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ORIGINAL ARTICLE

# The relationship between vitamin D deficiency, body composition, and physical/cognitive functions

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## Abstract

*Summary* Vitamin D deficiency is still an important subject due to its significant effects on various tissues and functions. We found a relationship between vitamin D deficiency and increase in adipose tissue thicknesses. This situation reveals the importance of vitamin D supplementation, the harms of weight gain and obesity, and the importance of a balanced diet.

**Purpose** Although the relationship between vitamin D (VitD) levels with body composition and physical/cognitive functions have been investigated in various studies, however, there is no study evaluating all these parameters together. In accordance with, we aimed to evaluate the relationship between VitD deficiency with body composition (i.e., skin, subcutaneous fat, and muscle thicknesses) and physical/cognitive functions.

**Methods** A total of 203 adults (78 M, 125 F, aged 19–91 years) who had recent 25-OH-vitamin D measurements were included. Ultrasonographic (US) measurements (skin, subcutaneous fat, and muscle thicknesses) were made from the dorsum of the hand, and anterior sides of forearm, arm, and thigh. Handgrip strength, gait speed, Timed Up and Go Test, and Chair Stand Test were evaluated. Additionally, cognitive status was also evaluated with Mini-Mental State Exam.

**Results** Subjects were classified as VitD deficient group ($<20$ ng/ml, $N=125$) and control group ($\geq 20$ ng/ml, $N=78$). The groups were not significantly different as regards age, gender, and anthropometric measurements (all $p>0.05$). Subcutaneous fat tissues were thicker in the VitD deficient group (all $p<0.05$). All the other US measurements and functional/cognitive tests were not significantly different between the groups (all $p>0.05$). According to linear regression analyses, body mass index (BMI) was independently related with all subcutaneous fat thicknesses in both genders, and VitD deficiency was related with all subcutaneous fat thicknesses in females and anterior forearm subcutaneous fat thickness in males (all $p<0.05$).

**Conclusion** We imply that together with BMI, VitD deficiency is independently related with increased regional subcutaneous fat tissue. We also underscore the role of US measurements for evaluation of body composition in related clinical scenarios.

**Keywords** Fat · Muscle · Sarcopenia · Skin · Ultrasound

## Introduction

Vitamin D (VitD) is a fat-soluble vitamin (with a hormone-like function) that has a paramount impact on the neuro-musculoskeletal health. While its most well-known effects are related with the calcium metabolism and bone health, VitD plays important roles in many cellular and metabolic cycles of almost all the systems [1]. VitD also has structural and functional effects on skin, subcutaneous fat, muscle, and many other tissues [2]. VitD levels are affected by various factors such as skin type, gender, body mass index (BMI), physical activity, alcohol intake, latitude, season, and VitD receptor polymorphism [2, 4, 5] whereby $<20$ ng/ml is generally accepted as deficiency [2–4].

Despite several molecular and clinical studies, there are very few studies examining the relationship between VitD levels and skin thickness. For instance, measuring skin thickness (the sum of skin and subcutaneous fat tissue) with caliper was found to be positively related with VitD levels in postmenopausal women [6]. However, caliper measurements are person-dependent and have individual- and site-specific difficulties [7]. In this regard, ultrasound (US) appears to be an appropriate tool for evaluating body composition,

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especially measuring skin, subcutaneous fat thickness, and muscle separately [7–9].

The relationship between VitD and adipose tissue has been studied in various populations, especially in obese individuals. The majority of studies have shown that there is an inverse relationship between VitD levels with BMI and adipose tissue mass [10–12]. In addition, higher doses of VitD replacement are suggested in obese individuals [10–13]. Although there are different theories that may cause this situation, there is a large consensus that the main reason is volume-induced dilution due to adipose tissue size [10–12]. In the pertinent literature, the number of studies examining the nature of tissues (regardless of BMI) is few [11, 12]. Since BMI is a ratio obtained from weight and height of the individuals, it cannot clearly reveal the problems caused by VitD deficiency. Instead, BMI changes may be related to adipose or muscle tissues [12, 13]. Furthermore, although the possible effects of VitD deficiency on adipose or muscle tissues are known; practical, harmless, and detailed methods such as US are needed in clinical management [11, 13, 14]. Studies examining the relationship between muscle mass and VitD are generally performed on the elderly population because of the possible risks in terms of sarcopenia. According to a meta-analysis [14], VitD replacement has a positive effect on overall muscle strength (not muscle mass). In another meta-analysis [15], it was reported that VitD replacement had no effect on muscle strength in individuals with a VitD level > 10 ng/ml. In addition, many studies have shown that grip strength tends to decrease with VitD deficiency [16, 17], and the effects of VitD on physical and cognitive function have been frequently studied [18, 19].

Despite all these studies, the impact of VitD deficiency on body composition and physical/cognitive functions has not been fully elucidated. Accordingly, in this study, we aimed to explore the relationship between VitD deficiency and body composition (i.e., skin, subcutaneous fat, and muscle thicknesses) and physical/cognitive functions.

## Methods

This cross-sectional study was performed at physical and rehabilitation medicine department in a tertiary care university hospital. A total of 203 subjects (78 M, 125 F, aged 19–91 years) who had recent VitD measurements (within the last 5 days) were included. Subjects who had hypothyroidism, renal/hepatic failure, neuromuscular, rheumatic, and dermatological disorders were excluded. All eligible participants were informed of the study procedure and were enrolled if they consented to participate. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Hacettepe University Ethics Committee (ethics number: GO 20/189). Written informed consent was obtained from all subjects/patients.

Demographic data, smoking/alcohol habits, exercise status, comorbidities (e.g., hypertension and diabetes mellitus), and VitD intake were noted. Current 25-OH VitD values were recorded from the medical records. According to the relevant literature, subjects were classified into two groups according to their 25-OH VitD levels; deficient group (< 20 ng/ml) and normal/control group (≥ 20 ng/ml) [3, 4]. Weight, height, and waist/hip circumferences were measured (to the nearest 0.1 kg and 1 cm, where appropriate).

### Ultrasonographic measurements

Skin, subcutaneous fat, and muscle thicknesses were measured using a 5–12-MHz linear probe (Logiq P5, GE, Medical Systems) from the dominant limbs. Ultrasonographic measurements were made from the dorsum of the hand, forearm, arm, and thigh at the localizations given in Table 1. All measurements were performed using sufficient amount of gel to avoid any tissue compression. Skin (sum of epidermis and dermis), subcutaneous fat (distance between dermis and muscle), and muscle (distance between the two fasciae) thicknesses were measured in short-axis views (Fig. 1). All measurements were performed from the dominant hand side of the body by a physiatrist (YD) who had more than 3 years of experience in musculoskeletal US.

### Physical function evaluations

*Muscle strength* was assessed by hand grip strength from the dominant side using a Jamar hydraulic hand dynamometer (Baseline Hydraulic Hand dynamometer Irvington, NY, USA). Measurements were repeated three times and the highest value was recorded. Participants were instructed

**Table 1** Ultrasonographic measurement regions/sites

| Region | Site |
|---|---|
| Hand (dorsal) | Midpoint, between the 3rd metacarpophalangeal and wrist joints |
| Anterior forearm | 1/3 proximal, between the wrist distal line and the antecubital fossa |
| Anterior arm | 1/3 distal, between the acromion and antecubital fossa |
| Anterior thigh | Midpoint, between the ASIS and the superior pole of patella |

*ASIS*, anterior superior iliac spine

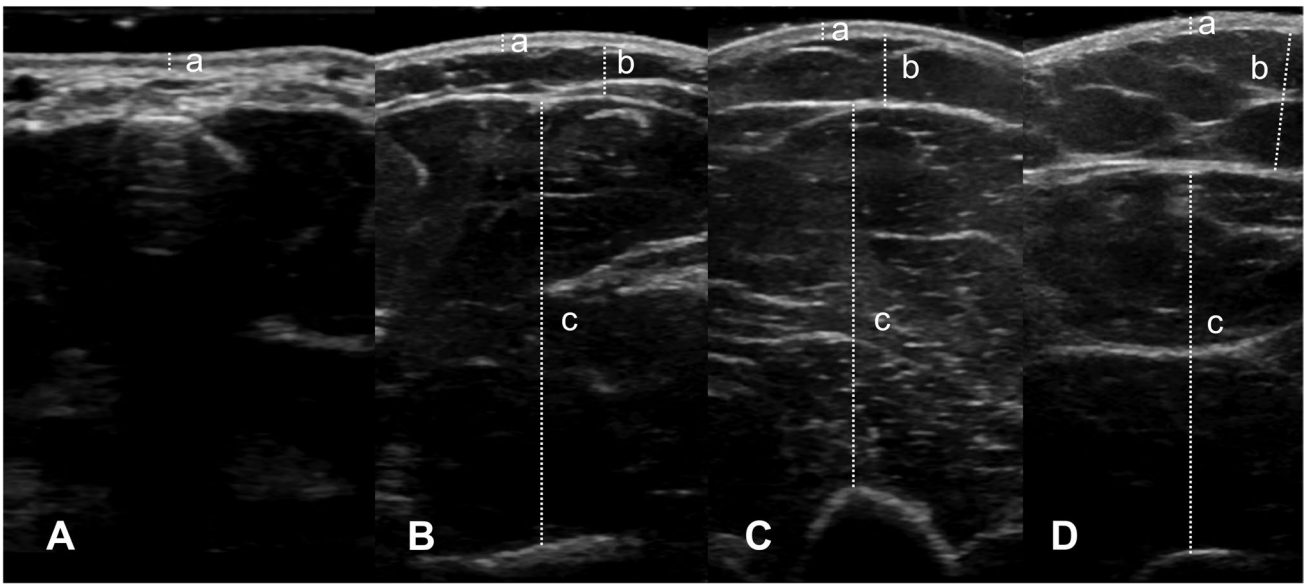


**Fig. 1** Axial ultrasound images of the anterior hand (**A**), forearm (**B**), arm (**C**), and midthigh (**D**) regions show skin (**a**), subcutaneous fat (**b**), and muscle (**c**) thickness measurements

to be in sitting position as their shoulders were adducted and neutrally rotated, elbows were flexed at 90°, and the forearms/wrists were kept in neutral position.

*Physical performance* was assessed by Timed Up and Go Test (TUG), Chair Stand Test (CST), and gait speed. For gait speed, subjects were asked to walk at normal speeds on a 6-m track on a flat surface, and the time was recorded with a stopwatch. For speed calculation (m/s), time was divided by distance. During TUG test, subjects sat in a chair placed at the beginning of a 3-m track on flat ground with a marked endpoint. After the test was described to the patients, the stopwatch was started when the patient got up with the command given. After walking 3 m at normal walking speed, the subject turned back and sat down again, and the stopwatch was stopped and the test was completed. For CST, subjects were asked to sit on a standard-height chair with a straight back and to place their hands crossed over their shoulders. The time it took to sit down and get up five times as fast as possible was recorded. Three measurements were obtained for all performance tests and the mean values were used for analyses.

### Cognitive function assessment

The revised Turkish version of Mini-Mental State Examination (MMSE) (classic and for illiterate patients) was administered. It consists of five main parts, i.e., orientation, immediate memory, attention/concentration, delayed recall, and language sections. The highest total score is 30, and low scores are associated with poor cognitive function.

### Statistical analysis

Statistical analyses were performed using SPSS 24.0 (Statistical Package for Social Sciences). Numerical variables are expressed as mean ± standard deviation (SD), and categorical variables as number ($n$) and percentage (%). Normal distribution was examined with the Kolmogorov Smirnov test. Comparisons for numerical variables were performed by Student's $t$ or Mann–Whitney $U$ tests, where appropriate. Categorical variables were compared by chi-square test. As gender was found to be a significant predictor for fat measurements, gender-specific multivariate linear regression analyses (with backward selection) were performed for fat measurements. A $p$ value of $< 0.05$ was accepted as statistical significance.

## Results

Comparisons of the clinical findings are given in Table 2. The two groups were not significantly different as regards age, gender, anthropometric measurements, smoking, and comorbidities (all $p > 0.05$). Although exercise frequency was less in the VitD deficient group, it did not reach statistical significance ($p = 0.052$). While the percentage of using VitD treatment in the VitD deficient group was 5.6%, it was more common (44.9%) in the control group ($p < 0.001$). The rate of measurement in the months with more sun exposure (April–October) was also less (25.6%) in the deficient group than in the control group (52.6%) ($p < 0.001$).

**Table 2** Comparison of the demographic and clinical variables of the groups ($N=203$)

| | Vitamin D deficient group ($N=125$) | Control group ($N=78$) | $p$ |
|---|---|---|---|
| Number | 125 | 78 | |
| Age (year) | 49.7 ± 14.8 | 51.1 ± 16.0 | 0.729 |
| Gender, male | 48 (38.4) | 30 (38.5) | 0.993 |
| Weight (kg) | 74.9 ± 11.8 | 72.3 ± 13.0 | 0.148 |
| Height (cm) | 165.0 ± 9.2 | 164.5 ± 10.7 | 0.737 |
| BMI (kg/m$^2$) | 27.6 ± 4.4 | 26.8 ± 4.5 | 0.088 |
| Smoking | 40 (32.0) | 20 (25.6) | 0.334 |
| Exercise | 53 (42.4) | 44 (56.4) | 0.052 |
| Circumference (cm) | | | |
| Waist | 94.9 ± 14.9 | 92.3 ± 16.8 | 0.137 |
| Hip | 103.0 ± 11.9 | 101.9 ± 14.5 | 0.471 |
| Comorbidities | | | |
| Hypertension | 32 (25.6) | 17 (21.8) | 0.538 |
| Diabetes mellitus | 14 (11.2) | 11 (14.1) | 0.540 |
| Obesity | 29 (23.2) | 18 (23.1) | 0.984 |
| 25-OH vitamin D (ng/ml) | 11.9 ± 4.4 | 30.0 ± 11.8 | **< 0.001** |
| Date (April–October) | 32 (25.6) | 41 (52.6) | **< 0.001** |
| Using vitamin D treatment | 7 (5.6) | 35 (44.9) | **< 0.001** |
| US measurements (mm) | | | |
| *Skin thickness* | | | |
| Hand | 1.3 ± 0.2 | 1.3 ± 0.2 | 0.633 |
| Forearm | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.875 |
| Arm | 1.5 ± 0.2 | 1.5 ± 0.2 | 0.335 |
| Anterior thigh | 1.9 ± 0.3 | 1.8 ± 0.3 | 0.116 |
| *Fat thickness* | | | |
| Forearm | 6.8 ± 3.6 | 5.6 ± 2.8 | **0.021** |
| Arm | 8.2 ± 4.1 | 7.0 ± 3.3 | **0.040** |
| Anterior thigh | 18.6 ± 8.2 | 16.7 ± 6.5 | **0.047** |
| *Muscle thickness* | | | |
| Forearm | 36.6 ± 4.9 | 35.7 ± 4.9 | 0.226 |
| Arm | 33.4 ± 5.4 | 32.7 ± 5.5 | 0.527 |
| Anterior thigh | 42.0 ± 7.9 | 40.6 ± 10.3 | 0.350 |
| Functional tests | | | |
| Grip strength (kg) | 35.2 ± 11.7 | 35.2 ± 12.4 | 0.929 |
| Gait speed (m/sec) | 1.2 ± 0.2 | 1.2 ± 0.2 | 0.400 |
| TUG (sec) | 8.1 ± 2.5 | 8.1 ± 3.0 | 0.583 |
| CST (sec) | 10.9 ± 3.7 | 10.8 ± 4.0 | 0.532 |
| MMSE | 28.7 ± 1.5 | 28.6 ± 1.7 | 0.451 |

Data are given as mean ± standard deviation or $n$ (%)

Bold indicates statistically significant $p$ values

*BMI*, body mass index; *CST*, Chair Stand Test; *TUG*, Timed Up and Go Test; *MMSE*, Mini-Mental State Examination; *sec*, second

Subcutaneous fat tissue thicknesses at anterior forearm, arm, and thigh regions were thicker in the VitD deficient group than those of the control group (all $p<0.05$). All the other US measurements and functional/cognitive tests were not significantly different between the groups (all $p>0.05$).

According to linear regression analyses (Tables 3 and 4); BMI was independently/positively related with all subcutaneous fat thicknesses in both genders. Furthermore, VitD deficiency was related with all subcutaneous fat thicknesses in females and with anterior forearm subcutaneous fat

**Table 3** Linear regression analysis for subcutaneous fat thickness in females ($N=125$)

| Dependent variable | Independent variable* | $\beta$ | $R$ | $R^2$ | $p$ |
|---|---|---|---|---|---|
| Anterior forearm | BMI | 0.501 | 0.629 | 0.395 | **< 0.001** |
| | Vitamin D deficiency | 0.195 | | | **0.010** |
| Anterior arm | Age | 0.231 | 0.644 | 0.415 | **0.006** |
| | BMI | 0.460 | | | **< 0.001** |
| | Vitamin D deficiency | 0.184 | | | **0.014** |
| Anterior thigh | BMI | 0.573 | 0.624 | 0.389 | **< 0.001** |
| | Vitamin D deficiency | 0.165 | | | **0.024** |

A $p$ value of < 0.05 was accepted as statistical significance

$\beta$, standardized coefficients; $R$, correlation coefficient; $R^2$, coefficient of determination; *BMI*, body mass index

*Independent variables included age, BMI, smoking and exercise statuses, presence of vitamin D deficiency, season (April–October vs. November–March), and using vitamin D treatment

**Table 4** Linear regression analysis for subcutaneous fat thickness in males ($N=78$)

| Dependent variable | Independent variable* | $\beta$ | $R$ | $R^2$ | $p$ |
|---|---|---|---|---|---|
| Anterior forearm | BMI | 0.331 | 0.456 | 0.208 | **0.003** |
| | Vitamin D deficiency | 0.218 | | | **0.048** |
| Anterior arm | BMI | 0.542 | 0.586 | 0.344 | **< 0.001** |
| | Vitamin D deficiency | 0.174 | | | 0.077 |
| Anterior thigh | Age | − 0.278 | 0.581 | 0.337 | **< 0.001** |
| | BMI | 0.553 | | | **0.020** |

A $p$ value of < 0.05 was accepted as statistical significance

$\beta$, standardized coefficients; $R$, correlation coefficient; $R^2$, coefficient of determination; *BMI*, body mass index

*Independent variables included age, BMI, smoking and exercise statuses, presence of vitamin D deficiency, season (April–October vs. November–March), and using vitamin D treatment

thickness in males (all $p<0.05$). Age was negatively related with anterior thigh fat thickness ($p<0.001$).

## Discussion

This study showed that regional subcutaneous fat measurements seemed to be independently and positively increased with BMI and presence of VitD deficiency. We observed that VitD deficiency did not have any association with skin and muscle thicknesses, or physical and cognitive functions. To the best knowledge of the authors, this is the first study evaluating the relationship among VitD, cognition, physical function, skin, subcutaneous fat, and muscle tissues.

According to our results, VitD deficiency was present in about 60% in both genders. Vitamin D deficiency is reported around 40% in the USA and Europe [3, 20, 21]. Regardless of our rate being higher than the literature, it is noteworthy that VitD deficiency is high in the adult population [21]. Herein, individual differences might stem from clothing, working, and living conditions in different countries, as well as from the current pandemic [22]. On the other hand, the mean values being 11.9 ± 4.4 ng/ml and 30.0 ± 11.8 ng/ml in our study groups suffice to represent VitD deficiency and normality.

In our study, although there were no differences regarding BMI values and presence of obesity between the groups, adipose tissues were found to be thicker in all the measured three regions in the deficient group. Inverse but weak relationship between VitD levels and BMI or the fact that VitD deficiency being more common in overweight and obese individuals has been previously reported [10, 23, 24]. In this context, it has been suggested that overweight/obese individuals go out less for social reasons and that they also prefer more closed clothes [10, 25]. According to another theory, decreased bioavailability of VitD accumulating in body fat compartments may be the reason for obesity-related VitD deficiency.

Since VitD is fat-soluble and stored in the adipose tissue, it is important to reveal its actual relationship with this tissue. In this regard, basal (after UV-B exposure) and after oral replacement, VitD levels were compared in normal weight (BMI ≤ 25 kg/m$^2$) and obese individuals whereby lower baseline values as well as 57% lower increase in VitD levels after UV-B exposure were reported in obese subjects [26]. The authors emphasized that oral VitD replacement may be more effective in terms of bioavailability, which is

sequestered to a larger adipose tissue. As emphasized in many other articles, although the most important source of VitD in humans is synthesis from the skin, in individuals with high BMI, oral replacement may be more effective in eliminating its deficiency [27]. Furthermore, higher doses are also advised for replacement and maintenance in obese individuals [27].

In light of our study results, significant correlations between VitD levels and adipose tissue thicknesses show that regional evaluations and direct fat tissue measurements can be superior to BMI values. Yet, although BMI values and presence of obesity were not different between the groups, there were significant differences between adipose tissue thicknesses — necessitating regional measurements in VitD deficiency. In the presence of (for instance) decreased muscle tissue and increased adipose tissue, i.e., with no change in BMI, regional US evaluation will be important to reveal such a situation. Likewise, Ata et al. [28] found an inverse relationship between VitD deficiency and trochanteric subcutaneous tissue thickness by using US. In this aspect, considering the concepts of sarcopenia and sarcopenic obesity in addition to VitD deficiency, the importance of regional US evaluation can be better understood [29].The use of US in dermatology allows more detailed examination of the skin [9, 30]. Herewith, when our study was planned, US data examining the relationship between VitD level and skin thickness was not present in the relevant literature. In the only study exploring the relationship between skin thickness and VitD, caliper was used [6]. Although our skin thicknesses were consistent with the literature [9], we found no difference between the groups. However, a preliminary study including 116 adults, skin thicknesses were found to be higher in the VitD deficient group [28]. Enrollment of more patients and detailed examination of clinical factors such as VitD usage and season are the superior aspects of our study. Herewith, there might also be some confounding effects due to the COVID-19 pandemic (quarantine, use of prophylactic VitD, change in the patient population, etc.) which might have affected our skin measurements.

The negative effects of VitD deficiency on muscle mass/strength are well known [31]. According to a meta-analysis [14], regardless of age, VitD replacement has a positive effect on global muscle strength, which may be more important in older adults (> 65 years of age) and individuals with severe deficiency (< 12 ng/ml). Another meta-analysis on the adults reported that VitD replacement had no effect on muscle strength in individuals with VitD levels > 10 ng/ml, and only a few studies has reported that VitD replacement can increase proximal muscle strength in case of deficiency [15].

However, most of the studies examined this relationship in the elderly population as they have higher risk for sarcopenia (i.e., loss of muscle mass/function). In addition, previous studies have shown that the loss of muscle mass accelerated after the 5th decade [32]. Similarly, deterioration in physical/cognitive functions becomes noticeable and measurable in advanced ages [33]. In our study, we found no difference between the groups regarding muscle thicknesses and physical/cognitive functions. Of note, mean age of our study population was about 50 years and this might make the results understandable. Similarly, although the tests used to evaluate physical/cognitive function are sufficient for the elderly, they may need to be diversified for younger populations.

Concerning exercise status, we found a borderline insignificant relationship with VitD deficiency. In the current literature, it has been reported that subjects who routinely exercise have higher VitD levels, and that out-door activities are more significantly associated with higher VitD levels [34, 35]. In our study, exercise status (based on declaration) and the presence of short-term sudden changes in exercise habits due to the pandemic might have affected our results, as well. Since VitD levels vary according to seasons, we have also noted the dates when VitD levels were measured. In line with the literature [1, 3, 5, 36], and as expected, the VitD deficient group included fewer subjects whose blood levels were tested during the sunny months or those receiving VitD therapy.

### Limitations

First, the study design was cross-sectional, and the study sample could have included more males and older subjects. Second, although the body composition was assessed from regional sites (specifically/separately for skin and muscle), subcutaneous fat measurements could have been performed from other adipose-rich regions such as trochanter and abdomen. Third, increased awareness as regards VitD supplementation during the pandemic might have also confounded our results, despite being an otherwise favorable health issue. Nonetheless, our results represent the first comprehensive analyses on the association between VitD deficiency, body composition, and physical/cognitive functions.

## Conclusions

We imply that VitD deficiency — in addition to increased BMI — is independently related with increased regional subcutaneous fat tissue. We also underscore the potential role of US measurements for the evaluation of body composition in pertinent clinical scenarios. Indisputably, further longitudinal studies assessing the effects of VitD treatment on body composition (especially for older subjects) are awaited.

## Declarations

**Conflicts of interest** The authors declare no competing financial interests. Yahya Doğan, Murat Kara, Mehmet Ali Culha, Levent Özçakar and Bayram Kaymak declare that they have no conflict of interest. Authors confirm that their study's involvement with human subjects complies with the Declaration of Helsinki. Written informed consents were obtained from all patients.

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