the MDDRA classification to aid referral to medical services. In cases of doubt, participants with ADRs will be referred for medical attention.

Participant follow-up

If participants withdraw from the study, the reason for withdrawal will be recorded. Participants will be encouraged to report when any adverse effects that persist beyond withdrawal have been resolved.

Auditing

The independent data monitoring committee will perform auditing and review of the study activity through the inbuilt auditing and monitoring feature in the research database. In particular, the committee will be responding to queries raised by the study facilitators and managing any discrepancies in study records or data. The data monitoring committee will meet after completing Part 1a and 1b and part way through Part 2. Trial results will be reviewed with respect to primary and secondary outcome measures and decisions made regarding any dose changes for subsequent parts. In addition, side effects, trial withdrawals, and trial conduct will be reviewed. Reports detailing the presentation of adverse events, participant drop out will be sent to the data monitoring committee via traceable means. External auditors will be afforded full access to electronic and written study records if required.

Discussion

TransVitD aims to assess the ability of a vitamin D phosphate transdermal patch to safey improve vitamin D status in healthy volunteers. It is anticipated that supplementation via the skin can use the transport machinery,

i.e., the vitamin D-binding protein, present in the tissue to facilitate effective absorption [12]. The study adds to the limited amount of pilot clinical work that suggests that transdermal vitamin D could be a useful means to deliver this important vitamin [22]. As far as we are aware, no other vitamin D phosphate-based transdermal patch formulation has been tested in a double-blinded, placebo-controlled human clinical trial.

After pilot dose escalation, a double-blind, placebo-controlled supplementation trial will be initiated to investigate the blood serum 25(OH)VD_3 levels after applying a vitamin D phosphate transdermal patch. This is a standard means to determine the effectiveness of vitamin D dosing during an interventional trial. However, the study will also measure both local and systemic VDBP concentrations. Recent work on vitamin D generation by UV-irradiation in VDBP knock-out animals has shown that the VDBP is essential to carry vitamin D synthesised in the skin out of the tissue and into the systemic circulation [14, 23]. Therefore, it was hypothesised in this work that the VDBP could be important in the absorption and transport of vitamin D administered into the skin using a transdermal patch. In the second part of the study, we added genetic analysis to search for associations between various biochemical processes that could improve or diminish an individual's response to vitamin D in order to try and understand the positive or negative impact of VDBP facilitating the absorption after penetration into the skin.

The trial faces some challenges, such as anticipating the initial vitamin D status of the trial participants and monitoring the vitamin D intake from food and sunlight. However, we have implemented validated questionnaires and monitoring strategies to overcome these. Other potential covariates such as ethnicity, sex, age, weight, and % body fat of the participants will also be monitored to enable post-trial data processes to account for these factors. The long half-life and distribution of vitamin D in body fat complicates the blood sampling regimen. Therefore, we have scheduled a participant sampling window of 2 weeks to allow sufficient time for vitamin D, relevant metabolites, and vitamin D-binding protein to reach steady state levels. Initial dosing was calculated considering a relative 15% transdermal bioavailability measured in rats, with the caveat that this could be reduced to 1.5% in humans, given the reduction in skin permeability compared to rats. There is no literature that provides accurate allometric scaling from humans to animals for vitamin D absorption. As a consequence, the main focus of this work was to optimise the vitamin D phosphate transdermal dose. The study design allows up to five different opportunities to get an appropriate dose and dosing interval in humans.

Relevant clinical data detailing the extent of transdermal delivery of vitamin D phosphate is required to confirm the preclinical evidence which shows the efficacy of transdermal supplementation. The results of this study may promote further investigation into transdermal delivery of phosphate esters due to their ability to modify the physicochemical properties of the parent drug transiently prior to reverting back to the parent upon esterase metabolism in the skin in a manner that enhances bioavilability.

Abbreviations

ISF	Interstitial fluid
KCL	King's College London
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
TEWL	Transepidermal water loss
VDBP	Vitamin D-binding protein
VDP	Vitamin D phosphate
UVA	ultraviolet radiation
UVB	ultraviolet radiation B

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08711-8.

Supplementary Material 1.

Dissemination policy

It is intended that the results of this study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. Following trial competition and the publication of results, data requests may be submitted to the researchers at the Institute of Pharmaceutical Science, King's College London. In addition, we will send a summary of the study results to study participants at their request.

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use

Delegated researchers will take samples with the appropriate training in sample collection techniques. All samples will be labelled in such a way that the participant and date of the collection can be identified beyond the unique study participant number if required. All biological samples will be stored at −20 or −80°C in accordance with the human tissue act where appropriate. If samples are to be used in future studies outside of the scope of this trial, Research Ethics Committee approval will be sought.

Name and contact information for trial sponsor

King's College London, Guy's Campus, King's College London. LONDON, SE1 4UL. Email: rec@kcl.ac.uk.

Trial status

Study recruitment started on 23rd Oct 2023. Recruitment is currently ongoing and is expected to complete in Dec 2024.

Authors' contributions

TH: writing draft, editing; PA: writing draft, editing; CL: writing draft, editing; QG: writing draft, editing; KL: conceptualisation, BC: editing; AD: writing draft, editing, supervision; RM: writing draft, editing; MAA writing draft, editing, supervision; SAJ: Conceptualisation, writing draft, editing, supervision.

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Data availability
The principal investigator SAJ has access to the full trial data and materials and they can be made available upon request.

Declarations

Ethics approval and consent to participate
Research ethics approval
This clinical trial has been approved by the King's College, London Research Ethics Committee (KCL-REC) [Reference Number: HR/DP-22/23-34078, Study Title: TransVitD]. Any protocol amendments will be submitted to King's College REC for approval. The trial will comply with good clinical practice guidelines over the reporting of adverse events (AEs), serious adverse events (SAEs), and suspected serious adverse reactions (SUSARs) as well as providing the REC with progress reports and final study report.
Patient or participant protocol involvement
There was no patient or participant involvement in the study protocol design.
Protocol amendments
If amendments to this protocol are required, approval will be sought from the KCL-REC and updated protocol will be made available on request. This protocol version is 1.5, 10th October 2024. The PI will notify the trial sponsor, funders, and trial centre clinical teams of any protocol changes. In addition, a copy of the revised protocol will be added to the Investigator Site File and the consent forms and clinical trial registry entry will be amended to reflect the changes.

Consent for publication
All participants have agreed to the publication of these results.

Competing interests
SAJ is the inventor of the vitamin D phosphate patent WO2022084669A1. MAA and SAJ have received funding to develop and test the vitamin D patch in preclinical studies from Vitamax Patch Wholesaler LLC.

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