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Effect of Uv-C Enrıched Food Consumptıon on Bıochemıcal Parameters in Adults wıth Vıtamın D Deficıency: A Randomızed Controlled Trıal

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ABSTRACT

Background: Mushrooms naturally contain low levels of vitamin D, while bread is not a natural source of this vitamin. The aim of this study was to determine the effect of consuming UV-treated foods on serum vitamin D levels on individuals with vitamin D deficiency. **Methods:** In this triple-blind, randomized, placebo-controlled study, 35 participants with serum 25(OH)D<30µg/l were randomly divided into four groups. The groups were as follows: Group 1, enriched mushroom (50 µg Vitamin D) + regular bread; Group 2, enriched mushroom (40 µg vitamin D) + enriched bread (10 µg vitamin D); Group 3, normal mushroom + regular bread; and Group 4, normal mushroom + regular bread + supplement (50 µg vitamin D). At the commencement of the study, and at weeks 2, 4 and 6, as well as one month after the study's conclusion, collected three tubes of blood from each participant. The initial blood sample was analyzed for calcium and parathormone levels. The remaining tubes of blood were then dispatched to the central laboratory for analysis of 25(OH)D and 25(OH)D2. **Results:** At the end of 6 weeks, no significant difference was found in the increase of serum 25(OH)D concentrations between enriched groups and supplement group, while the increase in control group was found to be significantly lower than the increase in the other groups (P<0.001). **Conclusions:** The bioavailability of vitamin D2 obtained from oyster mushrooms enriched with UV-C application and enriched whole wheat bread in humans is effective in correcting 25(OH)D deficiency, and the results after 6 weeks were no different from those obtained with the same amount of vitamin D3 supplementation.

Introductıon

Vitamin D is a fat-soluble vitamin essential for bone health and has important roles in non-skeletal health, organ function and disease prevention (Moulas and Vaiou, 2018). The main natural source of vitamin D is dermal synthesis through exposure to UV-B light. Only a small fraction of the required amount is obtained from dietary unfortified foods (Holick, 2017).

There are two forms of vitamin D: cholecalciferol or vitamin D3 and ergocalciferol or vitamin D2 (Moulas and Vaiou, 2018).

Vitamin D3 is produced in the skin of

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vertebrates by a non-enzymatic mechanism through the action of ultraviolet (UV) radiation (290-315 nm) on the precursor molecule 7-dehydrocholesterol (Moulas and Vaiou, 2018). Vitamin D2 is produced by irradiation of the plant sterol ergosterol (Holick, 2017). Both cholecalciferol and ergocalciferol are rapidly absorbed by the intestine (Silva and Furlanetto, 2018). After dermal production of cholecalciferol, cholecalciferol and ergocalciferol from plant foods enter the circulation from the intestine bound to vitamin D binding protein (DBP). Vitamin D in the skin, which is produced endogenously, is bound by DBP, and after dissociation, it can be taken up by target cells predominantly via the vitamin D receptor (VDR) and reach its target sites intracellularly (Schümmer *et al.*, 2021). After production or absorption, cholecalciferol is transported to the liver where it is enzymatically hydroxylated to 25-hydroxy vitamin D3 [25(OH)D3], the major circulating vitamin D compound (Christakos *et al.*, 2016). In a second enzymatic hydroxylation step, 25(OH)D3 is hydroxylated in the kidney to 1,25-dihydroxy vitamin D3 [1,25(OH)2D3], the hormonal form of vitamin D (Moulas and Vaiou, 2018). 25(OH)D is converted into 1,25(OH)2D in other tissues, especially in the kidney, and forms its most active form. 25(OH)D is at least a thousand times more abundant in the blood than 1,25(OH)2D. Therefore, the vitamin D level that should be measured in blood is 25(OH)D (Şenkal *et al.*, 2018). Both forms of vitamin D are responsible for maintaining serum 25(OH)D levels in humans (Koyyalamudi *et al.*, 2011).

Vitamin D has been reported to be responsible for three mechanisms. The first is that it is the only hormone known to stimulate proteins involved in intestinal calcium absorption, and it also stimulates intestinal phosphate absorption. The second mechanism is its role in increasing blood calcium concentrations, especially in the absence of intestinal calcium absorption (Suda *et al.*, 2003). The third mechanism takes place in the distal renal tubule in the kidney (Aksoy, 2000). When serum calcium is elevated, it suppresses bone destruction by decreasing parathormone hormone (PTH) secretion. Thus, reabsorption from the kidney decreases and urinary excretion increases (Derneği, 2022).

Currently, <20 ng/ml is considered deficient, 20-30 ng/ml is considered insufficient, and ≥30 ng/ml is considered sufficient (Holick *et al.*, 2011). Vitamin D intoxication is considered when the level is above 150 ng/ml (Derneği, 2019). Also. the European Food Safety Authority (EFSA) has set the adequate intake for all population groups at 600 IU (15 µg) for adults and 400 IU (10 µg) for infants (Bresson *et al.*, 2016).

Biological enrichment or biofortification refers to an increase in the nutritional content of a plant or animal-based food by adding vitamins during food processing, rather than adding vitamins by cultivating or genetic engineering, or by adding another food that is rich in a nutrient (Moulas and Vaiou, 2018, Sakhaei *et al.*, 2019). Biofortification represents a simple, noninvasive, and convenient strategy to enhance nutritional intake and may provide long-term preventive health benefits (Neill *et al.*, 2023).

Mushrooms have been consumed since ancient times due to their nutritional and medicinal properties. They have been reported to exhibit notable health-promoting activities, including antioxidant, antitumor, antimicrobial, anti-inflammatory, anti-tyrosinase, immunomodulatory, antiatherogenic, and hypoglycemic effects. The fruiting bodies of fresh or processed mushrooms are known to be rich in sterols, particularly ergosterol, which can be converted to vitamin D2 by UV irradiation. Techniques such as gamma irradiation and electron beam irradiation of mushrooms have been reported as a safe and cost-effective method commonly used to extend shelf life before or after harvest, and preserve quality, nutritional value (proteins, sugars, and vitamins), and bioactive composition (phenolics including flavonoids and aroma compounds) (Taofiq *et al.*, 2017). Exposure to sunlight or UV radiation has been recognized as an excellent source of vitamin D2 due to their high concentrations of the vitamin D precursor (ergosterol)and provitamin D2

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(Keegan *et al.*, 2013). Vitamin D content increased in mushrooms which were exposed to UV light after harvest, and vitamin D accumulation was measured after cold storage, cooking, and drying (Kohn, 2016). While vitamin D2 levels in UV-exposed mushrooms increase, if consumed before the expiration date, they can remain above 10 µg/100 g fresh weight. This is higher than most foods containing vitamin D (Cardwell *et al.*, 2018). Finally, vitamin D2 contents in mushrooms have been reported to depend on the duration of UV exposure, irradiation intensity, and UV source (sunlight, pulsed or continuous UV lamps, selected wavelengths (UV-A, UV-B, UV-C)) as well as the distance between the tissue surface and the emission source (Sommer *et al.*, 2023).

Mushroom consumption worldwide has increased significantly over the last four decades. They have been identified as the only non-supplemented, non-animal food that can provide significant amounts of the vitamin, and have the potential to be the only species that is a significant source of vitamin D in a single serving (Cardwell *et al.*, 2018). The cell membranes of bread yeast (*Saccharomyces cerevisiae*) also contains high amounts of ergosterol (35 mg/g dry cell weight). These high levels of ergosterol make baker's yeast an ideal target for UV irradiation (Schümmer *et al.*, 2021).

It has been determined that this approach is applicable for controlling vitamin D deficiency, particularly in individuals who do not consume animal-based foods, by applying it to inexpensive food like mushroom and staple foods like bread, thereby reducing costs and making it accessible to a wider population (Mehrotra *et al.*, 2014).

Vitamin D–enriched foods are proposed as a potential alternative to dietary supplements. Therefore, this study aimed to evaluate the efficacy of foods enriched with vitamin D via UV-C radiation in comparison with conventional vitamin D supplementation, using a novel methodological approach.

Materials and Methods

Study design and participants

This study was a 6-week, prospective, randomized, 4-arm, triple-blind, placebo-controlled trial which investigated the bioavailability of vitamin D2. The trial was initiated on February 8, 2023. Participants were recruited from hospital staff who were willing to adhere to their assigned dietary intervention and attend study visits as scheduled. Inclusion criteria were: age 18–65 years, body mass index ≤35 kg/m², serum 25(OH)D <30 µg/l, not pregnant or lactating, no vitamin D or fish oil supplementation within the previous month, no history of kidney disease or nephrolithiasis, no use of corticosteroids or anticoagulants, no solarium use, and fish consumption ≤1 serving per week. Eligible participants meeting these criteria were enrolled in the study.

Study protocol

In the first phase of this triple-blind, randomized study, 48 eligible volunteers were assigned to one of four groups using computer-assisted randomization: Group 1, enriched mushroom (50 µg Vitamin D) + regular bread; Group 2, enriched mushroom (40 µg vitamin D) + enriched bread (10 µg vitamin D); Group 3, normal mushroom + regular bread; and Group 4, normal mushroom + regular bread + supplement (50 µg vitamin D3)

The study employed a triple-blind design. Participants were unaware of their group allocation. The personnel responsible for preparing and serving the soups were separate, and all ingredients were labeled with coded identifiers, ensuring that neither the preparers nor servers knew the contents. The principal investigator monitored consumption without knowledge of the product assignments. Following the study, an independent academic disclosed the correspondence between codes and products. In addition, compliance was directly observed by the researcher, with all soup and bread consumption recorded in the hospital cafeteria.

Oyster mushrooms (Pleurotus ostreatus) and whole wheat bread were used in this research. Fresh mushrooms were sourced daily from wholesale suppliers, and bread from local vendors.

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UV-C enrichment of mushrooms and bread was performed daily in Beko laboratories, and the prepared materials were delivered to the hospital each morning. Each mushroom serving weighed 50 g (dry weight). Moreover, vitamin D3 content in mushroom soup was 0.203±0.025 µg/g (50.63±6.14 µg/250 g) for group 1 and 0.161±0.023 µg/g (40.14±5.73 µg/250 g) for group 2. UV-C–treated bread contained 0.266±*0.025* µg/g (10.65±1.01 µg/40 g), with a daily bread serving of 40 g per participant.

Ingredients, including enriched and unfortified mushrooms, bread, milk, butter, flour, salt, and black pepper, were delivered daily to the hospital cafeteria in four coded boxes according to group allocation. Soups and breads were also freshly prepared and provided to the participants on weekdays and weekends for six weeks. In group 4, daily dose of 50 µg vitamin D3 supplement was added to the unfortified mushroom soup. Food consumption records were collected by a dietitian at the beginning, midpoint, and end of the study. After one month, blood samples were collected to evaluate changes in biochemical parameters.

Sample size estimation

The sample size was determined based on the study by Urbain *et al.*(Urbain *et al.*, 2011). Using G*Power analysis with reference to serum vitamin D values in the mushroom, supplement, and placebo groups, an effect size of 0.798, an alpha level of 0.05, and a statistical power of 0.80 were applied, resulting in a minimum of 7 participants per group (total n=28). To account for potential dropouts, 75% additional participants were recruited, yielding an initial sample of 48 participants. The study concluded with 35 participants, ensuring that each group contained at least 7 individuals.

Biochemical analyses

Serum 25(OH)D, 25(OH)D3 (µg/l), calcium (mg/dl), and parathormone (pg/ml) levels were measured at baseline, the 2nd, 4th, and 6th weeks, and one month after study completion. Three blood samples were also collected from each participant in the mornings at each time point.

The first tube was used for calcium and parathormone analysis at the University of Health Sciences Fatih Sultan Mehmet Training and Research Hospital. After collection, samples were centrifuged at 4100 rpm for 10 minutes using a Nüve NF1200R centrifuge and analyzed in the hospital biochemistry laboratory. Calcium concentrations were measured using the Calcium Gen 2 assay with two-point calibration on the Roche Cobas c 501 analyzer, while parathormone levels were determined using the Elecsys PTH assay on the Roche Cobas e 601 analyzer.

The remaining tubes were sent on the same day, at 4 °C, via blood transport vehicle to the central laboratory of Istanbul Göztepe Prof. Dr. Süleyman Yalçın City Hospital for 25(OH)D and 25(OH)D*3* analysis. After centrifugation under the same conditions, vitamin D levels were quantified using the vitamin D assay on the Roche Cobas e 601 analyzer.

Ethical considerations

This trial approved by the Fatih Sultan Mehmet Education and Research Hospital Clinical Trials Ethics Committee (Approval No: 2023/13, Date: 26.01.2023), and concluded on March 22, 2023, following a 6-week intervention. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants prior to enrollment. The clinical trial is registered under NCT06098820 https://clinicaltrials.gov/study/NCT06098820.

Data analysis

In the study, participants' food consumption records were calculated using the “BeBİS 9” program, and the “SPSS 22.0” package program was used for statistical analysis. Descriptive statistics were provided as counts and percentages for categorical variables, and as mean, standard deviation, minimum, and maximum for numerical variables. In dependent groups, the analysis of numerical variables was performed using repeated measures analysis of variance when the difference met the normal distribution condition, and using the Friedman test when the condition was not met. Subgroup analyses in the nonparametric test were

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performed using the Wilcoxon test and interpreted with Bonferroni correction. Relationships with numerical variables were examined using Pearson Correlation analysis when the parametric test condition was met, and Spearman Correlation Analysis when the parametric test condition was not met. Finally, the statistical alpha significance level was set at P-value <0.05.

Results

The baseline characteristics of the study population are summarized in **Table 1**. No significant differences were observed between the groups at baseline, indicating comparable demographic and biochemical profiles.

There was no statistically significant difference between the groups in terms of baseline vitamin D concentrations. Vitamin D values measured at week 2 in group 4 were significantly higher than those in group 1 and group 3 (P=0.007), and vitamin D values measured at weeks 4 and 6 in group 3 were significantly lower than those in group 2 and group 4 (P=0.001 and P<0.001, respectively). The vitamin D values measured at week 10 in group 3 were significantly lower than group 4 (P=0.035, **Table 2**). Accordingly, there was no significant difference between the baseline vitamin D2 between the groups. There was no statistically significant difference between the groups in terms of serum calcium at baseline, week 2, week 4, week 4, week 6, and week 10 (**Table 2**).

In terms of parathormone measurements at baseline, group 4 had significantly lower parathormone measurements than group 1 and group 3 (P=0.047). At week 4, the parathormone value of group 3 was found to be statistically significantly higher (P=0,012) than group 2 and group 4. In addition, at week 6, the parathormone value of the group 3 was statistically significantly higher (P=0.001) than the other groups (**Table 2**).

Within-group analyses demonstrated that in group 1, serum vitamin D levels showed a non-significant increase at week 2, followed by significant increases at weeks 4 and 6 (P<0.001), and a non-significant decrease at week 10. In group 2, a non-significant increase was observed at week 2, whereas weeks 4 and 6 showed significant increases compared to baseline (P=0.003), followed by a non-significant decline at week 10. In group 3, no significant change in vitamin D levels was detected over time. Moreover, in group 4, vitamin D levels increased significantly at week 2 (P<0.001), continued to rise significantly at weeks 4 and 6 compared to week 2 (P<0.001), and decreased significantly at week 10 compared to week 6 (P<0.001, **Figure 1**). Compared with baseline values, serum vitamin D levels increased 1.7-fold in group 1, 2.2-fold in group 2, and 2.3-fold in group 4. Regarding vitamin D3, group 1 exhibited significant increases at each two-week interval up to week 6 (P<0.001). In group 2, vitamin D3 levels increased significantly compared to baseline at weeks 2, 4, and 6 (P<0.001), with no significant difference between weeks 2 and 4; a significant decrease was observed at week 10 compared to week 6 (P<0.001, **Figure 2**). Relative to baseline, vitamin D3 levels increased 10.9-fold in group 1 and 13.9-fold in group 2.

No significant differences in serum calcium levels were observed between groups at baseline or at weeks 2, 4, 6, and 10 (**Table 2**). However, within-group analyses showed that in group 1, serum calcium levels increased significantly at week 6 compared to baseline, week 2, and week 4 (P=0.005), followed by a significant decrease at week 10 compared to week 6 (P=0.005). In group 2, a significant increase was observed at week 6 compared to baseline, week 2, and week 4 (P<0.001), with no significant change at week 10. Moreover, in group 3, a significant increase was detected between weeks 4 and 6 (P=0.034). In group 4, serum calcium levels increased significantly between weeks 4 and 6, compared to the other time points (P=0.003, **Figure 3**).

At baseline, PTH levels in group 4 were significantly lower than those in groups 1 and 3 (P=0.047). At week 4, PTH levels in group 3 were significantly higher than those in groups 2 and 4 (P=0.012), and at week 6, group 3 continued to exhibit significantly higher PTH levels compared to the other groups (P=0.001, **Table 2**). Within-group analysis revealed that in group 1, PTH levels

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decreased significantly at weeks 2 and 6 compared to baseline (P<0.001) and increased significantly at week 10 compared to week 6 (P<0.001). In group 2, PTH levels decreased significantly at week 6 compared to baseline (P=0.044), with no significant difference between weeks 6 and 10. No significant temporal changes in PTH were observed in groups 3 and 4 (**Figure 4**).

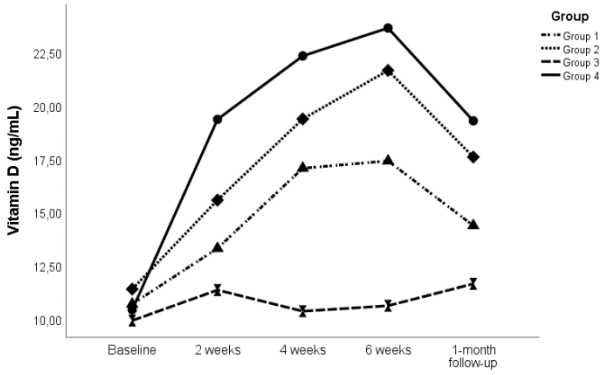

Figure 1. Time course of the mean changes in serum 25(OH)D over the 6-week study period and in 1-month follow-up.

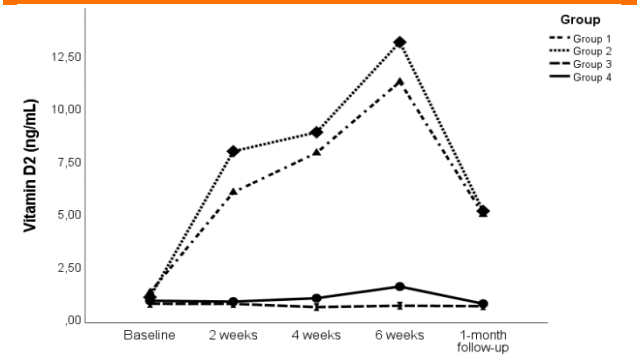

Figure 2. Time course of the mean changes in serum 25(OH)D2 over the 6-week study period and in 1-month follow-up.

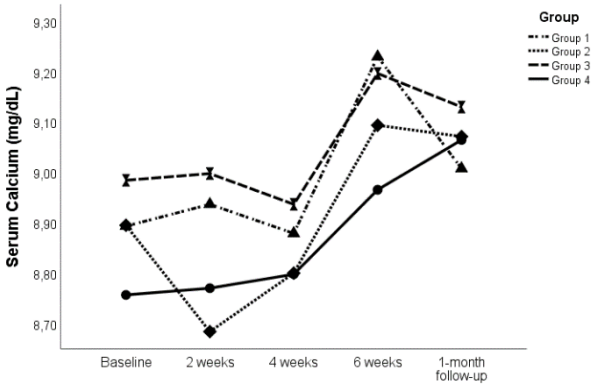

Figure 3. Time course of the mean changes in serum calcium over the 6-week study period and in 1-month follow-up.

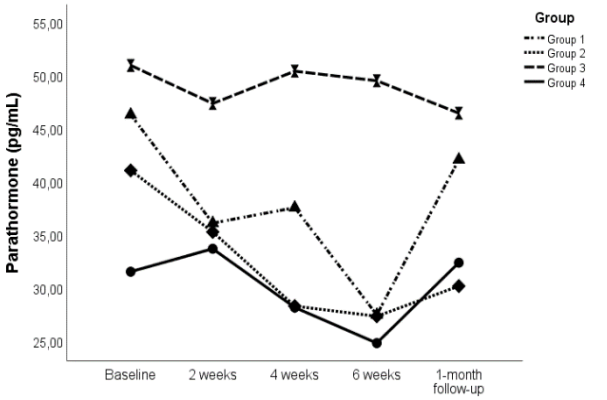

Figure 4. Time course of the mean changes in serum parathormon over the 6-week study period and in 1-month follow-up.

Table 1. Baseline characteristics of the participants and dropouts (n = 35).

Variables	Group 1 (n=9)	Group 2 (n=8)	Group 3 (n=9)	Group 4 (n=9)	P-value[a]
Male/Female (n)	4:5	3:5	4:5	5:4	0.966
Age (y)	45.2±13.3[b]	48.4±11.3	41.8±10.1	44.8±6.2	0.646
Body mass index (kg/m^2)					
Male	27.40±4.36	25.20±2.44	30.05±4.01	28.18±4.43	0.491
Female	31.12±5.86	29.02±3.96	27.62±2.20	27.10±4.24	0.495
Dietary vitamin D intake (µg/d)	3.99±3.36	4.79±3.72	4.03±1.7	4.27±1.94	0.932
Study dropout	3 at week 3	2 at week 1 2 at week 5	1 at week 2 2 at week 5	3 at week 4	

[a]: *One-way analysis of variance or Chi-square test was used for group comparisons;* [b]: *Mean±SD.*

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Table 2. Laboratory values in the four study arms.

Variables	Group 1(n=9)	Group 2(n=8)	Group 3(n=9)	Group 4(n=9)	P-value
Vitamin D (µg/l)					
Baseline	10.2(8.4-11.2)	9.9 (5.2-17.7)	9.7(6.5-14.1)	10.5(5.6-12.4)	0.959
2 weeks	13.3±2.4^{a}	15.57±4.9^{ab}	11.4±3.3^{a}	19.4±6.9^{b}	0.007
4 weeks	17.1±3.6^{ab}	19.4±6.0^{a}	10.4±4.1^{b}	22.3±7.9^{a}	0.001
6 weeks	17.4±3.0^{ab}	21.7±6.7^{a}	10.6±3.0^{b}	23.7±7.9^{a}	<0.001
1-month follow	14.4±2.8^{ab}	17.6±5.1^{ab}	11.6±3.1^{a}	19.3±9.1^{b}	0.035
Vitamin D2 (µg/l)					
Baseline	1.14(0.91-1.73)	0.99(0.54-1.44)	0.70(0.50-0.75)	0.72(0.50-0.94)	0.055
2 weeks	6.39(5.28-6.89)^{a}	7.99(6.94-8.95)^{a}	0.70(0.57-0.79)^{b}	0.54(0.52-0.89)^{b}	<0.001
4 weeks	7.86(6.79-8.49)^{a}	9.05(6.93-10.47)^{a}	0.54(0.50-0.54)^{b}	0.50(0.50-1.07)^{b}	<0.001
6 weeks	12.45(8.04-13.97)^{a}	13.81(10.62-14.58)^{a}	0.50(0.50-0.68)^{b}	1.73(0.63-2.11)^{b}	<0.001
1-month follow	4.51(3.56-6.40)^{a}	5.44(4.50-5.76)^{a}	0.50(0.50-0.66)^{b}	0.56(0.50-0.99)^{b}	<0.001
Serum Calcium (mg/dl)					
Baseline	8.89±0.22	8.90±0.26	8.98±0.60	8.76±0.27	0.640
2 weeks	8.89(8.73-9.19)	8.59(8.43-9.08)	8.83(8.52-9.17)	8.75(8.70-8.84)	0.393
4 weeks	8.81(8.59-9.19)	8.80(8.62-8.85)	8.80(8.73-8.92)	8.77(8.54-9.08)	0.960
6 weeks	9.23±0.28	9.09±0.30	9.20±0.46	8.97±0.20	0.322
1-month follow	8.93(8.89-9.14)	9.12(8.91-9.24)	9.07(8.77-9.32)	9.04(8.94-9.24)	0.803
Parathormone (pg/ml)					
Baseline	42.3(41.4-43.9)^{a}	34.6(20.7-56.9)^{ab}	51.8(38.8-63.6)^{a}	30.6(21.8-41.1)^{b}	0.047
2 weeks	36.09±10.80	35.26±17.47	47.34±15.75	33.67±10.51	0.164
4 weeks	37.53±12.92^{ab}	28.27±12.81^{b}	50.39±20.90^{a}	28.14±11.14^{b}	0.012
6 weeks	27.45±4.80^{a}	27.32±11.38^{a}	49.48±19.45^{b}	24.79±8.79^{a}	0.001
1-month follow	42.06±9.59	30.16±15.37	46.43±13.35	32.36±15.73	0.057

Descriptive statistics are expressed as mean±standard deviation or median (25th - 75th percentile). One-way analysis of variance or Kruskal-Wallis H test was used for group comparisons. Similar letters in the same row indicate statistical similarity, different letters indicate statistical difference. $p<0.05$.

Discussion

In this study, it was found that both UV-C-enriched foods (source of vitamin D2) and dietary supplements (source of vitamin D3) significantly increased serum 25(OH)D levels in individuals with vitamin D deficiency compared to group 3 (placebo). The most noteworthy finding of the study was that there was no statistically significant difference in serum vitamin D increase between the group consuming foods enriched with vitamin D2 and the group receiving vitamin D3 supplementation. All the participants with vitamin D deficiency at baseline (<25 nmol/l) were found to have increased 25(OH)D levels to adequate levels.

A review of the literature reveals that some studies contradict the findings of this research. For example, Vaughan *et al.* reported that food fortification provided a lower increase in 25(OH)D compared to oral supplements and that D2 supplementation was less effective than D3 (Vaughan *et al.*, 2021). They suggested that vitamin D3 is superior to vitamin D2 in increasing serum 25(OH)D concentration. Armas *et al*. in one of the most striking studies comparing the efficacy of D2 and D3, administered a single dose of 50,000 IU of vitamin to healthy volunteers (Armas *et al.*, 2004). Although similar increases were observed in both groups during the first 3 days, the increase in the D3 group continued until day 14 and peaked, while levels in the D2 group rapidly declined, returning to baseline values by day 14. As a result, the effectiveness of vitamin D2 was reported to be less than one-third that of D3. Similarly, Tripkovie *et al.* reported that vitamin D3 is more effective than D2 due to its longer half-life and higher affinity for DBP (Tripkovic *et al.*, 2012). The studies reported that although

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mushroom-derived vitamin D2 intake increased serum 25(OH)D2 levels, it did not have a significant effect on total vitamin D or muscle function (Hidalgo-Sanz *et al.*, 2023, Nieman *et al.*, 2014, Stepien *et al.*, 2013). Furthermore, other studies emphasized that D2 intake increased serum 25(OH)D2 while decreasing 25(OH)D3 levels (Bailer *et al.*, 2022, Cashman *et al.*, 2016, Mehrotra *et al.*, 2014, Stephensen *et al.*, 2012); therefore, the expected increase in total vitamin D levels was not achieved. However, in this study, total vitamin D levels in the fortified food groups showed a similar increase to the supplement group, and this adverse effect was not observed.

The results of this study are consistent with other studies that argue that D2 and D3 have similar effects in terms of bioavailability (Biancuzzo *et al.*, 2010, Holick *et al.*, 2008). Similar results have been obtained in studies that examined the effect of the food matrix. For example, Urbain *et al*. found that UV-irradiated mushroom soup increased serum 25(OH)D levels to a similar extent as supplements. In this study, foods enriched with UV-C technology were also found to be at least as effective as pharmacological supplements in terms of bioavailability (Urbain *et al.*, 2011). This proves that vitamin D2 is effectively absorbed and metabolized when consumed within a food matrix. In a study on athletes, also reported that UV-B-treated mushroom powder significantly increased 25(OH)D levels (Shanely *et al.*, 2013). Furthermore, van den Heuvel *et al*. confirmed the efficacy of vitamin D2, stating that although vitamin D3 is superior in increasing total vitamin D3 concentration, both vitamin D2 and D3 have similar positive effects on calcitriol levels, the active form (van den Heuvel *et al.*, 2024).

The main reason for the discrepancy between the results of the current study and those that found D3 to be superior may be “dosage frequency.” A comprehensive meta-analysis revealed that D3 is superior in high-dose single (bolus) applications, but the difference in effect between D2 and D3 disappears in daily doses (Balachandar *et al.*, 2021). In this study, participants consuming foods and supplements “daily” eliminated the disadvantage of vitamin D2's short half-life in the blood and achieved a serum increase as effective as D3. This finding suggests that when vitamin D2 is added to a regular dietary routine, it can be a strong alternative to D3, especially for vegetarians D2 (Cardwell *et al.*, 2018).

Finally, this study also observed an increase in calcium and a decrease in PTH accompanying the increase in vitamin D. As expected, the rise in serum 25(OH)D levels increased calcium absorption, which in turn suppressed PTH secretion. This physiological response is consistent with the study by (Malik *et al.*, 2022) on rats, which showed that UV-B-treated mushrooms reduced PTH and increased calcium. The decrease in PTH levels in the D2 group is important because it shows that vitamin D from fortified foods is not just a circulating molecule in the blood, but also regulates calcium homeostasis in a biologically active form.

At 4 weeks after the end of this study, there was no significant change in serum 25(OH)D levels in groups 1 and 2, whereas a significant decrease was found in group 4 at week 10 compared to week 6. In vitamin D2 measurements, there was a statistically significant decrease in serum 25(OH)D levels in group 1 and group 2 at week 10 compared to week 6. The decrease in total 25(OH)D levels when supplementation was not taken in the 4-week period from the end of the study to the 10th week showed that blood serum levels were directly affected by supplementation, but this decrease was not observed in the enriched groups, which positively showed that vitamin D2 from enriched foods had an effect on the maintenance of blood serum 25(OH)D levels. In the long term, fortified foods may have a protective effect on serum 25(OH)D levels.

One of the most important strengths of this study is that environmental factors affecting vitamin D levels were strictly controlled. The fact that data were collected during the February-March period, when solar UVB rays (due to the zenith angle effect) do not trigger vitamin D synthesis in our country, eliminated seasonal effects as a

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confounding factor. Furthermore, the fact that participants worked in the same workplace, had similar socioeconomic conditions, and benefited from institutional nutrition services which ensured high group homogeneity by standardizing dietary intake and lifestyle factors. The effect size of 0.947 and 99% power achieved through statistical analyses demonstrated the sample adequacy and reliability of the study's results, while conducting the analyses in a state hospital laboratory increased the accuracy of the data. Nevertheless, certain limitations should be acknowledged. Although initially designed with 48 participants, the study was completed with 35 individuals due to attrition (job changes, health reasons, voluntary withdrawal). Smoking and alcohol consumption were not controlled as potential confounders. Moreover, the intervention period was limited to six weeks, and longer-term follow-up would be necessary to determine sustained efficacy and clinical outcomes.

Conclusion

This study demonstrated the efficacy of different short-term (6 weeks) replacement regimens in individuals with vitamin D deficiency and their effects on biochemical parameters. The treatment protocols applied (group 1, group 2, and group 4) were statistically and significantly effective in increasing serum 25(OH)D levels compared to the placebo/control group (group 3). In contrast, no significant improvement in vitamin D levels was observed in participants in group 3 throughout the study. The approximately 10- to 14-fold increase in serum 25(OH)D2 levels observed in groups 1 and 2 indicates that the treatment administered to these groups (ergocalciferol-based) was successfully absorbed. PTH levels, one of the most important indicators of the biological efficacy of vitamin D replacement, showed significant decreases in the treated groups (especially groups 1 and 2). This proves that the treatments administered were effective in preventing or suppressing secondary hyperparathyroidism. Conversely, the fact that PTH values in group 3, which did not receive treatment, remained significantly higher than in other groups at week 6, confirms the metabolic risks of untreated deficiency. Although statistically significant increases in serum calcium levels were observed in the treated groups at week 6, these values remained within physiological limits and did not lead to acute hypercalcemia. This finding supports the safety of the administered doses in the short term. At the 1-month follow-up (10th week) after discontinuation of treatment, a decrease in vitamin D levels and a tendency for PTH levels to rise again (especially in group 1) were observed in all treatment groups. This situation emphasizes the importance of maintenance doses and the necessity of continuous treatment in vitamin D deficiency therapy. In summary, this study demonstrates that replacement therapy with appropriate doses and duration rapidly improves biochemical parameters in symptomatic or asymptomatic vitamin D deficiency, but long-term follow-up and maintenance therapy planning are necessary to preserve the gains.

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Authors' contributions

Dalbudak D: Conceptualization, Data curation, Investigation, Writing, Review & Editing. Seylam Küşümler A: Methodology, Editing. Eryılmaz M, Kömürlü E, Coşkun CK: Methodology, Reediting.

Conflicts of Interest

The authors declared no conflict of interest.

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