Archives of Dermatological Research (2025) 317:348
https://doi.org/10.1007/s00403-025-03837-4

ORIGINAL PAPER

Can the use of vitamin D-fortified sunscreen cream be the solution to the vitamin D deficiency pandemic?

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Received: 30 August 2024 / Revised: 3 January 2025 / Accepted: 18 January 2025

Abstract

Current approaches to vitamin D supplementation are generally limited to its oral intake. In this experimental study, the effects of applying vitamin D-fortified sunscreen creams to the skin on the absorption, and therefore levels of serum vitamin D metabolites were investigated. Forty 8-week-old male Wistar Albino rats were used in the study. Eight rats (Group B) were sacrificed to determine the baseline values of biochemical parameters. The remaining 32 rats were randomly divided into 4 equal groups as follows: Group S, only the back skin of the rats were shaved; Group SD, only vitamin D_3 diluted with sunflower oil was applied to the shaved area; Group SC, only sunscreen cream was applied to the shaved area; and Group SDC, sunscreen cream fortified with vitamin D_3 was applied to the shaved area. Serum 25(OH)D_3 and 24,25$(OH)_2D_3$ levels were determined at the end of 8 weeks. Mean (±SD) serum 25(OH)D_3 levels of groups B, S, SD, SC, and SDC were determined as 17.7±5.7, 13.5±3.1, 54.1±13.0, 19.6±2.7, 67.2±16.5 ng/mL, respectively. There were statistically significant differences in serum 25(OH)D_3 values between groups S and SD ($p<0.001$) and between groups SC and SDC ($p=0.002$). A positive correlation was found between serum 25(OH)D_3 and 24,25$(OH)_2D_3$ parameters ($r=0.772$; $p<0.001$). With this study, it was concluded that vitamin D-fortified sunscreen cream increases serum vitamin D levels by exerting transdermal activity. Further studies are required to confirm this observations.

Keywords Vitamin D · Sunscreen cream · Rat · Transdermal · Cancer

Introduction

Forms of vitamin D can be partially ingested through diet (vitamin D_2 and vitamin D_3) or synthesized via exposure of skin to sunlight (vitamin D_3) [1]. Upon exposure to sunlight, epidermal 7-dehydrocholesterol (7-DHC) is converted into provitamin D_3 by sunlight (UVB) at a wavelength of 290–315 nm. The conversion of provitamin D_3 to vitamin D in the skin takes about 8 h. Therefore, this process is slower than oral vitamin D intake. However, while all of the vitamin D produced in the skin binds to vitamin D binding protein (DBP) and reaches the liver, only about 60% of vitamin D taken orally through diet or supplementation binds to DBP [2]. Vitamin D entering the circulation is first converted to 25-hydroxyvitamin D (25(OH)D) by the action of hepatic hydroxylase. The circulating 25(OH)D level is an indicator of vitamin D intake from the skin through diet and exposure to sunlight. The 25(OH)D is converted to its active form, 1,25 dihydroxyvitamin D (1,25$(OH)_2$D), mainly by the kidneys in case of need. Vitamin D metabolism is controlled by parathyroid hormone [3]. Vitamin D

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Published online: 06 February 2025

contributes to bone mineralization, mainly by regulating calcium phosphorus homeostasis. In addition, vitamin D receptors (VDRs) is present in many tissues and organs (pancreatic, skin, pituitary, breast, colon and prostate cancer cells and immune cells). Vitamin D is thought to exert favorable protective effects in cases of immunodeficiencies, certain types of cancer, type 2 diabetes and cardiovascular diseases. Although the extraskeletal effects of vitamin D are not entirely clear, the popularity of vitamin D is increasing day by day [4, 5]. Similarly, keratinocytes in the epidermis also possess VDRs. The presence of $1,25(OH)_2D$ in the skin contributes to the integrity of the skin barrier, inhibition of proliferation, regulation of immunity, suppression of tumor formation, and hair follicle formation via VDRs [6].

Although vitamin D can be both exogenously ingested and endogenously synthesized in the body, it is estimated that more than half of the world's population is vitamin D deficient [7]. It is not possible to meet the daily vitamin D requirements from natural foods that are not fortified with vitamin D. The majority of vitamin D is synthesized endogenously in our body through the effects of ultraviolet B (UVB) radiation coming from the sun [8]. Factors such as working or living indoors, clothing style, use of a sunscreen creams, air pollution, season, latitude all affect vitamin D synthesis [9, 10]. Vitamin D intake of 15–20 µg (600–800 IU) per day is recommended to achieve a target serum 25(OH)D level of 50 nmol/L [11]. In order to overcome vitamin D deficiency, various foods and beverages are still being enriched with vitamin D. However, it has been reported that only commonly consumed vitamin D-fortified foods can be effective against vitamin D deficiency [12]. In addition, biliary obstruction, celiac disease, chronic pancreatitis and gastric bypass surgery etc. have been shown to decrease bioavailability of oral vitamin D. These disadvantageous conditions can negate the benefits of vitamin D-fortified foods [13]. Recently, microencapsulation studies have been carried out using nanotechnological developments to increase the bioavailability of vitamin D supplements added to foods [14]. Instead of direct fortification of foods with vitamin D, biofortification studies are carried out by adding vitamin D to animal feeds [15]. It has been also reported that the application of vitamin D-containing patches to the skin increases serum 25(OH)D levels by exerting transdermal activity [16].

The use of sunscreen creams, which are preferred both for cosmetic purposes and to protect the skin from the harmful effects of sunlight, is gradually increasing and becoming widespread. In addition, regular use of sunscreen creams is recommended in public education campaigns against many types of skin cancer caused by exposure to ultraviolet radiation from the sun [17]. A study conducted in Denmark covering approximately 20 years, reported that the use of sunscreen creams has gradually increased. Despite being a Northern European country, an increase was observed both in the frequency, and amount of the sunscreen cream with higher protection factor (SPF) values [18]. Similarly, studies conducted in Norway [19] and the USA [20] reported that the use of sunscreen creams is becoming more widespread. Some in vitro studies performed under laboratory conditions have reported that intensive sunscreen use (2 mg/cm^2) decreases cutaneous synthesis of vitamin D under UVB radiation [17, 21]. In contrast, studies with actual sunlight exposure have reported that sunscreen creams with high UVA and low UVB protection factors do not adversely affect vitamin D synthesis. In fact, these creams have been observed to increase vitamin D synthesis due to their UVB transmitting ability. In recent years, the use of broad-spectrum sunscreen creams that provide both UVA and UVB protection (2 mg/cm^2 intensity and at least 15 SPF) has been recommended [11, 22, 23].

In this experimental study, the beneficial effects of vitamin D-fortified sunscreen creams on the levels of serum vitamin D metabolites were examined. It is thought that adding vitamin D to sunscreen creams would be a good alternative way for improving vitamin D intake due to reasons such as the increasing widespread use of sunscreen creams, the potential of the ingredients of sunscreen creams to facilitate dermal penetration, and the binding of all dermally applied vitamin D by DBP. In addition, although a controversial issue, it was aimed to contribute to an increase in vitamin D intake through use of vitamin D-fortified sunscreen cream against the potential inhibitory effect of sunscreen creams per se on cutaneous vitamin D synthesis. This research, designed as an experimental animal model, may be an alternative model for the elimination of vitamin D deficiency common in people.

Methods

Experimental animals

In this study, 40 male 8-week-old (200–250 g) Wistar Albino rats were used. Experimental animals were obtained from Balıkesir University Experimental Animal Production, Care and Research Center (Balıkesir, Turkey). All rats were housed in a room under constant ambient temperature (21 ± 3 °C) and fed with vitamin D-poor rat food and water intake ad libitum in alternating 12 h light, and 12 h dark cycles during the 4-week pre-experimental adaptation and 8-week experimental period. All study groups were housed in the same room and maintained under identical conditions (e.g., temperature, humidity) throughout the experiment. The experimental protocol was approved by the Balıkesir

University Local Ethics Committee for Animal Experiments (2023/2–3).

Materials

In this study, Devit-3 IM/oral ampoule (300,000 IU/ml) and Devit-3 oral drops (50,000 IU/15 ml) (Deva Pharmaceutical Co., Turkey) with the same vitamin D form (cholecalsiferol) were used as vitamin D source. Taking advantage of the difference in concentrations of these two products, namely, Devit-3 ampoules were used to be added to the sunscreen cream and Devit-3 drops were used for direct application to the shaved area.

Commercially available sunscreen cream (Sebamed Sun Care Multi Protect SPF 30 Sun Cream, Sebapharma GmbH & Co. KG, Germany) was preferred in our study. This is a broad spectrum cream that provides protection against both UVA and UVB radiation. It contains titanium dioxide as a UV reflector and organic ingredients such as octocrylene, diethylamino hydroxybenzoyl hexyl benzoate, avobenzone, bemotrizinol and iscotrizinol as UV absorbers. Apart from UV blockers, sunscreen cream contains water, glycerin, benzyl alcohol as solvents or moisturizers; lecithin, glyceryl stearate, stearyl alcohol, cetyl palmitate as emulsifiers; citric acid, sodium hydroxide as pH regulators; and cellulose, cellulose gum, xanthan gum, silica for viscosity control.

Experimental protocol

Forty rats were randomly divided into 5 groups with 8 experimental animals in each group (Table 1):

Basal group (B): It was formed to determine the levels of biochemical parameters at the beginning of the experiment.

Shaved-only group (S): Only back skin of the rats was shaved regularly for eight weeks without application of anything to the shaved areas.

Table 1 Summary of the experimental protocol

Groups	*n*	Daily doses	Method of Application	Application period
B	8	-	-	0
S	8	-	-	8 weeks
SD	8	5 µg vitamin D_3 /60 µl Devit3 Drops	Dermal	8 weeks
SC	8	160 mg sunscreen cream (2 mg/cm^2)	Dermal	8 weeks
SDC	8	5 µg vitamin D_3 /160 mg sunscreen cream (2 mg/cm^2)	Dermal	8 weeks

n: Number of rats, **B**: Basal group, **S**: Shaved-only group, **SD**: Shaved group treated with Devit-3 drops **SC**: Shaved group treated with topical application of sunscreen cream **SDC**: Shaved group treated with topical application of vitamin D-fortified sunscreen cream

Shaved group treated with vitamin D_3 (SD): Back skin of the rats was shaved, and only vitamin D_3 (Devit-3 drops) was applied to the shaved areas for eight weeks.

Shaved group treated with topical application of sunscreen cream (SC): Back skin of the rats was shaved, and only sunscreen cream was applied regularly to the shaved areas for 8 weeks.

Shaved group treated with topical application of vitamin D-fortified sunscreen cream (SDC): Back skin of the rats was shaved, and sunscreen cream fortified with vitamin D_3 was applied to the shaved areas for 8 weeks.

Immediately following the formation of the experimental groups, rats in group B were sacrificed after their serum samples were taken under general anesthesia. The samples were stored at -40 °C until the time of analysis. For the remaining four groups, the back skin area of the rats (approximately 80 cm^2) was shaved every other day. A specialized shaving machine designed for animals was utilized. Shaving and the application of cream were performed with careful attention to the animals' comfort. Sunscreen cream at a concentration of 2 mg/cm^2 (recommended concentration) was applied to the shaved area daily in a circular motion with a gloved fingertip. Animals were taken to their cages 20 min after application of sunscreen cream [22, 24]. While only back skin of the rats was shaved in group S, 60 µl of Devit-3 drops (containing 5 µg vitamin D_3) were applied daily to the shaved areas in group SD. Devit-3 drops, which contain sunflower oil, were preferred because they contained a natural compound and research studies have shown that fatty acids (especially oleic acid) increase intradermal penetration, and do not irritate the skin [25]. In group SC, only 160 mg (2 mg/cm^2) sunscreen cream was applied to the shaved areas every day. In group SDC, 160 mg (2 mg/cm^2) sunscreen cream containing 5 µg vitamin D_3 was applied to the shaved areas.

Collection of samples

At the end of 8-weeks of experimental period, rats were anesthetized with 80 mg/kg ketamine and 8 mg/kg xylazine at doses adjusted to their body weights. Under deep anesthesia, cardiac puncture was performed and 5 ml of blood samples were collected in gel tubes. Blood samples were centrifuged at 1300xg for 15 min and the separated serum samples were collected in Eppendorf tubes and stored at -40 °C until the time of analysis.

Biochemical measurements

Serum aspartate transaminase (AST), alanine transaminase (ALT), calcium and phosphorus levels in samples obtained

Table 2 Vitamin D metabolites are shown along with respective precursor/product ions and collision energy

Analytes	Precursor (m/z)	Product (m/z)	Collision Energy (eV)	Polarity
$25(OH)D_3$	401,3	257,2	15	Positive
$25(OH)D_3$	401,3	159,3	30	Positive
$24,25(OH)_2D_3$	417,3	381,3	15	Positive
$24,25(OH)_2D_3$	417,3	159,2	30	Positive
$25(OH)D_3$-d3	404,3	257	15	Positive
$24,25(OH)_2D_3$-d6	423,3	159,3	30	Positive

were measured using commercial kits (Beckman Coulter, USA) in an autoanalyzer (AU 680 Beckman Coulter, USA).

Plasma vitamin D metabolites ($25(OH)D_3$, $24,25(OH)_2D_3$) were measured by The Thermo Scientific™ TSQ Quantum™ Access MAX LC-MS/MS triple quadrupole mass spectrometer using a commercial kit (EqC Lab. Tech. and R&D Inc., Turkey).

In LC-MS/MS analyses, protein precipitation was performed after adding internal standard solution to plasma. The supernatant obtained from samples and centrifuged at 10,000xg was vialed for analysis. For chromatographic purification, the samples were placed in the HPLC device. Mobile phase flow rate was 0.6 mL/min and sample volume was 20 µL. For mass spectrometric analysis, atmospheric pressure chemical ionization (APCI) method was used. Capillary temperature was set at 250ºC, vaporizer temperature at 270ºC, sheath and auxillary (aux) gas pressure at 50 and 15 arb, respectively. Within-run, between-run and between day precisions (CV%) for $25(OH)D_3$ and $24,25(OH)_2D_3$ were 3.0%, 3.4%, 4.9%, 3.4%, 3.9% and 4.4% respectively. Both vitamin metabolites were observed to be linear in the range of 2.1–200 ng/mL. The most sensitive multiple reaction monitoring (MRM) transition with the most sensitive precursor and product ions was selected for LC-MS/MS analysis. The mass spectrometry parameters of the analytes and the selected MRM transitions are given in Table 2.

Statistical analysis

Statistical analyses were performed using IBM SPSS 25.0 package program. Scatter plots (histograms) and Shapiro-Wilk test were used to investigate whether the data were normally distributed. Intergroup comparisons were made with the One-Way ANOVA test when the assumption of homogeneity of variances was met. Post-hoc analysis of the groups were evaluated with Tukey test. When the variances were not homogeneous, Welch test was used and Tamhane T2 analysis was applied for post-hoc analysis of the groups. Chi-square test or Fisher's exact test was used to evaluate categorical data. Pearson correlation analysis was employed to evaluate the relationship between the parameters. $p<0.05$ was accepted as the level of statistical significance.

Results

The blood sample of one rat from the SDC group was excluded from the analysis due to hemolysis and a total of 39 rats were included in the evaluation.

Biochemical findings

Mean (±SD) and minimum-maximum values of serum AST, ALT, calcium, phosphorus are given in Table 3. A statistically significant intergroup difference was found as for serum levels of AST ($p<0.001$), ALT ($p=0.015$), calcium ($p<0.001$) and phosphorus ($p=0.011$).

Mean (±SD) serum $25(OH)D_3$ levels of groups B, S, SD, SC, and SDC were determined as 17.7±5.7, 13.5±3.1, 54.1±13.0, 19.6±2.7, 67.2±16.5 ng/mL, respectively. For serum $25(OH)D_3$ values the difference between the groups was statistically significant ($p<0.001$; Welch Test). When the mean (±SD) serum $25(OH)D_3$ values of the groups were subjected to post-hoc analyses (Fig. 1), the mean (±SD) serum $25(OH)D_3$ value of the group S was significantly lower than those of groups SD ($p<0.001$) and SDC ($p=0.001$). Similarly, the mean (±SD) serum $25(OH)D_3$

Table 3 Mean (±SD) and minimum-maximum values of remarkable biochemical parameters by groups

	Groups					
	B (*n*:8)	S (*n*:8)	SD (*n*:8)	SC (*n*:8)	SDC (*n*:7)	*p*
AST (U/L)	110.13±12.22 94–129	135.88±10.70 119–150	142.50±14.42 117–168	122.75±8.99 110–134	114.71±15.06 95–143	*<0.001*[a]
ALT (U/L)	55.50±6.82 46–65	62.88±3.60 56–68	68.63±5.97 60–78	60.25±5.20 53–69	58.43±10.00 49–71	*0.015*[b]
Ca (mg/dL)	12.29±0.24 11.8–12.5	10.38±0.24 10.1–10.8	10.35±0.19 10.1–10.7	10.39±0.17 10.2–10.6	10.27±0.17 10-10.5	*<0.001*[a]
P (mg/dL)	8.71±1.15 6.7–10.3	7.74±1.00 6.3–9.2	7.61±0.78 6.6–8.7	8.48±0.73 7.5–9.7	9.20±0.99 7.6–10.7	*0.011*[a]

[a]ANOVA Test, [b]Welch Test. **B**: Basal group, **S**: Shaved-only group, **SD**: Shaved group treated with Devit-3 drops, **SC**: Shaved group treated with topical application of sunscreen cream, **SDC**: Shaved group treated with topical application of vitamin D-fortified sunscreen cream

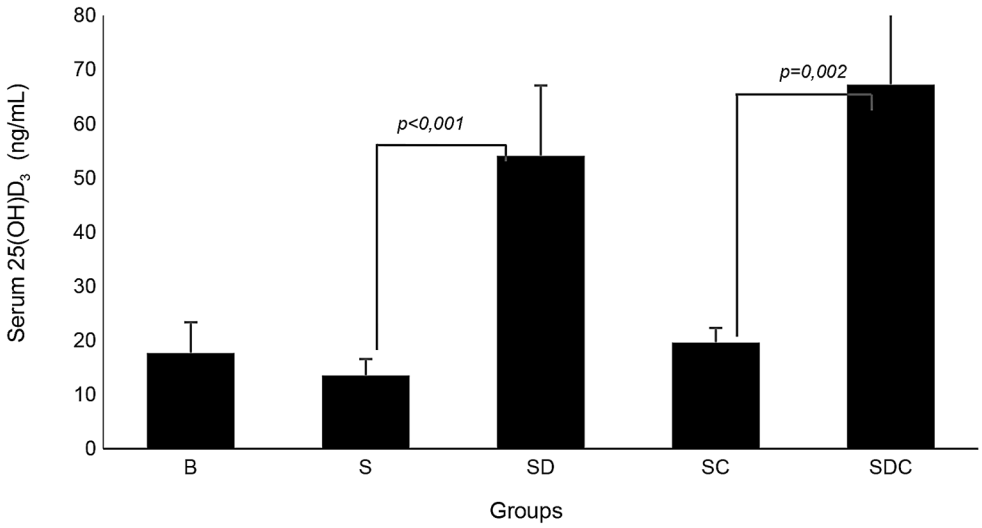

Fig. 1 Comparison of serum $25(OH)D_3$ levels between grups. B: Basal group, S: Shaved-only group, SD: Shaved group treated with Devit-3 drops, SC: Shaved group treated with topical application of sunscreen cream, SDC: Shaved group treated with topical application of vitamin D-fortified sunscreen cream

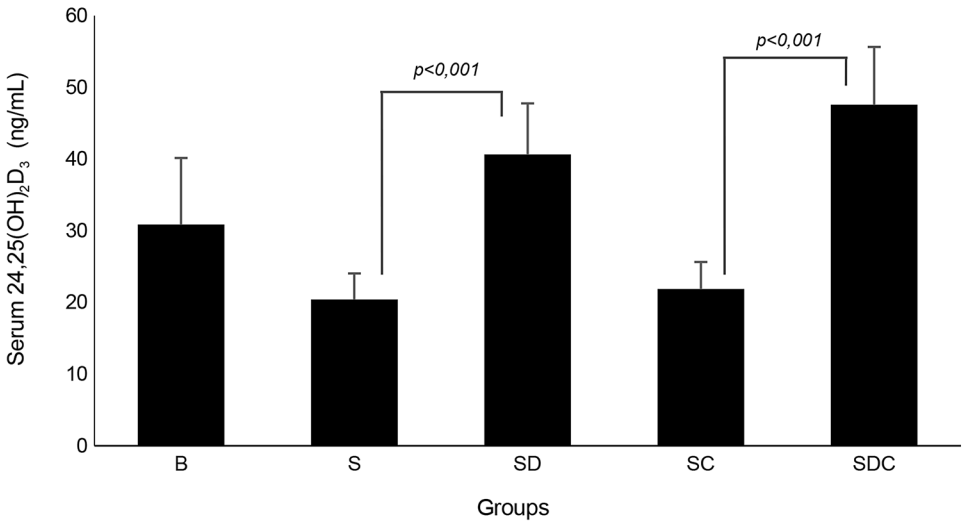

Fig. 2 Comparison of serum $24,25(OH)_2D_3$ levels between grups. B: Basal group, S: Shaved-only group, SD: Shaved group treated with Devit-3 drops, SC: Shaved group treated with topical application of sunscreen cream, SDC: Shaved group treated with topical application of vitamin D-fortified sunscreen cream

value of the group SC was significantly lower than those of groups SD ($p=0.001$) and SDC ($p=0.002$). Although the mean (±SD serum $25(OH)D_3$ value of the group SDC was higher than that of group SD, this intergroup difference was not statistically significant ($p=0.712$). The mean (±SD) serum $25(OH)D_3$ value of Group B was significantly lower than those of groups SD ($p<0.001$) and SDC ($p=0.001$).

Mean (±SD) serum $24,25(OH)_2D_3$ levels of groups B, S, SD, SC, and SDC were determined as 30.9±9.3, 20.4±3.6, 40.7±7.1, 21.9±3.8, 47.6±8 ng/mL, respectively. For serum $24,25(OH)_2D_3$ values the difference between the groups was statistically significant ($p<0.001$; ANOVA). When the groups were subjected to post-hoc analyses regarding serum $24,25(OH)_2D_3$ values (Fig. 2), the mean (±SD) serum $24,25(OH)_2D_3$ value of the group S was significantly lower than those of the groups SD ($p<0.001$) and SDC ($p<0.001$). Similarly, the mean (±SD) serum $24,25(OH)_2D_3$ value of the group SC was significantly lower than those of the groups SD ($p<0.001$) and SDC ($p<0.001$). In addition, the mean (±SD) serum $24,25(OH)_2D_3$ value of the group B was significantly lower than those of groups SD ($p=0.047$) and SDC ($p<0.001$). Although the mean (±SD) serum $24,25(OH)_2D_3$ value of the group SD was lower than that of the group SDC, this intergroup difference was not statistically significant ($p=0.290$).

Discussion

In our review, no similar study was found in the literature that examined serum vitamin D values by using vitamin D-fortified sunscreen cream. Based on some previous experimental studies, Wistar Albino rats were used in our study due to their suitability for shaving and cutaneous application of sunscreen cream [26, 27]. Although rat skin is more permeable than human skin, it is the most similar to

human skin among rodents [28]. It has also been reported that cholecalciferol given to Wistar rats is metabolized by their organisms [29].

Serum parameters of AST and ALT are enzyme biomarkers used to monitor structural integrity and damage of the liver [30]. Serum AST and ALT variables were analyzed to ensure that the liver, which plays a major role in 25(OH)D synthesis, is not dysfunctional. In addition, serum calcium and phosphorus values were analyzed to observe potential vitamin D toxicity. Hypercalcemia and relatively high serum phosphorus levels are expected in vitamin D toxicity [31]. Ignoring statistical differences between groups, these biochemical values were evaluated according to the reference ranges determined for animals with similar characteristics [32–35]. Serum AST, calcium and phosphorus values of the rats were found to be within their normal reference ranges regardless of the group studied. However, serum ALT values of some animals were at or above the upper limit of the reference range. It has been reported in the literature that serum ALT/AST ratio is an index to determine liver pathology and this ratio should be less than 1 [30]. In our study, ALT/AST ratios of all animals were less than 1. Therefore, we have confirmed in this study that hepatic function of the rats were not impaired without any risk of vitamin D toxicity.

Group B, which was terminated at the beginning of the study, was created to demonstrate the vitamin D deprivation of the rats during the study period of 8-weeks. Any statistically significant difference was not observed between the B group and the S group in terms of serum vitamin D levels and it was understood that the experiments had been conducted under stable conditions. In this study, the S group served as the control group for the SD group and the SC group served as the control group for the SDC group. In addition, in the SD group, oral vitamin D_s drops (Devit-3 drops) of the same company (DEVA Pharma, Istanbul, Turkey), containing the same form of vitamin D (cholecalciferol) ready diluted in sunflower seed oil (Devit-3 ampoule) were used. Thus we intended to show the difference between applications of sunscreen cream per se and vitamin D-fortified sunscreen cream.

A statistically and clinically significant difference (approximately 3-fold) was noted between vitamin D-treated (SD, SDC) and untreated groups (B, S, SC) in terms of mean serum 25(OH)D_3 values. Based on this finding, transdermal administration may serve as an effective alternative route for vitamin D intake. The presence of DBP in the skin supports transdermal efficacy by binding and delivering vitamin D. Furthermore, transdermal administration may bypass the negative impact of gastrointestinal diseases that cause fat malabsorption, which hinder vitamin D absorption. Nevertheless, limited research exists on the transdermal delivery of vitamin D. In an ex vivo study using porcine skin, vitamin D_3 successfully penetrated the skin [25]; however, in a similar ex vivo study using human skin, vitamin D_3 penetration was not observed [36]. In another study using rat epidermal keratinocytes, a vitamin D phosphate analog demonstrated transdermal activity and increased DBP levels in keratinocytes [37].

In our study, serum 25(OH)D_3 levels were significantly increased in both SD and SDC groups. The reason for the increase in serum vitamin D levels in the SD group is thought to be related to the fact that the sunflower oil in the commercial preparation increases its penetration. In related studies, it has been reported that some fatty acids increase transdermal permeability of vitamin D [38, 39].

Recently, studies on the use of transdermal patches for the delivery of vitamin D have been conducted at an increasing rate. At the same time, the ingredients that increase the transdermal permeability of vitamin D in these applications are being investigated. In an in vitro study using artificial skin, a transdermal patch containing vitamin D_3 diluted in various solutions was produced and its permeability was monitored. Vitamin D_3 permeability increased in the presence of ethanol and acetone [40]. In similar laboratory studies, substances such as lecithin, isopropyl palmitate, ethoxyglycol, grain alcohol, dodecylamine were reported to increase transdermal permeability of vitamin D [25, 36]. It is extremely important for the penetration of vitamin D_3, which is highly hydrophobic, to ensure its aqueous solubilization without losing its activity [41]. The sunscreen cream used in our study contains water, glycerin, benzyl alcohol as solvent or moisturizer; lecithin, glyceryl stearate, stearyl alcohol, cetyl palmitate as emulsifier; citric acid, sodium hydroxide as pH regulator; cellulose, cellulose gum, xanthan gum, silica for viscosity control. The presence of multiple solvents and emulsifiers in the sunscreen cream used in the study provides an advantage for transdermal penetration of vitamin D_3 (SDC group). In addition, active vitamin D metabolites (such as calcitriol), which are more hydrophilic than cholecalciferol, penetrate better through the skin [13]. Although highly hydrophobic vitamin D (cholecalciferol) was used in our study, the increase in serum 25(OH)D_3 level is thought to be due to the ingredients present in sunscreen cream (SDC group). However, the potential of sunscreen creams with different contents to negatively affect vitamin D activity should be taken into consideration.

The body areas where people commonly apply sunscreen in daily life (e.g., face, neck, hands, arms, lower legs, and feet) account for approximately 25% of the total body surface area [42]. In this study, following the human model, approximately one-fourth (80 cm^2) of the rats' body surface area was shaved, and sunscreen cream was applied [43]. However, determining the precise amount of vitamin D-fortified sunscreen cream required for humans remains

challenging. Further studies involving human participants are necessary to address this issue.

The number of studies investigating the long-term effects of transdermal vitamin D application is limited. In humans, an aloe vera-based cream containing vitamin D3 has been shown to increase serum 25(OH)D levels after 3 to 4 months of use [44, 45]. Another study examined the effects of a transdermal patch containing cholecalciferol on 30 healthy adults over a specific follow-up period. At the end of the study, it was reported that serum 25(OH)D levels gradually increased every week [16]. These studies motivate to test sunscreen cream containing added vitamin D in humans. In the transdermal patch study, skin rash and irritation were reported on the skin of some participants depending on the patch size [16]. However, in our study, performed on rats any skin rash, irritation or allergic reaction was not observed during the 8-week application.

The 24, 25 dihydroxy vitamin D_3 ($24,25(OH)2D_3$) is formed as an end product of the vitamin $25(OH)D_3$ metabolism. Serum $24,25(OH)_2D_3$ levels increase as a result of catabolism of both $25(OH)D_3$ and $1,25(OH)_2D_3$ metabolites [46]. Therefore, in our study, it is expected that serum $24,25(OH)_2D_3$ levels also increase with increasing serum 25(OH)D3 levels due to transdermal vitamin D_3 intake. In addition, it has been reported that vitamin D metabolite ratio (VMR) (serum $24,25(OH)_2D$ / serum 25(OH)D) reflects vitamin D sufficiency independent of changes in DBP [47]. In our study, serum $24,25(OH)_2D_3$ values increased similarly to serum $25(OH)D_3$ values. This is an indication that transdermally administered vitamin D is metabolized. In addition, VMR values were found to be lower in the vitamin D-treated groups (especially SDC group) compared to the untreated groups (S and SC groups) in our study. This phenomenon was interpreted as a decrease in $25(OH)D_3$ catabolism versus $1,25(OH)_2D_3$ saturation.

The strength of this study is that while searching for transdermal applications such as sunscreen patches, creams and strips as an alternative to oral vitamin D intake, this study is the first in the literature to demonstrate transdermal uptake of vitamin D-fortified sunscreen cream, which has amphipathic properties thanks to its ingredients. The use of sunscreen cream is strongly recommended against erythema formation, skin burns and cancer risk. Our study may contribute to the treatment of vitamin D deficiency, by fortifying sunscreen creams -which are nowadays used increasingly, and prevalently- with vitamin D supplements. Although not entirely clear, it may be possible that sunscreen creams mediate vitamin D uptake against the potential of sunscreen creams to inhibit or reduce cutaneous vitamin D synthesis. Since vitamin D-fortified sunscreen cream facilitates transdermal penetration of vitamin D, hydrophobic vitamin D absorption may be more effective compared to other transdermal applications. Considering the disadvantages of its oral intake (malabsorption, GIS diseases, inability to bind with DBP), transdermal application of vitamin D supplemented sunscreen cream seems advantageous in terms of its favorable bioavailability.

The potential benefits of transdermal vitamin D may extend beyond this. While the skin serves as a strong barrier, it also facilitates the delivery of certain molecules. Lipophilic vitamin D can more easily penetrate the stratum corneum and reach underlying tissues. Additionally, keratinocytes in the epidermis can synthesize vitamin D3 from 7-DHC and convert it into its active form, $1,25(OH)_2D_3$. Epidermal $1,25(OH)_2D_3$ contributes to maintaining skin barrier integrity and regulating immunity through its interaction with VDR in keratinocytes [6].

This study has several important limitations. For effective transdermal vitamin D intake, sunscreen creams should be used at recommended doses and frequencies. Although the use of sunscreen creams is becoming increasingly common, the dosages and frequency of application may be insufficient for adequate vitamin D intake [48]. Additionally, sunscreen creams are used less frequently in fall and winter due to a lower risk of sunburn. Therefore, transdermal vitamin D intake through sunscreen may not be a viable alternative during fall and winter, when vitamin D requirements are higher. Transdermal efficacy of vitamin d may be lower for human skin compared to the rat skin due to the different characteristics of rat and human skin (such as the number of pores). Further studies with similar designs in human subjects are recommended. It is also known that environmental factors such as heat, light, and oxygen affect vitamin D stability and reduce its bioavailability [49]. Under UV radiation exposure from the sun, vitamin D in sunscreen may undergo photodegradation, potentially diminishing its transdermal efficacy. Moreover, the formulation of sunscreen creams may provide protection against the degradation of vitamin D. Including a study group with UV exposure could have strengthened the findings of this study. Vitamin D application via sunscreen cream may be considered a prophylactic approach rather than a therapeutic intervention for vitamin D deficiency.

In conclusion, this study demonstrated that vitamin D incorporated into sunscreen cream exhibits transdermal activity, leading to increased serum vitamin D levels. This approach may represent an alternative route for vitamin D supplementation; however, further studies are required to confirm these findings.

Acknowledgements We are very grateful to Dr. Elif Aksöz, Dr. Mustafa Hilmi Yaranoğlu for their technical assistance and/or discussions. We would also like to thank medical translator and interpreter Dr. Gürkan Kazancı for his assistance in proofreading of the manuscript.

Author contributions HK: conceptualization, design, material preparation, data collection, analysis and manuscript preparation. ÜP: conceptualization, design, manuscript editing and manuscript review. ÖB: conceptualization, design, material preparation and analysis. ES: material preparation and data collection. GT: data collection and analysis. All authors read and approved the final manuscript.

Funding No funding was received for conducting this study.

Data availability Data of the present study can be requested from the corresponding author upon reasonable request.

Declarations

Ethical approval Balıkesir University, Local Ethics Committee for Animal Experiments, Decision Number: 2023/2–3.

Competing interests The authors declare no competing interests.

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