



# Does 25(OH)D deficiency affect muscle elasticity, physical function, pain, and quality of life? A shear-wave elastography-based cross-sectional study

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## Abstract

**Summary** In this cross-sectional study of 80 young adults, 25(OH)D deficiency (< 20 ng/mL) was associated with lower SWE-measured muscle elasticity (FCR, BB, and TA), and reduced fingertip pinch strength. When women and men were evaluated separately, muscle elasticity values were lower in the low 25(OH)D group in both sexes. Muscle elasticity showed strong correlations with 25(OH)D and accurately discriminated deficiency (AUC 0.935–0.957) in this young cohort.

**Background** Vitamin D is a key biological regulator in maintaining bone mineralization and muscle function by regulating calcium and phosphorus metabolism, promoting muscle protein synthesis, and supporting muscle cell growth and differentiation.

**Aim** To assess muscle elasticity using shear-wave elastography (SWE) in young adults with low and adequate 25-hydroxyvitamin D [25(OH)D] and explore the diagnostic usefulness of SWE in distinguishing 25(OH)D deficiency.

**Design** A cross-sectional study.

**Setting** Physical Medicine and Rehabilitation and Radiology Departments in the Selcuk University Faculty of Medicine Hospital.

**Population** Eighty participants (18–45 years) were included in two groups regarding 25(OH)D levels.

**Methods** Participants with serum 25(OH)D levels (< 20 ng/mL and those with ≥ 20 ng/mL) were classified as Groups I (*n* = 40) and II (*n* = 40). Muscle elasticity of flexor carpi radialis (FCR), short head of biceps brachii (BB), and tibialis anterior (TA) was measured using SWE (kPa).

**Results** Group I consisted of 21 women and 19 men, while Group II consisted of 22 women and 18 men. The groups were similar in demographic characteristics, physical activity, and SF-36 scores. FCR-kPa, BB-kPa, and TA-kPa were lower in Group I (*p* < 0.001). When women and men were evaluated separately, FCR, BB, and TA muscle elasticity values were significantly lower in both sexes in the low 25(OH)D group (all *p* < 0.001). Fingertip pinch strength was lower in the dominant and non-dominant hands in Group I (*p* = 0.001; *p* = 0.023). AUC values for predicting 25(OH)D deficiency were FCR: 0.94 (cut-off 23.5 kPa), BB: 0.957 (26.5 kPa), and TA: 0.935 (23.75 kPa). Serum 25(OH)D showed a strong positive correlation with elasticity measurements (*r* = 0.870–0.916; *p* < 0.001).

**Conclusion** Low 25(OH)D levels were found to be associated with lower elasticity values, as measured by SWE, in FCR, BB, and TA muscles in women and men.

**Keywords** Muscle elasticity · Pain · Physical function · Quality of life · Shear-wave elastography · 25(OH)D deficiency

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## Introduction

Vitamin D, a key biological regulator in maintaining bone mineralization and muscle function by regulating calcium and phosphorus metabolism [1], promotes muscle protein synthesis and supports muscle cell growth and

differentiation [2]. In the study by Bass et al. it was revealed that disruption of vitamin D signaling leads to muscle atrophy by disrupting the regulation of protein synthesis and gene expression in muscle cells [3]. In the study, the researchers reported that VDR suppression decreased the cross-sectional area of fast-twitch Type IIb muscle fibers and led to muscle atrophy. These findings support the possible mechanisms underlying the muscle loss observed in 25-hydroxyvitamin D [25(OH)D] deficiency [3]. Additionally, vitamin D regulates calcium homeostasis in skeletal muscle through both genomic and non-genomic mechanisms, and vitamin D has been reported to improve muscle contraction by promoting calcium transport in the sarcoplasmic reticulum [4, 5]. It is suggested that impaired calcium uptake resulting from 25(OH)D deficiency alters muscle contraction kinetics, prolonging the relaxation phase and contributing to muscle weakness [5]. 25(OH)D deficiency is defined as serum 25(OH)D < 20 ng/mL (50 nmol/L) under the guidelines by the Endocrine Society [6].

However, given the relationship between 25(OH)D and skeletal muscle mass and functional performance, findings from clinical human studies are quite heterogeneous. In various community-based cohorts, generally weak, often insignificant, and inconsistent associations have been stated between dietary vitamin D intake or serum 25(OH)D levels and performance tests, such as muscle mass, hand grip strength, and walking speed [7]. Therefore, evaluating the effects of 25(OH)D on muscle function solely through mass or functional tests may not fully reflect the alterations in underlying tissue characteristics.

However, recent developments in sonoelastography have made it possible to quantitatively evaluate the elastic properties of tissues [8]. Shear-wave elastography (SWE) is a sonoelastography method that measures the elastic properties of soft tissues by evaluating the particle displacement created in the tissue by an acoustic wave through an ultrasound transducer [9] and, in recent years, has been widely utilized to evaluate many musculoskeletal diseases, including muscle and tendon pathologies [10–13]. In previous studies, while the relationship between 25(OH)D and muscle health was evaluated through parameters such as muscle thickness, fat tissue characteristics, muscle strength, physical performance, and muscle fiber cross-sectional area, muscle elasticity was not directly examined [3, 14]. To our knowledge, the relationship between 25(OH)D deficiency and muscle elasticity assessed by SWE has yet to be sufficiently investigated, suggesting that more detailed and quantitative studies are required to investigate the effects of 25(OH)D deficiency on the mechanical properties of muscle tissues.

Therefore, the present study primarily aims to compare the elasticity values of the flexor carpi radialis (FCR), short head of biceps brachii (BB), and tibialis anterior (TA)

muscles, measured by SWE, in young adults with low and sufficient 25(OH)D levels, and to reveal possible biomechanical differences associated with 25(OH)D deficiency. Also, our secondary objectives are to examine the relationships between muscle elasticity parameters, hand grip and thumb pinch strength, lower extremity gait performance, generalized pain area and pain intensity, physical activity level, and quality of life (QoL), and to evaluate their potential diagnostic value in differentiating 25(OH)D deficiency.

## Material and Method

### Design of study and ethical approval

This cross-sectional study was conducted in collaboration with the Department of Physical Medicine and Rehabilitation and the Department of Radiology of a tertiary hospital. Approval was obtained from the Local Ethics Committee of the Medical Faculty at Selcuk University (Registration number and date: 2024/577-11/21/2024). Written informed consent was obtained from all participants, and the study was conducted under the principles of the Helsinki Declaration.

### Participants

According to the a priori power analysis, the target sample size was set at 40 participants per group. Consecutively recruited participants who met the inclusion and exclusion criteria were enrolled, and recruitment was terminated when 40 participants were reached in each group. Serum 25(OH)D levels were measured as part of routine clinical evaluation at the relevant outpatient clinic, and results from blood samples taken in the morning following an overnight fast within the last five days were used in the study. All participants' samples were evaluated using the chemiluminescence immunoassay method in the same biochemistry laboratory, using the same analysis system [Beckman Coulter UniCel DxI 800 Access immunoassay analyzer (USA)] and commercial kits from the same manufacturer. Under 25(OH)D levels, those with <20 ng/mL were categorized as the low 25(OH)D group (Group I,  $n = 40$ ), and those with  $\geq 20$  ng/mL were categorized as the sufficient 25(OH)D group (Group II,  $n = 40$ ) [6, 15]. Then the patients were evaluated with an ultrasound.

### Exclusion criteria

Patients with neuromuscular disorders, using medications causing rhabdomyolysis, with a history of acute trauma, and those with thyroid and parathyroid dysfunction, chronic

kidney and liver failure, those who received vitamin D supplementation within the last 3 months, and those with rheumatological and dermatological diseases were excluded from the study.

### Demographic data

Demographic characteristics of patients, such as age, sex, height, weight, body mass index (BMI), marital status, smoking status, educational level, comorbidities, and professional data, were recorded.

### Measurements of muscle elasticity

The FCR, BB, and TA muscles were selected because they are representative upper- and lower-extremity muscles commonly engaged during activities of daily living. The medical history of all patients and healthy volunteers was examined by two blinded and independent radiologists, unaware of their clinical parameters, each with nine and 18 years of experience. Participants were instructed to avoid strenuous activities for at least 24 h before imaging. Grayscale and SWE examinations were performed using a US device equipped with a high-frequency (14 MHz) linear array transducer (Mindray Resona I9, Mindray Medical Systems, Shenzhen, China). Imaging was performed in the supine normoanatomical position of patients on their dominant extremity, and participants were instructed to remain immobile to avoid artifacts; additionally, ample coupling gel was applied to minimize subcutaneous soft tissue variations; the transducer was placed perpendicular to the midline of the thickest portion of the FCR, BB, and TA muscles, and the cross-sectional area of the FCR, BB, and TA muscles was measured from their midline portions. Then, three different measurements were taken from the midline of the muscles, and the average of the region of interest values was used to obtain SWE and kPa (kilopascals) values (Fig. 1). The US probe was held still for 3–5 seconds to prevent artifacts, and after the SWE images were fixed, the images were saved to the archive. Gray-scale US and SWE parameters were the same for all participants.

### Assessment of physical function

Physical function was assessed with the determination of grip strength, lower extremity performance, and physical activity level.

#### Grip strength

Dominant hand grip strength was measured with a manual hydraulic hand dynamometer (Baseline®) [16]. Dominant and non-dominant grip strengths of the patients were

evaluated separately. Each participant was tested while sitting with shoulder adduction, elbow 90° flexion, and forearm in a neutral position, and each measurement was repeated three times with one-minute intervals, and the average value was used in the analysis [17]. Then, the measured strength was recorded in kilograms/force.

Thumb muscle strength measurements of the patients were performed using “Mechanical pinch strength (Baseline®)” by evaluating dominant and non-dominant thumb muscle strengths separately. Lateral (LPS), palmar (PPS), and fingertip (TPS) muscle strength assessments were implemented in the same position. The measurements were done three times at 10-second intervals, and the average value was used in the study. The measured strengths were recorded in kilograms/force [18, 19].

### Performance of lower extremities

During the 6-meter walking time, participants were asked to walk six meters at a normal pace, and to calculate the walking time over a distance of six meters, the average time of two trials was recorded in seconds [20].

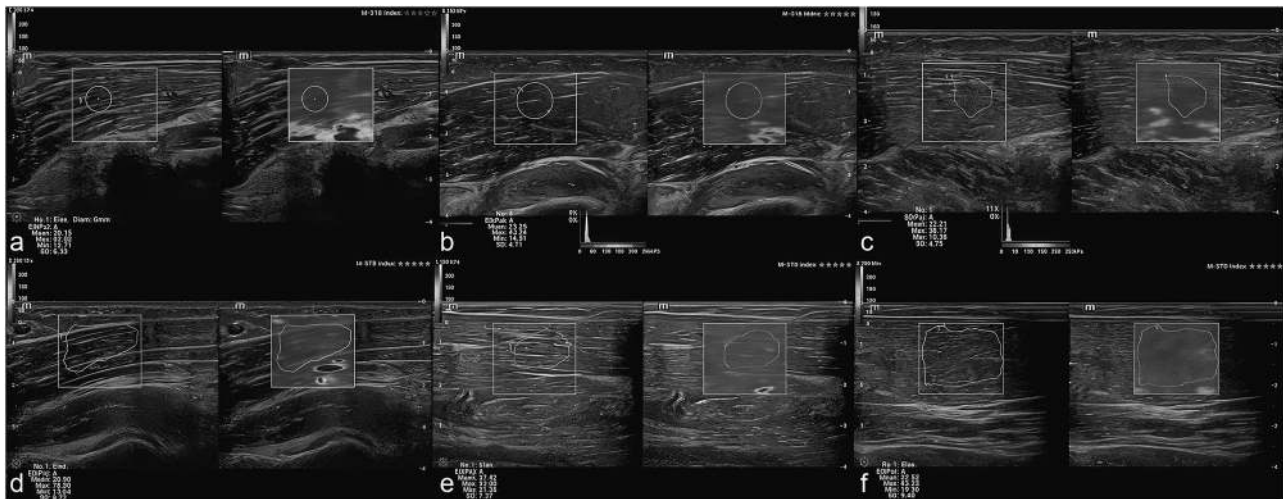
### Assessment of physical activity level

To assess physical activity levels through the International Physical Activity Questionnaire (IPAQ-Long Form), for which the validity and reliability were tested in Turkish [21], participants were asked about the duration and frequency of walking, moderate, and vigorous physical activity performed in the last seven days. These durations were multiplied by the relevant MET (Metabolic Equivalent) values to calculate the total physical activity level (MET–minutes/week), and based on the obtained scores, participants were divided into three categories: low physical activity (< 600 MET–min/week), moderate physical activity (600–3000 MET–min/week), and high physical activity (> 3000 MET–min/week).

### Assessment of pain

#### Pain Drawing

Participants were asked to mark painful areas and indicate the intensity of pain in each area using a grading system of symbols (X=mild, XX=moderate, XXX=severe, and XXXX=unbearable pain) on a standard body diagram, including 45 anatomical regions [22, 23]. These 45 anatomical regions were grouped into 13 main regions: head, neck, shoulder girdle, upper extremities, back, lower back, forearm, hip, thigh, lower extremities, chest, abdomen, and feet, and for each main region, pain frequency and intensity were analyzed separately.



**Fig. 1** Representative SWE images from participants with different serum 25(OH)D concentrations (ng/mL). **a** FCR muscle: 25(OH)D = 10 ng/mL, kPa = 20.35. **b** BB muscle: 25(OH)D = 13 ng/mL, kPa = 23.25. **c** TA muscle: 25(OH)D = 12 ng/mL, kPa = 22.21.

**d** FCR muscle: 25(OH)D = 29 ng/mL, kPa = 28.90. **e** BB muscle: 25(OH)D = 39 ng/mL, kPa = 37.42. **f** TA muscle: 25(OH)D = 33 ng/mL, kPa = 32.52. BB Biceps brachii, FCR Flexor carpi radialis, SWE Shear-wave elastography, TA Tibialis anterior

## Measurement of quality of life

**Short Form Health Survey (SF-36)** This scale, for which the validity and reliability of the Turkish version were established [24], was developed to assess the QoL of individuals. SF-36 consists of a total of 36 items and eight sub-dimensions: physical function, physical role limitation, emotional role limitation, body pain, social functioning, mental health, vitality (energy level), and general health perception. Scores obtained from each subscale range from 0 to 100, and higher scores indicate better health status.

**Statistical Analysis** The sample size was calculated using G\*Power (v.3.1.9.7) with the assumptions of  $\alpha = 0.05$ , power = 0.90, and Cohen's  $d = 0.80$  for the two-tailed independent  $t$ -test, and  $\geq 34$  (total  $\geq 68$ ) were found to be required for each group. The sample size was exceeded by including 40 individuals in each group (total 80), and sufficient statistical power was achieved by considering a possible loss of 10–15%.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The distribution characteristics of continuous variables were analytically evaluated using the Shapiro–Wilk test. Continuous variables exhibiting a normal distribution were summarized as mean  $\pm$  standard deviation; those not normally distributed were summarized as median (25th–75th percentile), and categorical variables were presented as numbers and percentages.

Demographic and clinical characteristics were compared between 25(OH)D groups, both in the entire sample and

separately for women and men. Continuous variables were presented as median (25th–75th percentile), and groups were compared using the Mann–Whitney U test. Fisher's exact test was used to compare two-category variables, and the Fisher–Freeman–Halton exact test was used to compare three-category IPAQ physical activity level.

To assess the potential impact of sex-related differences on muscle elasticity measured by SWE, analyses were conducted separately for women and men. While the variables with normal distribution were compared using the independent samples  $t$ -test, those with non-normal distribution were compared using the Mann–Whitney U test. Additionally, the multivariate analysis of covariance (MANCOVA) was performed to evaluate the independent effects of 25(OH)D status and sex on muscle elasticity measured by SWE. FCR-kPa, BB-kPa, and TA-kPa were included in the model as dependent variables. While 25(OH)D status and sex were included as fixed factors, age and BMI were included as covariates. The 25(OH)D status  $\times$  sex interaction term was added to the model to assess whether 25(OH)D status showed an effect on muscle elasticity measured by SWE in terms of sex difference. The magnitude of the difference between women and men was also assessed using Cohen's  $d$ .

Unadjusted comparisons between groups in terms of handgrip strength, pinch strength, 6-meter walk time, and pain outcomes were performed using the Mann–Whitney U test in the total sample. Each outcome variable was then analyzed using a separate two-factor ANCOVA model where 25(OH)D status and sex were fixed factors, age and BMI were covariates, and the 25(OH)D status  $\times$  sex interaction was included. Simple effect analyses with

**Table 1** Demographic and clinical characteristics according to 25(OH)D status, overall and stratified by sex

| Parameters                                            | Group I Total<br>(n = 40) | Group II Total<br>(n = 40) | Total test    | Group I Women<br>(n = 21) | Group II Women<br>(n = 22) | Women<br>test | Group I Men<br>(n = 19) | Group II Men<br>(n = 18) | Men test      |
|-------------------------------------------------------|---------------------------|----------------------------|---------------|---------------------------|----------------------------|---------------|-------------------------|--------------------------|---------------|
| Age (years), median (25th–75th percentile),           | 25.5 (22.0–32.5)          | 25.0 (22.0–29.0)           | $p = 0.508^1$ | 29.0 (22.5–33.5)          | 23.5 (21.8–27.3)           | $p = 0.020^1$ | 24.0 (22.0–27.0)        | 27.5 (23.8–35.3)         | $p = 0.107^1$ |
| BMI, median (25th–75th percentile), kg/m <sup>2</sup> | 25.4 (22.1–29.7)          | 24.2 (20.9–26.1)           | $p = 0.059^1$ | 25.0 (20.4–28.6)          | 21.9 (18.7–25.7)           | $p = 0.088^1$ | 26.5 (22.6–30.9)        | 25.4 (23.5–26.3)         | $p = 0.354^1$ |
| Married, n (%)                                        | 17 (42.5)                 | 14 (35.0)                  | $p = 0.647^2$ | 13 (61.9)                 | 4 (18.2)                   | $p = 0.005^2$ | 4 (21.1)                | 10 (55.6)                | $p = 0.045^2$ |
| Smokers, n (%)                                        | 7 (17.5)                  | 6 (15.0)                   | $p = 1.000^2$ | 2 (9.5)                   | 1 (4.5)                    | $p = 0.607^2$ | 5 (26.3)                | 5 (27.8)                 | $p = 1.000^2$ |
| Physically active profession n (%)                    | 9 (22.5)                  | 16 (40.0)                  | $p = 0.148^2$ | 3 (14.3)                  | 5 (22.7)                   | $p = 0.698^2$ | 6 (31.6)                | 11 (61.1)                | $p = 0.103^2$ |
| Comorbidity, n (%)                                    | 12 (30.0)                 | 8 (20.0)                   | $p = 0.439^2$ | 8 (38.1)                  | 5 (22.7)                   | $p = 0.332^2$ | 4 (21.1)                | 3 (16.7)                 | $p = 1.000^2$ |
| IPAQ physical activity level n (%)                    |                           |                            |               |                           |                            |               |                         |                          |               |
| Low                                                   | 18 (45.0)                 | 14 (35.0)                  |               | 13 (61.9)                 | 7 (31.8)                   |               | 5 (26.3)                | 7 (38.9)                 |               |
| Moderate                                              | 16 (40.0)                 | 18 (45.0)                  | $p = 0.669^3$ | 5 (23.8)                  | 10 (45.5)                  | $p = 0.133^3$ | 11 (57.9)               | 8 (44.4)                 | $p = 0.748^3$ |
| High                                                  | 6 (15.0)                  | 8 (20.0)                   |               | 3 (14.3)                  | 5 (22.7)                   |               | 3 (15.8)                | 3 (16.7)                 |               |
| 25(OH)D, median (25th–75th percentile), ng/mL         | 12.4 (10.0–16.0)          | 25.0 (22.0–30.0)           | $p < 0.001^1$ | 12.0 (8.7–13.9)           | 22.5 (21.0–27.2)           | $p < 0.001^1$ | 13.6 (11.0–16.0)        | 27.8 (24.6–30.8)         | $p < 0.001^1$ |

<sup>1</sup>Mann–Whitney U test, <sup>2</sup>Fisher's exact test, <sup>3</sup>Fisher–Freeman–Halton exact test, BMI Body mass index, IPAQ International Physical Activity Questionnaire, ng/mL Nanogram/milliliter

Bonferroni correction were used to compare 25(OH)D groups within each sex. The relationships between serum 25(OH)D and variables were evaluated using Pearson's correlation for normally distributed variables and Spearman's correlation for non-normally distributed variables. The analyses of receiver operating characteristic (ROC) curves were performed to evaluate the diagnostic performance of muscle elasticity parameters in distinguishing 25(OH)D deficiency and to determine the most appropriate cut-offs. To identify the independent determinants of muscle elasticity, multiple generalized linear model analyses were carried out by including potential confounders such as age, sex, BMI, serum 25(OH)D level, muscle cross-sectional area, and IPAQ physical activity levels; in all analyses, a two-tailed  $p < 0.05$  was considered statistically significant.

## Results

In the present study, including a total of 80 participants [43 women (53.8%) and 37 men (46.2%)], median 25(OH)D levels were measured as 12.4 (10.0–16.0) ng/mL in Group I and 25.0 (22.0–30.0) ng/mL in Group II. The demographic and clinical characteristics of the participants are presented in Table 1, both for the total sample and by sex. Except for the expected difference in serum 25(OH)D levels, no significant differences were found between the two groups in terms of demographic and clinical characteristics in the total sample. In subgroup analyses by sex, the median age of women participants in Group I was significantly higher than in Group II ( $p = 0.020$ ). Marital status distribution differed between the 25(OH)D groups in both women ( $p = 0.005$ ) and men ( $p = 0.045$ ). In contrast, within the women and men subgroups, no significant differences were found between the two 25(OH)D groups in terms of BMI, smoking, working in a physically active occupation, presence of additional diseases, and IPAQ physical activity level (all  $p > 0.05$ ) (Table 1).

Considering muscle elasticity measurements, FCR-kPa, TA-kPa, and BB-kPa values were significantly lower in Group I (all  $p < 0.001$ ) (Table 2). However, no significant difference was observed between the groups in terms of muscle cross-sectional area (all  $p > 0.05$ ).

Given the separate evaluations between women and men, muscle elasticity values measured by SWE were found to be significantly lower in Group I compared to Group II in both women and men. FCR-kPa, BB-kPa, and TA-kPa values in both sexes were all significantly lower in Group I, compared to Group II (all  $p < 0.001$ ). In contrast, muscle cross-sectional area values showed no significant difference between the 25(OH)D groups in each sex. Based on these findings, the MANCOVA analysis was performed to

**Table 2** Comparison of dominant-extremity muscle elasticity and cross-sectional area according to 25(OH)D status in the overall cohort and by sex

| Parameters                                | Group I Total<br>(n = 40) | Group II Total<br>(n = 40) | Total test                    | Group I Women<br>(n = 21) | Group II Women<br>(n = 22) | Women Test                   | Group I Men<br>(n = 19) | Group II Men<br>(n = 18) | Men test                      |
|-------------------------------------------|---------------------------|----------------------------|-------------------------------|---------------------------|----------------------------|------------------------------|-------------------------|--------------------------|-------------------------------|
| FCR-kPa, median<br>(25th–75th percentile) | 21.5<br>(19.5–23.0)       | 26.5 (24.5–28.0)           | Z = -6.804,<br>$p < 0.001^1$  | 20.0 (18.0–21.0)          | 25.0 (24.0–26.0)           | Z = -5.443,<br>$p < 0.001^1$ | 23.0<br>(22.0–24.0)     | 27.5<br>(27.0–29.0)      | Z = -5.153,<br>$p < 0.001^1$  |
| BB-kPa, mean $\pm$ SD                     | 23.40 $\pm$ 2.56          | 30.38 $\pm$ 3.56           | t = -10.066,<br>$p < 0.001^2$ | 22.10 $\pm$ 2.55          | 28.41 $\pm$ 3.00           | t = -7.418,<br>$p < 0.001^2$ | 24.84 $\pm$ 1.68        | 32.78 $\pm$ 2.60         | t = -11.090,<br>$p < 0.001^2$ |
| TA-kPa, median (25th–75th<br>percentile)  | 21.0<br>(19.0–22.5)       | 26.0 (24.0–28.0)           | Z = -6.729,<br>$p < 0.001^1$  | 19.0 (17.5–20.5)          | 24.0 (24.0–25.25)          | Z = -5.206,<br>$p < 0.001^1$ | 22.0<br>(21.0–24.0)     | 27.0<br>(26.0–28.75)     | Z = -5.235,<br>$p < 0.001^1$  |
| FCR area, mean $\pm$ SD                   | 419.38 $\pm$ 28.57        | 415.08 $\pm$ 28.71         | t = 0.671,<br>$p = 0.504^2$   | 397.38 $\pm$ 14.97        | 395.77 $\pm$ 14.74         | t = 0.355,<br>$p = 0.725^2$  | 443.68 $\pm$ 18.20      | 438.67 $\pm$ 23.48       | t = 0.729,<br>$p = 0.471^2$   |
| BB area, median (25th–75th<br>percentile) | 445.0<br>(422.5–468.5)    | 437.5<br>(421.0–471.5)     | Z = -0.375,<br>$p = 0.707^1$  | 424.0<br>(417.0–436.0)    | 424.5<br>(417.0–432.0)     | Z = -0.451,<br>$p = 0.652^1$ | 470.0<br>(459.0–493.0)  | 473.0<br>(464.8–492.0)   | Z = -0.563,<br>$p = 0.574^1$  |
| TA area, mean $\pm$ SD                    | 422.48 $\pm$ 30.79        | 419.18 $\pm$ 32.11         | t = 0.469,<br>$p = 0.640^2$   | 397.67 $\pm$ 13.65        | 396.91 $\pm$ 14.61         | t = 0.175,<br>$p = 0.862^2$  | 449.90 $\pm$ 18.30      | 446.39 $\pm$ 26.01       | t = 0.476,<br>$p = 0.637^2$   |

<sup>1</sup>Mann-Whitney U test, <sup>2</sup>Student's *t*-test, Area: Ultrasonographic cross-sectional area of the muscle (mm<sup>2</sup>), BB Biceps brachii, FCR Flexor carpi radialis, kPa Muscle elasticity value (kilopascals), SD Standard deviation, TA Tibialis anterior

evaluate the independent and combined effects of 25(OH)D status and sex on muscle elasticity.

In the age- and BMI-adjusted MANCOVA model, the 25(OH)D status was found to have a significant multivariate effect on muscle elasticity parameters measured by SWE (Pillai's trace = 0.691,  $F = 53.691$ ,  $p < 0.001$ , partial  $\eta^2 = 0.691$ ). Sex also showed a significant multivariate effect on muscle elasticity parameters (Pillai's trace = 0.352,  $F = 13.009$ ,  $p < 0.001$ , partial  $\eta^2 = 0.352$ ), and the FCR-kPa, BB-kPa, and TA-kPa values were higher in men than in women. Based on raw averages, these differences were 3.03 kPa for FCR-kPa, 3.38 kPa for BB-kPa, and 2.91 kPa for TA-kPa. Given the magnitude of the difference between sexes through Cohen's *d*, moderate-to-large effect sizes were found at 0.86 for FCR-kPa, 0.77 for BB-kPa, and 0.80 for TA-kPa. However, partial eta-squared ( $\eta^2$ ) was used as the primary effect size indicator in evaluating the sex effect within the model.

Furthermore, the 25(OH)D status  $\times$  sex interaction was found to be significant at the multivariate level (Pillai's trace = 0.132,  $F = 3.662$ ,  $p = 0.016$ , partial  $\eta^2 = 0.132$ ); however, in univariate analyses, this interaction was not significant separately for FCR-kPa, BB-kPa, or TA-kPa. These findings indicate that the association between the low 25(OH)D status and decreased muscle elasticity is present in both sexes, and the group effect is not significantly sex-dependent in terms of individual muscles.

While grip and lateral/palmar pinch strengths were found to be similar between the groups, fingertip pinch strength was significantly lower in both the dominant and non-dominant hands in the low 25(OH)D group ( $p = 0.001$  and  $p = 0.023$ , respectively) (Table 3). However, no difference was found between the two groups in terms of the 6-meter walking time (Table 3). Additionally, given IPAQ physical activity levels, no significant difference was also detected between the two groups in terms of physical activity levels ( $p = 0.669$ ). Given the assessment of pain in body regions, the total number of pain areas ( $p = 0.038$ ) and the total pain intensity score ( $p = 0.002$ ) were significantly higher in the low 25(OH)D group (Table 3). In age- and BMI-adjusted sex-specific analyses, the total pain intensity score was lower in women with 25(OH)D levels  $\geq 20$  ng/mL ( $p = 0.017$ ); no significant differences were found in other strength and gait parameters. In men, the group with 25(OH)D levels  $\geq 20$  ng/mL had higher dominant and nondominant fingertip pinch strengths ( $p = 0.013$  and  $p = 0.004$ , respectively), and a lower total pain intensity score ( $p = 0.001$ ). No significant differences were found in other outcome variables (Table 3). However, the 25(OH)D status  $\times$  sex interaction was not statistically significant in any of the evaluated outcome variables (all  $p > 0.05$ ). Therefore, the differences observed in the sex-specific analyses were not interpreted as a sex-specific relationship.

**Table 3** Comparison of handgrip strength, pinch strength, pain parameters, and 6-meter walking time according to 25(OH)D status in the total sample and by sex

| Parameters                          | Group I Total<br>( <i>n</i> = 40) | Group II Total<br>( <i>n</i> = 40) | Total test              | Group I<br>women( <i>n</i> = 21) | Group II women<br>( <i>n</i> = 22) | Women test <sup>a</sup> | Group I Men<br>( <i>n</i> = 19) | Group II Men<br>( <i>n</i> = 18) | Men test <sup>a</sup>   | 25(OH)D status<br>× sex <i>p</i> -value <sup>b</sup> |
|-------------------------------------|-----------------------------------|------------------------------------|-------------------------|----------------------------------|------------------------------------|-------------------------|---------------------------------|----------------------------------|-------------------------|------------------------------------------------------|
| <b>Jamar grip strength, kg</b>      |                                   |                                    |                         |                                  |                                    |                         |                                 |                                  |                         |                                                      |
| Dominant hand                       | 21.25 (18–38)                     | 30 (20–40)                         | <i>p</i> = 0.390        | 18 (12–20)                       | 20 (13.75–21.25)                   | <i>p</i> = 0.321        | 40 (27–45)                      | 40 (33.75–45)                    | <i>p</i> = 0.190        | 0.779                                                |
| Nondominant hand                    | 20 (14–34)                        | 20 (20–35)                         | <i>p</i> = 0.339        | 14 (10–19)                       | 20 (13.75–20)                      | <i>p</i> = 0.128        | 34 (27–42)                      | 35 (30–40.5)                     | <i>p</i> = 0.690        | 0.454                                                |
| <b>Fingertip pinch strength, kg</b> |                                   |                                    |                         |                                  |                                    |                         |                                 |                                  |                         |                                                      |
| Dominant hand                       | 5 (3.5–7)                         | 5.5 (5–7.5)                        | <b><i>p</i> = 0.001</b> | 4 (3–5)                          | 5 (5–5.25)                         | <i>p</i> = 0.149        | 7 (5–7)                         | 7.5 (7.38–10)                    | <b><i>p</i> = 0.013</b> | 0.396                                                |
| Nondominant hand                    | 4.5 (3–7)                         | 5 (4–7.5)                          | <b><i>p</i> = 0.023</b> | 3.5 (3–4.75)                     | 5 (2.88–5)                         | <i>p</i> = 0.442        | 7 (4–7)                         | 7.5 (6.5–10)                     | <b><i>p</i> = 0.004</b> | 0.109                                                |
| <b>Lateral pinch strength, kg</b>   |                                   |                                    |                         |                                  |                                    |                         |                                 |                                  |                         |                                                      |
| Dominant hand                       | 7 (5–9.5)                         | 7.5 (5–10)                         | <i>p</i> = 0.086        | 7 (5–7)                          | 6.25 (5–7.5)                       | <i>p</i> = 0.422        | 9 (7–10)                        | 10 (7.5–10)                      | <i>p</i> = 0.154        | 0.622                                                |
| Nondominant hand                    | 7 (5–8)                           | 7.5 (5–10)                         | <i>p</i> = 0.093        | 5 (4.75–7)                       | 5 (5–7.5)                          | <i>p</i> = 0.333        | 7 (7–10)                        | 10 (7.5–10)                      | <i>p</i> = 0.118        | 0.630                                                |
| <b>Palmar pinch strength, kg</b>    |                                   |                                    |                         |                                  |                                    |                         |                                 |                                  |                         |                                                      |
| Dominant hand                       | 7 (5–7)                           | 7.5 (5–8.75)                       | <i>p</i> = 0.290        | 5 (4.5–6.25)                     | 5 (5–7.5)                          | <i>p</i> = 0.610        | 7 (7–10)                        | 8.75 (7.5–10)                    | <i>p</i> = 0.371        | 0.762                                                |
| Nondominant hand                    | 5.5 (4.75–7)                      | 5 (5–7.5)                          | <i>p</i> = 0.522        | 5 (4–6.25)                       | 5 (4–5.63)                         | <i>p</i> = 0.859        | 7 (5.5–10)                      | 7.5 (6.88–10)                    | <i>p</i> = 0.250        | 0.475                                                |
| Total number of pain<br>areas       | 2 (1–3)                           | 1 (0–2)                            | <b><i>p</i> = 0.038</b> | 2 (1–4)                          | 2 (1–2)                            | <i>p</i> = 0.522        | 2 (0–3)                         | 1 (0–1.25)                       | <i>p</i> = 0.107        | 0.407                                                |
| Total pain intensity<br>score       | 6 (1–8)                           | 2 (0–4)                            | <b><i>p</i> = 0.002</b> | 4 (3–8)                          | 2 (2–4)                            | <b><i>p</i> = 0.017</b> | 4 (0–6)                         | 1 (0–2.25)                       | <b><i>p</i> = 0.001</b> | 0.221                                                |
| 6-m walking time, s                 | 5 (5–5)                           | 5 (5–6)                            | <i>p</i> = 0.090        | 5 (4.5–5)                        | 5 (4–6)                            | <i>p</i> = 0.411        | 5 (5–5)                         | 5 (5–6)                          | <i>p</i> = 0.130        | 0.585                                                |

Data are presented as median (25th–75th percentile). Total-sample *p*-values were obtained using the Mann–Whitney U test. <sup>a</sup>Sex-specific *p*-values represent age- and BMI-adjusted simple effects of 25(OH)D status. ANCOVA was used for hand grip, pinch-strength, walking outcomes, and pain outcomes. <sup>b</sup>*p*-values represent the 25(OH)D status × sex interaction in the age- and BMI-adjusted ANCOVA models. Significant *p*-values are shown in bold. *BMI* Body mass index, *25(OH)D* 25-hydroxyvitamin D, *kg* Kilogram, *s* Second, *6-m* 6-meter

Among the QoL subscale scores of SF-36, the scores for physical function ( $p = 0.344$ ), difficulties in physical role ( $p = 0.708$ ), emotional role ( $p = 0.955$ ), social functioning ( $p = 0.623$ ), pain ( $p = 0.639$ ), vitality ( $p = 0.311$ ), mental health ( $p = 0.740$ ), and general health ( $p = 0.277$ ) showed no significant differences between the two groups.

In the ROC curve, indicating the diagnostic performance of FCR-kPa values in predicting 25(OH)D deficiency, AUC was calculated as 0.94 [95% confidence interval (CI): 0.892–0.988;  $p < 0.001$ ], and the sensitivity of 82.5% and specificity of 93% were found at a cutoff value of 23.5 kPa. For BB-kPa, however, AUC was found to be 0.957 (95% CI: 0.917–0.997;  $p < 0.001$ ), and at a threshold value of 26.5 kPa, the sensitivity and the specificity were determined to be 87.5 and 90%, respectively. In the ROC analysis performed for TA-kPa, AUC was 0.935, with a CI of 0.884–0.986 at a cutoff value of 23.75 kPa, and the sensitivity and specificity rates were calculated as 85 and 90%, respectively (Fig. 2).

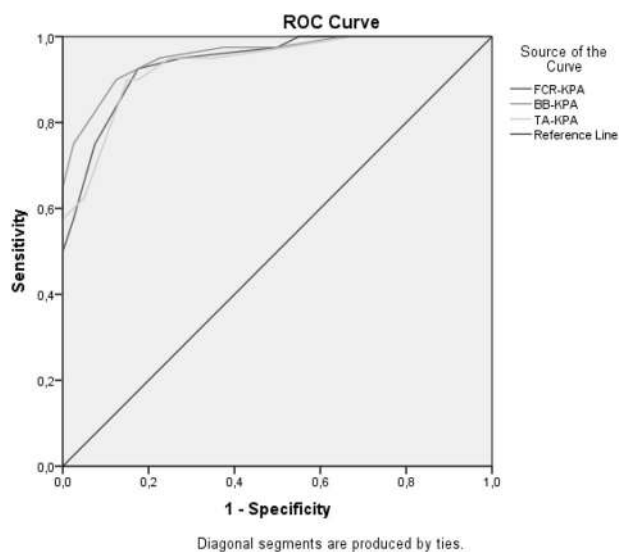
In the Spearman correlation analysis, significant and strong positive relationships were revealed between 25(OH)D levels and muscle elasticity measurements, and 25(OH)D levels showed a strong positive correlation with FCR-kPa ( $r = 0.880$ ;  $p < 0.001$ ). Similarly, the relationship between 25(OH)D level and BB-kPa was also quite strong ( $r = 0.916$ ;  $p < 0.001$ ), and a strong positive correlation was found between TA-kPa and 25(OH)D level ( $r = 0.870$ ;  $p < 0.001$ ). Given the evaluations performed separately between women and men, strong and positive correlations

were determined between serum 25(OH)D levels and muscle elasticity values measured by SWE in both sexes. Among women, strong positive correlations were found between serum 25(OH)D levels and FCR-kPa ( $r = 0.931$ ,  $p < 0.001$ ), BB-kPa ( $r = 0.930$ ,  $p < 0.001$ ), and TA-kPa ( $r = 0.863$ ,  $p < 0.001$ ). In men, strong positive correlations were also found between serum 25(OH)D levels and FCR-kPa ( $r = 0.848$ ,  $p < 0.001$ ), BB-kPa ( $r = 0.897$ ,  $p < 0.001$ ), and TA-kPa ( $r = 0.887$ ,  $p < 0.001$ ).

Generalized linear models were established to evaluate the independent determinants of FCR-kPa, BB-kPa, and TA-kPa. In all models, age, sex, BMI, category of physical activity, and serum 25(OH)D level were evaluated as determining variables. The area variable of the relevant muscle was also included in each muscle model, and the overall fit of the models was found to be acceptable (all omnibus likelihood ratio tests  $p < 0.001$ ). Multicollinearity was assessed among the independent variables, and the variance inflation factor (VIF) values were  $< 5$  for all variables.

In the FCR-kPa model, the overall effect was significant (likelihood ratio  $\chi^2(7) = 122.6$ ,  $p < 0.001$ ). In the model, sex and serum 25(OH)D level were independently associated with FCR-kPa; age ( $B = -0.004$ ;  $p = 0.910$ ), BMI ( $B = 0.007$ ;  $p = 0.860$ ), and FCR area ( $B = -0.006$ ;  $p = 0.579$ ) were not significant. Regarding sex, men had a mean FCR-kPa value that was 2.28 kPa higher than that in women ( $B = 2.280$ ; 95% CI 0.99–3.57;  $p = 0.001$ ). A strong positive association was observed between serum 25(OH)D levels and FCR-kPa ( $B = 0.349$ ; 95% CI 0.303–0.395;  $p < 0.001$ ). This coefficient indicated that clinically, every 10-unit increase in 25(OH)D levels corresponded to approximately a 3.5-unit increase in FCR-kPa. IPAQ physical activity levels demonstrated a tendency for FCR-kPa to be higher in the high-activity group, compared to the low-activity group; however, this relationship was borderline significant ( $B = 1.064$ ; 95% CI  $-0.06$ –2.19;  $p = 0.063$ ), and there was no significant difference between the moderate- and low-active groups ( $B = 0.444$ ; 95% CI  $-0.44$ –1.33;  $p = 0.325$ ).

In the BB-kPa model, the overall effect was significant (likelihood ratio  $\chi^2(6) = 139.8$ ;  $p < 0.001$ ). In this model, sex, IPAQ physical activity levels and serum 25(OH)D level were remarkable as independent determinants; age ( $B = 0.044$ ;  $p = 0.261$ ) and BMI ( $B = 0.023$ ;  $p = 0.593$ ) showed no significant relationship with BB-kPa. The BB-kPa value in men was found to be 1.95 kPa higher than in women ( $B = 1.950$ ; 95% CI 1.06–2.84;  $p < 0.001$ ), and there was a strong and positive association between serum 25(OH)D level and BB-kPa ( $B = 0.450$ ; 95% CI 0.400–0.501;  $p < 0.001$ ). In the group with a high level of IPAQ physical activity, the BB-kPa value was found to be higher, compared to the group with a low activity level



**Fig. 2** ROC curves for FCR-kPa, BB-kPa, and TA-kPa values. BB Biceps brachii, FCR Flexor carpi radialis, kPa Muscle elasticity value (kilopascals), ROC Receiver operating characteristic curve, TA Tibialis anterior. ROC curves used for FCR-kPa, BB-kPa, and TA-kPa measurements to predict 25(OH)D deficiency. Sensitivity and specificity values for the areas under the curve and cut-off values are given in the text

( $B = 1.322$ ; 95% CI 0.10–2.54;  $p = 0.034$ ). The difference between the moderate- and low-activity groups was not statistically significant ( $B = 0.725$ ; 95% CI 0.24–1.69;  $p = 0.140$ ).

In the TA-kPa model, the overall effect was also found to be significant (likelihood ratio  $\chi^2(6) = 123.5$ ;  $p < 0.001$ ). Consistent with other models, sex and serum 25(OH)D level were strong and independent predictors of TA-kPa, and age ( $B \approx 0.000$ ;  $p = 0.989$ ) and BMI ( $B = 0.015$ ;  $p = 0.695$ ) were not significant. Men had an average of 1.73 kPa, higher TA-kPa values than women ( $B = 1.729$ ; 95% CI 0.91–2.55;  $p < 0.001$ ). Serum 25(OH)D levels showed a strong association with TA-kPa ( $B = 0.356$ ; 95% CI 0.310–0.403;  $p < 0.001$ ), indicating that every 10-unit increase in 25(OH)D levels could be associated with approximately a 3.6-unit increase in TA-kPa. The group with high IPAQ physical activity levels tended to demonstrate higher TA-kPa values, compared to the low-activity group; however, while there was a borderline significant correlation between the IPAQ physical activity levels and TA-kPa values ( $B = 1.102$ ; 95% CI  $-0.03$ –2.23;  $p = 0.055$ ), the moderate activity group showed a significant positive correlation, compared to the low-activity group ( $B = 1.120$ ; 95% CI 0.23–2.01;  $p = 0.014$ ).

## Discussion

In this cross-sectional study, it was shown that the elasticity values of the FCR, BB, and TA muscles measured by SWE in young adults with low 25(OH)D levels were lower both in the total sample and in the women and men subgroups. In analyses adjusted for age and BMI, 25(OH)D status and sex were independently associated with muscle elasticity. The 25(OH)D status  $\times$  sex interaction, although significant at the multivariate level, was not significant in analyses of individual muscles; Therefore, it was considered that the relationship between low 25(OH)D and reduced muscle elasticity was found in both sexes, but did not show a clear and consistent sex-specific effect. In secondary outcomes, total pain intensity was higher in both sexes in the low 25(OH)D group, while the decrease in fingertip pinch strength remained significant only in men; however, as there was no significant 25(OH)D status  $\times$  sex interaction, this result was not interpreted as a men-specific relationship. Additionally, serum 25(OH)D level showed a strong positive correlation with muscle elasticity measurements, while SWE parameters showed high performance in distinguishing low 25(OH)D levels.

In previous studies, it was emphasized that the mechanical properties of muscles can differ according to sex. In the study in which healthy young adults were assessed with SWE by Lee et al., elasticity values in the

rectus femoris, biceps femoris, TA, and medial gastrocnemius muscles were reported to be higher in men than in women [25]. Similarly, Zhang et al. also showed that the elasticity of medial and lateral gastrocnemius muscles was higher in adult men than in adult women [26]. In our study, the higher FCR-kPa, BB-kPa, and TA-kPa values in men are consistent with those reported in the literature. However, in the low 25(OH)D group, the elasticity values of all three muscles investigated in our study were significantly lower in both women and men. Additionally, in age- and BMI-adjusted analyses, the independent effects of 25(OH)D status and sex on muscle elasticity support the idea that the association between low 25(OH)D and decreased muscle elasticity exists, independent of sex.

In previous studies, predictive investigations, mostly based on US or mechanical measurements, which examine the relationship between 25(OH)D status and the morphological and biomechanical properties of muscle and surrounding soft tissues, have been reported. In various studies evaluating adult individuals, subcutaneous fat tissue thickness was demonstrated to be higher in peripheral regions, such as the forearm, arm, thigh, and trochanteric region, in groups with 25(OH)D deficiency through conventional US [14, 27]. These findings suggest that there may be significant changes in peripheral body composition in 25(OH)D deficiency. On the other hand, it has been emphasized that one of the main mechanisms accompanying these morphological changes in 25(OH)D deficiency is proximal muscle atrophy, especially the loss of fast-twitch Type II muscle fibers. Thus, in histopathological studies, atrophy has been shown in Type II skeletal muscle fibers in adults [28]. It has been suggested that these structural changes may negatively affect the mechanical properties of the muscle, such as elasticity and force production.

It has been revealed that not only the thickness but also the mechanical properties of muscle tissue can be affected by 25(OH)D status [29, 30], and in another study performed elderly individuals using MyotonPRO, it was stated that increased elasticity was detected only in certain proximal muscles (nondominant quadriceps and hamstring) in the 25(OH)D -sufficient group, but muscle tone and tissue rigidity parameters showed no significant differences concerning 25(OH)D levels, and the diagnostic value of these biomechanical measurements in predicting 25(OH)D deficiency was limited [31]. Although revealing the relationship of muscle biomechanics to 25(OH)D, these findings suggest that the methods used may have several limitations in terms of sensitivity and measurement depth. Therefore, the capacity of SWE to measure muscle elasticity objectively and reproducibly provides a significant methodological advantage [32, 33]. However, no study directly evaluating the effect of 25(OH)D deficiency on muscle elasticity using SWE-based measurements has been encountered, based on

the literature. We consider that the current study, focusing on the evaluation of muscle elasticity with SWE, provides a unique contribution to the literature by going beyond morphological measurements and presenting the parameters of quantitative muscle stiffness sensitive to 25(OH)D status.

Evidence on the effects of vitamin D supplementation on muscle strength and physical performance is heterogeneous. In the study by Houston et al., although serum 25(OH)D levels with 2000 IU/day cholecalciferol treatment for 12 months in individuals aged 65–89 years with low functional capacity were significantly increased, no additional gain was revealed, compared to placebo in measures, such as leg muscle strength, hand grip strength, Short Physical Performance Battery score, Timed Up and Go and short-distance walking performance [34]. In contrast, Gupta et al. reported statistically significant but relatively small absolute increases in hand grip strength, lower extremity muscle strength, and 6-m walking time in a randomized controlled trial conducted in young adults with 25(OH)D deficiency [35]. In a retrospective, propensity score-matched study conducted in middle-aged and elderly individuals by Qi et al., as well as a statistically significant increase in both hand grip strength and hand pinch strength over time in the group receiving vitamin D supplementation, a certain degree of improvement was also witnessed in the control group due to health training and lifestyle interventions [36]. When these findings are considered together, it is seen that the relationship between 25(OH)D levels and muscle function can vary depending on age, baseline functional capacity, comorbidity, degree of deficiency, and end-point muscle strength used. Our study, however, is a cross-sectional study performed in relatively young adults without significant muscle weakness and examines the relationship between current 25(OH)D levels and concomitant muscle characteristics, but not the response to vitamin D supplementation. Unlike the studies by Gupta, Qi, and Houston, our assessment of muscle elasticity using SWE in addition to classic hand grip and pinch strength measurements allowed us to detect subclinical biomechanical changes associated with 25(OH)D deficiency before global muscle strength and walking performance deteriorated. Therefore, in contrast to the limited strength/performance changes reported in supplementation studies, the significant difference in muscle elasticity observed in our study is an expected finding, consistent with the design differences of the studies.

It is suggested that the prevalence and severity of chronic musculoskeletal pain may be higher in individuals with 25(OH)D deficiency, compared to those with adequate 25(OH)D levels [37]. In age- and BMI-adjusted sex-specific analyses, lower 25(OH)D levels were associated with higher total pain intensity in both sexes, while men also had lower dominant and non-dominant fingertip pinch strengths.

However, the lack of a significant 25(OH)D status  $\times$  sex interaction in ANCOVA models for pain and muscle strength outcomes does not support a clear sex-specific effect for these outcomes. While suggesting a possible relationship between 25(OH)D levels and pain intensity and some specific strength measures, these findings should not be considered a causal or generalizable outcome due to the cross-sectional study design and the limited sample size of the subgroups created according to sex. The fact that no difference was found between SF-36 pain subscale scores suggests that 25(OH)D may be associated with distribution and intensity of pain; however, the reflection of the deficiency on overall health-related QoL may depend on more complex and multifactorial determinants.

One of the most striking clinical findings of our study is that the AUC values in the ROC curves obtained for FCR-kPa, BB-kPa, and TA-kPa ranged from 0.94 to 0.96, and that cut-off values distinguishing 25(OH)D deficiency (25(OH)D < 20 ng/mL) with high sensitivity and specificity were identified for all three muscles. This level of diagnostic performance suggests that muscle elasticity measurements used to measure 25(OH)D deficiency, beyond being merely a research tool, could be considered a complementary monitoring method.

Furthermore, even when the effects of potential confounders such as age, BMI, physical activity level, and muscle cross-sectional area were statistically controlled in multivariate generalized linear models established for FCR-kPa, BB-kPa, and TA-kPa, it is noteworthy that serum 25(OH)D levels and sex remained independently and strongly associated with muscle elasticity parameters. This finding supports that the observed elasticity differences are not solely dependent on body composition or physical activity level, but 25(OH)D status may also be an independent determinant of muscle biomechanics.

Our study is one of the pioneering studies to quantitatively evaluate the effects of 25(OH)D deficiency on muscle biomechanics. The use of a comparative control group and the consideration of clinical characteristics that may affect the outcome, such as age, sex, BMI, and physical activity level, in the statistical analyses strengthen the internal validity of the findings. The fact that our samples were limited to individuals between 18 and 45 years of age allowed us to specifically observe the effect of 25(OH)D by eliminating age-related muscle changes. The fact that sex-specific analyses were performed and 25(OH)D  $\times$  sex interaction evaluated is one of the strengths of our study, as these analyses demonstrate that the observed relationship cannot be explained solely by the inherent differences in elasticity between men and women.

However, our study also has some limitations. The fact that the study was conducted in a single tertiary care center and on a sample of young adults may limit the

generalizability of the study to different age groups and community-based populations. Although planned prospectively, the cross-sectional design does not allow the study to establish the causality of the relationship between 25(OH)D levels and muscle elasticity and functional markers, nor does it allow for the reflection of this relationship on long-term clinical outcomes. The fact that muscle elasticity measurements were performed only on three selected muscles on the dominant side and with the same device may limit the generalization to other muscle groups and different laboratory conditions. Additionally, time spent outdoors and sun exposure were not directly measured; therefore, the potential contribution of lifestyle-related ultraviolet exposure to serum 25(OH)D status could not be evaluated separately by sex. Furthermore, all US examinations were evaluated by consensus by two radiologists, but the lack of calculating the inter-observer agreement statistics (e.g., kappa coefficient) is another limitation of our study.

## Conclusion

In conclusion, our study is among the pioneering studies quantitatively revealing the relationship between 25(OH)D deficiency and muscle elasticity parameters in young adults and evaluating the predictability of 25(OH)D deficiency with muscle elasticity measurements through USG. We consider that our findings need to be confirmed, and their implications should be better clarified for clinical practice with larger sample sizes, multicenter studies, and preferably longitudinal designs, including different age groups.

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## Declarations

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Conflict of interest** Lütüye Parlak, Nusret Seher, Funda Levendoğlu, Ezgi A. Tezcan, Esra Öz, Mehmet Sedat Durmaz, and İlknur A. Gezer declared no conflicts of interest.

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