

# The Differences in Parameters in Ultrasound Imaging and Biomechanical Properties of the Quadriceps Femoris with Unilateral Knee Osteoarthritis in the Elderly: A Preliminary Observational Study

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**Purpose:** Our study aims to evaluate differences in muscle parameters of the quadriceps muscles in patients with knee osteoarthritis (KOA) in older adults.

**Methods:** The study included 40 patients diagnosed with unilateral knee osteoarthritis in the KOA group (KG) and 40 asymptomatic elderly individuals in the control group (CG). Muscle ultrasonic mean echo intensity and shear modulus, as well as tone and stiffness of the rectus femoris (RF), vastus medialis (VM), and vastus lateralis (VL) were analyzed. Additionally, clinical correlations were performed.

**Results:** In the KG group, there were significant differences in echo intensity, shear modulus, and tone between the affected and unaffected sides for RF ( $p=0.003, 0.019, 0.014$ ), while VM showed significant differences in shear modulus and tone ( $p=0.006, 0.002$ ). Additionally, VL exhibited significant differences in echo intensity, shear modulus, and stiffness ( $p=0.007, 0.006, 0.010$ ). Compared to the CG group, the KG group showed significant differences in echo intensity of the affected side RF ( $p=0.001$ ). VM exhibited statistically significant differences in echo intensity and shear modulus ( $p < 0.001, p=0.008$ ), while VL showed statistically significant differences in echo intensity, tone, and stiffness ( $p < 0.001, p=0.028, p < 0.001$ ). The correlation results showed that patients with unilateral KOA, VM, and VL echo intensity were correlated with K-L grade ( $r = 0.443, p=0.004; r = 0.469, p=0.002$ ). The tone of VL was correlated with VAS and WOMAC ( $r = 0.327, p=0.039; r = 0.344, p=0.030$ ).

**Conclusion:** The parameters of the quadriceps femoris muscle exhibit asymmetry between the affected and unaffected sides in patients with unilateral KOA, as well as a difference between the dominant side of healthy older individuals and the affected side of KOA.

**Keywords:** knee osteoarthritis, quadriceps femoris, ultrasound, muscle tone, stiffness

## Introduction

Knee osteoarthritis (KOA) is a common degenerative joint disease among older individuals worldwide.<sup>1,2</sup> It is now commonly recognized as a chronic disorder affecting the entire joint, including the synovial membrane, cartilage, meniscus, subchondral bone, infrapatellar fat pad, ligament, and periarticular muscle.<sup>3–6</sup> With an aging population, the incidence of KOA will continue to rise. This poses a severe health threat, leading to higher medical costs and increased economic burdens for families and society.<sup>7</sup> The etiology of KOA is complex, involving not only the pathological changes in various structures around the joint but also alterations in the body's microenvironment.<sup>8,9</sup> Among them, atrophy and weakness of skeletal muscles around the knee joint are essential clinical symptoms of KOA and risk factors for its occurrence.<sup>10,11</sup> The quadriceps femoris muscle group is responsible for both knee extension and hip flexion, making it the primary knee extensor of the knee joint. Its crucial role in maintaining stability within the knee joint ensures normal movement and upright body posture. Improving the quadriceps femoris muscle can reduce the risk of KOA, alleviate pain in KOA patients, enhance physical function, and improve their quality of life.<sup>12,13</sup> Therefore, selecting an appropriate approach for assessing quadriceps femoris muscle alterations can guide knee osteoarthritis rehabilitation.

Ultrasound can display anatomical images of muscles, tendons, ligaments, joint capsules, and other soft tissues in real time while dynamically observing the morphology of muscles.<sup>14</sup> The ultrasound image of muscle can not only clearly show the cross-sectional area, thickness, and fiber length of the muscle as well as its pinnate angle but also indirectly reflect proportional changes in muscle structure by quantifying the echo intensity of the image.<sup>15,16</sup> The echo intensity of muscle fiber parenchyma is dark, while that of non-contractile tissue is bright in the ultrasound image of the muscle. Therefore, echo intensity can also reflect muscle composition and quality.<sup>17,18</sup> Naoki Akazawa et al used the echo intensity of quadriceps femoris to reflect muscle structure composition and found a negative correlation with daily activity ability.<sup>19</sup> Fukumoto et al discovered that changes in the echo intensity of quadriceps femoris could indicate differences in physical activity, allowing for the evaluation of muscle quality and function.<sup>20</sup> Ultrasound shear wave elastography (SWE) is a practical, non-invasive tool for measuring soft tissue and elastic properties in vivo.<sup>21</sup> It can quantify the stiffness of local areas of soft tissue by shear modulus. Previous studies have shown that shear modulus values can reflect quadriceps stiffness, which is related to muscle strength and proportional to contraction.<sup>22–24</sup> Additionally, Muscle tone and stiffness are the foundation for maintaining physiological function in muscles, playing a crucial role in joint stability and overall body function.<sup>25</sup> In recent years, using equipment to measure muscle properties instead of manual measurement has improved the reliability of measurements.<sup>26</sup> Myoton PRO is a convenient handheld device for measuring muscle mechanical properties among different populations and at various joint angles with good intra- and inter-rater reliability.<sup>27,28</sup>

Based on the reliability of ultrasound and Myoton PRO in assessing muscle parameters, we used these tools to compare differences in muscle echo intensity, shear modulus, tone, and stiffness between the affected and unaffected sides of patients with unilateral knee osteoarthritis. We also compared these parameters between the affected side of KOA patients and the dominant side of healthy older individuals. Additionally, we examined correlations between muscle parameters and clinical severity scores.

## Material and Methods

### Design

The study was designed as a preliminary observational study aimed at evaluating muscle differences between the affected and unaffected sides in KOA, as well as between patients with KOA and healthy older individuals. Additionally, it assesses the correlation between muscle parameters and clinical severity in KOA patients.

### Participants

The study included 40 asymptomatic volunteers and 40 patients with KOA. All the patients had unilateral KOA and were recruited from the outpatient department of Guangdong Provincial Second Hospital of Traditional Chinese Medicine. The inclusion criteria for KOA were as follows: 1) aged  $\geq 60$ , 2) meeting the clinical diagnostic criteria for KOA set by the

American College of Rheumatology,<sup>29</sup> 3) unilateral KOA with a Kellgren/Lawrence (K/L) grade  $\geq 2$ ,<sup>30</sup> 4) BMI  $\leq 30$  kg/m<sup>2</sup>. The exclusion criteria include the following: 1) lower extremity trauma or deformity, 2) presence of muscle-related diseases such as polymyositis, myasthenia gravis, and muscular dystrophy, 3) presence of neurological diseases such as stroke and Parkinson's disease, 4) other non-degenerative knee-related diseases like gout and rheumatoid arthritis, and 5) skin ulceration around the knee and thigh. Forty asymptomatic individuals were recruited as controls. The participants were selected from families of patients at Guangdong Provincial Second Hospital of Traditional Chinese Medicine and surrounding communities, meeting the following inclusion criteria: 1) aged  $\geq 60$ , 2) no history of lower limb deformity or trauma, 3) no history of knee joint-related diseases, and 4) no musculoskeletal or nervous system disorders. Demographic information, including name, sex, age, height, weight, and body mass index, was collected. In addition, patients in the KOA group were assessed for their Western Ontario McMaster University Osteoarthritis Index score (WOMAC), the visual analogue scale of pain, and K/L grade. In KOA patients, measurements of both lower limbs were taken, and the affected or healthy side was recorded. For asymptomatic individuals, the dominant side was determined by asking about their kicking preference and measured.<sup>31</sup> This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Guangdong Provincial Second Hospital of Traditional Chinese Medicine (No. YB01).

## Muscle Ultrasound Image Measurement

The Super Sonic Imaging color Doppler ultrasound diagnostic instrument (Aixplorer company, France) was used with an L15-4 array probe operating in muscle examination mode at 4–12 MHz frequency. The gain value and depth were set to the same level. The subject was positioned supine with both legs extended and muscles relaxed, and coupling gel was evenly applied to the probe before measurements were taken perpendicular to the skin. The measurement points were selected as follows: the RF was chosen at the middle and lower 1/3 of the upper margin of the anterior superior iliac spine and patella; The VM and VL were measured at the medial and lateral positions, respectively, 10 cm above the patella.<sup>32</sup> The optimal measurement location was determined when several muscle fascicles could be seen without disconnection through the image.<sup>22</sup>

## Measurement of Shear Modulus

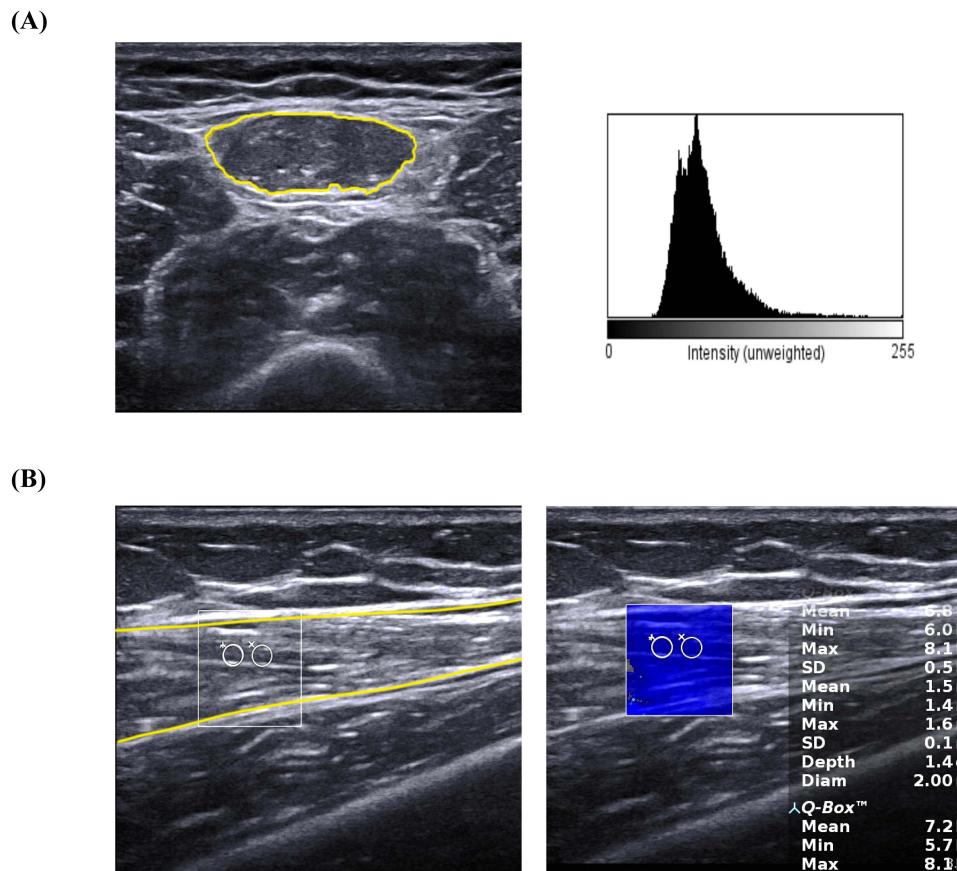
The ultrasound diagnostic instrument was switched to the SWE measurement mode, and the probe was rotated parallel to the muscle at the measurement point described above. A 1cm\*1cm square area (SWE-Box) was selected as the field of view for observation in the muscle image. Once a stable shear wave appeared within this field, the image was frozen, and the average shear modulus was measured using a supersonic imaging analysis plug-in (Q-Box). The measurement image is shown in [Figure 1](#).

## Measurement of Echo Intensity

Image J 1.8.0 was used for the quantitative analysis of ultrasound images. The recognizable muscle tissue on the image was selected using the polygon box tool, and the Region of Interest (ROI) outline was circled. Then, the “Measure” function in “Analyze” was used to calculate the average gray value of echo intensity in the selected area, which ranges from 0 to 255.<sup>33</sup> The measurement image is shown in [Figure 1](#).

## Measurement of Myoton PRO

The RF, VL, and VM biomechanical properties were measured using the Myoton PRO device (Tallinn, Estonia) by the same trained operator. Tone and stiffness values were recorded at the following points: For the rectus femoris muscle, two-thirds of the distance between the anterior superior iliac spine and the superior pole of the patella was selected.<sup>34</sup> The measurement points of vastus lateralis and vastus medialis are the most salient points of the muscle belly.<sup>28</sup> All subjects were required to rest for 5 min before measurement, during which the subjects were lying with knees extended and hip in the neutral position and were asked to relax their muscles and not speak to avoid any possible muscle contraction. In this study, Myoton PRO (Tallinn, Estonia) was used to evaluate the quadriceps tone and stiffness.



**Figure 1** The method for measuring ultrasound-related parameters of the rectus femoris muscle.

**Notes:** (A) depicts a schematic diagram of the cross-section of the rectus femoris muscle and the measurement of echo intensity. The histogram of the gray scale of the rectus femoris muscle in ImageJ software (0–255) is shown in the right image of part A. (B) shows a longitudinal section diagram of the rectus femoris muscle and illustrates the measurement of shear modulus.

## Clinical Severity

The Visual analogue scale (VAS) score was used to evaluate patients' pain. It is a widely used evaluation tool for pain in KOA patients. The score range is 0–10. The Western Ontario and McMaster University Osteoarthritis Index (WOMAC) evaluates knee joint function in pain, stiffness, and joint function.<sup>35</sup> It covers the primary symptoms and signs of arthritis affecting the entire knee. Higher scores indicate more severe knee dysfunction. This study recorded only total score values for pain, stiffness, and joint function. The Kellgren-Lawrence (K-L) classification is a commonly used clinical system for grading knee osteoarthritis based on imaging manifestations, with severity divided into five grades ranging from 0 to IV. Higher grades indicate more severe degrees of the condition.<sup>30</sup>

## Statistical Analysis

Statistical software SPSS (Version 26.0. Armonk, NY: IBM Corp) was used for statistical analysis. Shapiro–Wilk test was used to analyze the normality of the data, and the Levene test was used for homogeneity of variance. For the data with normal distribution and homogeneity of variance, the independent sample *t*-test was used to compare the two groups, and the Paired Student's *t*-test was used for intra-group comparison. Mann–Whitney *U*-test for between-group comparisons and the Wilcoxon test for within-group comparisons were used when data were not normally distributed or did not meet homogeneity of variance. Pearson's correlation test (normal distribution) or Spearman's (non-normal distribution) was used to analyze the correlation between muscle parameters and clinical severity. Numerical data were represented by frequency or percentage (%), and the comparison of rate or ratio was analyzed by chi-square test ( $\chi^2$ ). Statistical differences were considered significant at  $p < 0.05$ .

**Table 1** Characteristics of Participants

Characteristics	KG (n=40)	CG(n=40)	P value
<b>Age (year)</b>	66.78 ± 3.56	66.38 ± 3.19	0.598
<b>Gender (n)</b>			0.639
Male	13/40 (32.5%)	15/40 (37.5%)	
Female	27/40 (67.5%)	25/40 (62.5%)	
<b>Height (cm)</b>	159.63 ± 6.17	160.15 ± 6.08	0.703
<b>Weight (kg)</b>	61.25 ± 4.34	60.90 ± 4.62	0.728
<b>BMI (kg/m<sup>2</sup>)</b>	24.13 ± 2.39	23.85 ± 2.57	0.780
<b>VAS</b>	4.55 ± 1.54		
<b>WOMAC</b>	38.40 ± 9.18		
<b>K-L</b>			
II	18		
III	20		
IV	2		

**Notes:** Values are Mean ± SD or n or Percent (%).

**Abbreviations:** BMI, body mass index; KG, KOA patients' group; CG, control group; K/L, Kellgren/Lawrence.

## Results

### Participants Characteristics

Demographic results showed no significant differences in age, height, weight, or BMI between the two groups. The information of all participants is presented in [Table 1](#).

### Muscle Echo Intensity and Shear Modulus

As shown in [Table 2](#) and [Figure 2](#), the results showed that in the KG group, the echo intensity of RF and VL on the affected side was higher than that on the healthy side ( $p=0.003$ ,  $p=0.007$ ). The shear modulus of RF, VM, and VL on the affected side were lower than those on the healthy side ( $p=0.019$ ,  $p=0.006$ ,  $p=0.006$ ). Compared with CG, the echo intensity of RF, VM, and VL in the KG group was higher than in CG ( $p=0.001$ ,  $p<0.001$ ,  $p<0.001$ ). The shear modulus of VM in the KG was lower than that of CG ( $p=0.008$ ).

### Tone and Stiffness

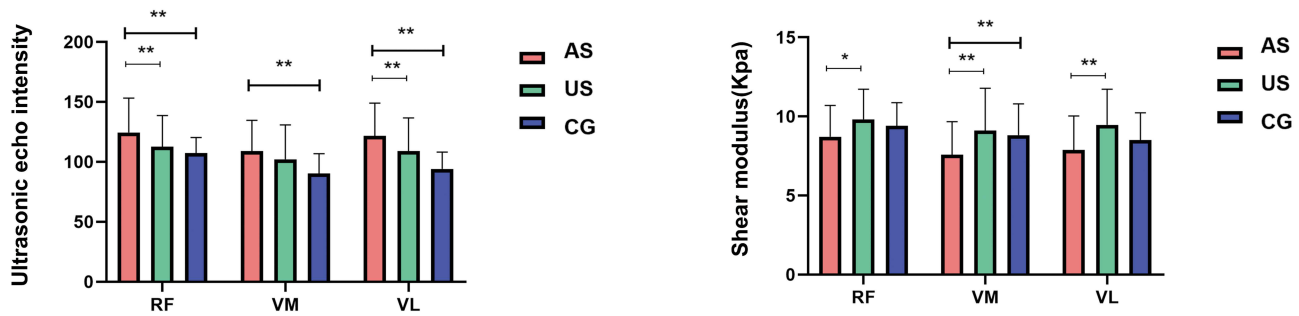
As shown in [Table 3](#) and [Figure 3](#), the results indicated that in KG, the tone of RF and VM on the affected side was higher than that on the healthy side ( $p=0.014$ ,  $p=0.002$ ), with the stiffness in VL on the affected side was higher than that

**Table 2** Muscle Ultrasound Parameters of the RF, VL, and VM Between Patients with Knee Osteoarthritis and Asymptomatic Subjects

Muscle ultrasound parameters		KG		P value (AS VS US)	CG	P value (DS VS AS)
		AS	US		DS	
Ultrasonic echo intensity	RF	124.44 ± 28.57	112.52 ± 26.10	0.003*	107.30 ± 12.98	0.001 <sup>▲</sup>
	VM	108.97 ± 25.60	102.17 ± 28.66	0.195	90.33 ± 16.38	<0.001 <sup>▲</sup>
	VL	121.58 ± 27.26	108.97 ± 27.53	0.007*	94.05 ± 14.11	<0.001 <sup>▲</sup>
Shear modulus (Kpa)	RF	8.72 ± 1.98	9.83 ± 1.89	0.019*	9.41 ± 1.45	0.077
	VM	7.58 ± 2.09	9.11 ± 2.68	0.006*	8.83 ± 1.96	0.008 <sup>▲</sup>
	VL	7.89 ± 2.15	9.47 ± 2.25	0.006*	8.51 ± 1.72	0.155

**Notes:** Values are mean ± standard deviation; AS compared to US \* $p < 0.05$ ; KG compared to CG, <sup>▲</sup> $p < 0.05$ .

**Abbreviations:** KG, KOA group; CG, control group; AS, affected side; US, unaffected side; DS, dominant side; RF, rectus femoris; VM, vastus medialis VL, vastus lateralis.



**Figure 2** Differences in Muscle ultrasonic echo intensity and Shear modulus of the RF, VM, and VL between patients with knee osteoarthritis and healthy subjects. **Notes:** \*\*Indicates  $p$  value < 0.01; \*Indicates  $p$  value < 0.05. **Abbreviations:** AS, Affected side; US, unaffected side; CG, control group.

in unaffected side ( $p=0.010$ ). Compared with CG, the tone and stiffness of VL in the KG group were higher than in CG ( $p=0.028$ ,  $p<0.001$ ).

### Relevance

In patients with unilateral KOA, VM and VL echo intensity were correlated with K-L grade ( $r = 0.443$ ,  $p=0.004$ ;  $r = 0.469$ ,  $p=0.002$ ). The tone of VL was correlated with VAS and WOMAC ( $r = 0.327$ ,  $p=0.039$ ;  $r = 0.344$ ,  $p=0.030$ ). All results are shown in Table 4.

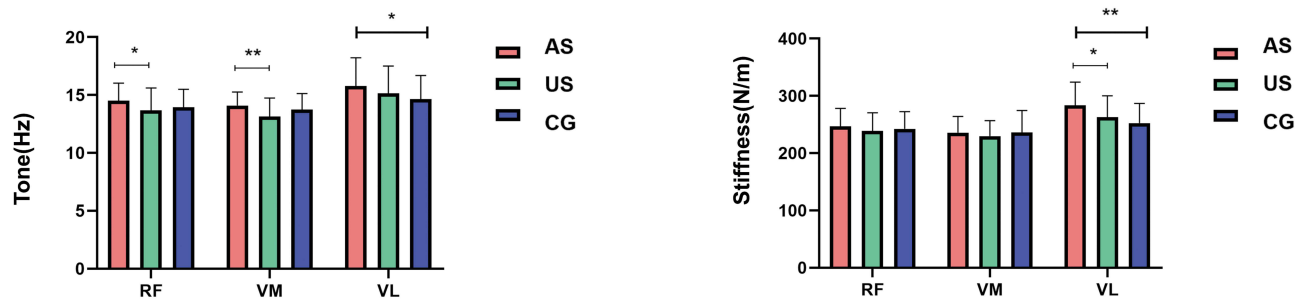
### Discussion

The ultrasonic evaluation results showed that the RF and VL echo intensity on the affected side of the KG were higher than that on the healthy side, while the RF, VM, and VL echo intensity in the KG were higher than those in the CG. These findings suggest differences in ultrasound images of muscles on the affected side of KOA patients, and an increase in echo intensity indicates muscle quality deterioration.<sup>18</sup> The shear modulus in RF, VM, and VL of the affected and healthy sides of the KG showed statistically significant differences. Additionally, there was a statistically significant difference in the shear modulus of VM between CG and KG. The shear modulus reflects muscle elasticity, with lower values indicating poorer muscle elasticity.<sup>36</sup> The evaluation results of Myoton PRO showed that the tone of RF and VM on the affected side was higher than that on the healthy in the KG, and the stiffness of VL on the affected side was also higher than that on the healthy side. In Addition, there were statistically significant differences in both tone and stiffness of VL between CG and KG. Tone and stiffness represent changes in muscle properties, and the two are often consistent. An increase in both also indicates a certain degree of decrease in muscle quality. Our study demonstrates asymmetries in muscle parameters between the affected and healthy sides of KOA patients and differences in muscle parameters on the

**Table 3** Muscle Biomechanical Properties of the RF, VL, and VM Between Patients with Knee Osteoarthritis and Asymptomatic Subjects

Muscle biomechanical property		KG		P value (AS VS US)	CG	P value (DS VS AS)
		AS	US		DS	
Tone(Hz)	RF	14.52 ± 1.52	13.68 ± 1.94	0.014*	13.94 ± 1.55	0.099
	VM	14.10 ± 1.17	13.15 ± 1.60	0.002*	13.74 ± 1.40	0.214
	VL	15.78 ± 2.46	15.15 ± 2.37	0.079	14.65 ± 2.04	0.028 <sup>▲</sup>
Stiffness(N/m)	RF	246.70±31.48	238.70±31.85	0.073	242.55±29.88	0.547
	VM	235.95±28.17	229.78±26.92	0.324	236.28±38.39	0.966
	VL	283.38±40.64	262.83±37.22	0.010*	252.60±34.29	<0.001 <sup>▲</sup>

**Notes:** Values are mean ± standard deviation; AS compared to US \* $p$  < 0.05; KG compared to CG, <sup>▲</sup> $p$  < 0.05. **Abbreviations:** KG, KOA group; CG, control group; AS, affected side; US, unaffected side; DS, dominant side; RF, rectus femoris; VM, vastus medialis VL, vastus lateralis.



**Figure 3** Differences in tone and stiffness of the RF, VM, and VL between patients with knee osteoarthritis and healthy subjects.

**Notes:** \*\*Indicates  $p$  value < 0.01; \*Indicates  $p$  value < 0.05.

**Abbreviations:** AS, Affected side; US, unaffected side; CG, control group.

affected side between healthy elderly individuals and KOA patients of the same age. Additionally, we found a correlation between muscle parameters and clinical symptoms in KOA patients. Therefore, knee osteoarthritis is associated with an imbalance in the muscle parameters of the lower extremities, highlighting the importance of monitoring changes in muscle quality in patients with KOA.

The degeneration in muscle quality around the knee joint increases the risk and may affect the progression of KOA.<sup>37</sup> In patients with osteoarthritis, the weakening of knee extension function increases the risk of symptoms and functional deterioration.<sup>38</sup> Previous studies have concluded that quadriceps femoris muscle mass is associated with symptomatic knee osteoarthritis (KOA), and in women, the decline of muscle strength co-occurs with the progression of KOA symptoms.<sup>39,40</sup> Berger et al found a correlation between the knee extensor muscle strength and the WOMAC score, indicating that KOA patients with severe WOMAC scores have weaker knee extensor strength.<sup>41</sup> Ruhdorfer et al further found that for every 1/20 unit increase in the WOMAC pain scale, age-adjusted isometric knee extensor and flexor strength decreased by about 2%.<sup>42</sup> Luc-Harkey et al found greater muscle strength, less pain, and better physical function performance in patients with KOA.<sup>43</sup> In addition, both men and women with knee extensor muscle weakness have an increased risk of developing knee osteoarthritis.<sup>44</sup> Enhancing knee extensor strength may help prevent the onset of this condition.<sup>38</sup> Shigeru Takagi et al found

**Table 4** Correlation of Muscle Ultrasound Parameters and Biomechanical Property with Clinical Scores in Patients with KOA

Muscle ultrasound parameters and property		KG (AS)					
		VAS		WOMAC		K-L grade	
		<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Ultrasonic echo intensity	RF	-0.038	0.817	-0.146	0.368	0.079	0.630
	VM	0.148	0.363	0.083	0.612	0.443	0.004*
	VL	0.312	0.050	0.189	0.243	0.469	0.002*
Shear modulus(Kpa)	RF	0.133	0.414	0.005	0.974	-0.108	0.506
	VM	0.263	0.101	0.262	0.102	0.301	0.059
	VL	-0.080	0.625	0.071	0.664	0.132	0.416
Tone(Hz)	RF	-0.069	0.674	-0.079	0.627	-0.093	0.567
	VM	-0.097	0.553	-0.168	0.301	0.087	0.595
	VL	0.327	0.039*	0.344	0.030*	0.192	0.235
Stiffness(N/m)	RF	-0.074	0.648	0.069	0.671	-0.030	0.854
	VM	-0.100	0.539	-0.151	0.352	-0.264	0.100
	VL	-0.051	0.754	0.068	0.676	0.266	0.097

**Notes:** \* $p$  < 0.05.

**Abbreviations:** VAS, Visual analogue scale; WOMAC, Western Ontario McMaster and Kmaster University Osteoarthritis Index; K-L grade, The Kellgren-Lawrence grade; AS, affected side; RF, rectus femoris; VM, vastus medialis VL, vastus lateralis.

that quadriceps weakness is associated with an increased risk of knee osteoarthritis, suggesting that improving quadriceps strength in both men and women may effectively prevent KOA.<sup>11</sup> Muscle-specific training interventions were observed to have beneficial effects on pain and function in KOA patients.<sup>45-47</sup> The quadriceps femoris is a shock absorber and joint stabilizer for the knee, crucial in maintaining stability. In knee osteoarthritis (KOA) patients, limb immobility can further exacerbate muscle atrophy, creating a vicious cycle. Strengthening the muscles surrounding the knee should be an integral part of the treatment plan for KOA patients. Based on the above, an accurate assessment of thigh muscle parameters can provide valuable information for preventing and rehabilitating KOA.<sup>47</sup>

Ultrasound and Myoton PRO are commonly used non-invasive quantitative muscle assessment tools in clinical practice. They have advantages in muscle assessment and can be combined in clinical practice.<sup>48</sup> Ultrasound echo intensity can be used to measure muscle composition, reflecting the quality of the muscle by focusing on changes in its internal structural components. The basic fundamental for measuring muscle quality through echo intensity is that skeletal muscle consists of contractile and non-contractile elements. Different tissues exhibit varying pixel intensities on an ultrasound image: muscle appears hypoechoic (black), while adipose and fibrous tissue appear hyperechoic (white). Hypoechoogenicity is associated with superior muscle quality, whereas hyperechogenicity is associated with fatty infiltration, disease, and muscle injury.<sup>18</sup> Studies have confirmed the feasibility of using ultrasound to predict inter-muscular fat content in animals by establishing equations.<sup>49,50</sup> Due to the difficulty in obtaining human specimens, it is currently possible to infer from animal studies that the structure of human muscles could also be estimated by ultrasound. Young et al established a calibration equation to calculate the percentage of adipose in muscle using echo intensity and subcutaneous fat thickness, while it is known that adipose affects the function of muscle.<sup>51-53</sup> Karapınar et al suggested that the increase in echo intensity indicates the increase in the percentage of intramuscular adipose tissue in the muscle and directly used the magnitude of echo intensity to indicate the fat content in the muscle.<sup>54</sup> The echo intensity of non-contractile and contractile tissue in muscles differs, which can reflect variations in muscle composition. Therefore, echo intensity changes can indicate muscle structure alterations closely related to muscle function. The study by Rech et al shows a negative correlation between EI and physical function and muscle strength.<sup>55</sup> Fukumoto et al discovered a negative relationship between echo intensity and muscle thickness and strength of the rectus femoris muscle while finding a positive correlation with age.<sup>56</sup> In the study by Akima et al, it was found that there was a correlation between physical function and age with both echo intensity and thickness of the quadriceps femoris muscle. In males, there was a significant positive correlation between age and quadriceps femoris echo intensity.<sup>57</sup> All of these suggest that echo intensity is a reliable parameter reflecting muscle quality and function, which can be used as a novel and practical method for muscle assessment.<sup>58</sup>

Currently, echo intensity has good diagnostic value for neuromuscular diseases and tendinopathy, and it can effectively reflect the abnormal soft tissues of the body. However, there have been few studies on its application in KOA.<sup>59-61</sup> The results of our study revealed differences in muscular echo intensity between healthy older adults and patients with KOA, as well as the limbs of patients with unilateral KOA, providing a quantitative reference for evaluating muscle quality. At the same time, we have also discovered that the echo intensity of VM and VL is related to the severity of KOA. The VM and VL muscles in patients with knee osteoarthritis are prone to abnormalities. Studies have reported that the VM and VL muscles are the most common sites for active or latent myofascial trigger points in elderly patients with knee osteoarthritis.<sup>62</sup> Moreover, muscle abnormalities are correlated with pain symptoms and disability in patients with KOA.<sup>63</sup> In our study, differences in echo intensity suggested the presence of muscle lesions in patients with knee osteoarthritis (KOA), which may be attributed to local soft tissue abnormalities, including infiltration of fat into muscle fibers, fibrosis, and abnormality in muscle fascia resulting in abnormal ultrasound images of muscles. This suggests that clinicians should pay more attention to the quality of quadriceps femoris, particularly in the VM and VL, when treating KOA.

Shear wave elastography (SWE) is frequently utilized in clinical practice to assess the stiffness of local soft tissues. Under ultrasound shear imaging, the probe captures the velocity of shear wave propagation in the tissue and converts it to the elastic modulus value to quantify the exact value of local tissue stiffness.<sup>36</sup> Xu et al used ultrasonic shear modulus to measure the stiffness of the quadriceps femoris during passive knee extension (0°-120°). The results showed that the shear modulus of the rectus femoris increased more rapidly than that of VM and VL, indicating a more significant change



in stiffness for the rectus femoris during knee extension.<sup>22</sup> Additionally, a robust linear relationship exists between muscle strength and shear modulus, making the shear modulus a sensitive indicator for assessing changes in muscle contraction and evaluating alterations in muscle strength. Kawai et al discovered that patients who had undergone knee surgery and experienced quadriceps femoris dysfunction exhibited lower muscle stiffness during contraction than healthy individuals by evaluating shear modulus.<sup>64</sup> Similarly, the shear modulus can capture changes in gastrocnemius muscle stiffness during contraction. Muscle stiffness in response to shear modulus was positively correlated with the level of muscle resistance. Furthermore, the knee and hip angle also affect the shear modulus of the quadricep.<sup>65,66</sup> These studies indicate that the shear modulus has become a commonly used application in muscle assessment as a non-invasive method of examining muscles. In our study, the shear modulus of the muscle on the affected side showed a downward trend compared to the dominant side of healthy elderly individuals and the healthy side of KOA patients, indicating a decrease in muscle elasticity and stiffness in KOA patients as measured by SWE. Although we could not determine the causal relationship between KOA and decline in muscle quality, it was at least partially confirmed that there was an influence between the two.

Maintaining muscle's normal physiological function is the most concerning and ultimate problem in clinical practice, including contraction and relaxation activities, limb movements, and maintenance of daily needs. An accurate assessment of muscle biomechanical parameters can evaluate muscle quality and provide references for disease prevention, clinical treatment, and rehabilitation. Myoton PRO (manufactured by Myoton Ltd, Estonia) is a convenient, hand-held meter for assessing muscle biomechanical properties that have previously demonstrated reliability in measuring quadriceps tone (frequency of natural oscillation) and stiffness.<sup>28,67</sup> Myoton PRO focuses on assessing the mechanical properties of muscles. Previous studies using Myoton PRO have investigated the symmetry of tone, stiffness, and elasticity in the lower limbs of healthy older men and found no statistically significant difference in muscle mechanical properties between their dominant and non-dominant muscles.<sup>26</sup> However, age and gender impact muscle properties.<sup>34</sup> Muscle tone and stiffness increase with age, while men exhibit higher levels of tone and stiffness than women.<sup>34,68</sup> The imbalance caused by pathological changes has also been observed in Myoton PRO in different diseases. In the case of Achilles tendinopathy, the stiffness of symptomatic tendons was significantly lower than that of asymptomatic ones.<sup>69</sup> Athletes with previous hamstring injuries exhibit higher muscle tone and stiffness levels than those without injuries.<sup>70</sup> Similarly, patients with chronic ankle instability are characterized by increased tone and stiffness in the peroneus longus and tibialis anterior muscles.<sup>71</sup> In unilateral limb disease, the biomechanical balance of muscles may also be affected. Patients with unilateral pathology often exhibit asymmetrical muscle tone and stiffness. Wu Z et al found that elderly patients with chronic low back pain on one side had higher tone and stiffness in the paravertebral muscles of the affected side.<sup>25</sup> Chang TT et al used Myoton PRO to find that patients with knee osteoarthritis exhibited higher stiffness in the vastus lateralis muscle at 60° and 90° knee flexion compared to healthy asymptomatic subjects.<sup>27</sup> This is consistent with our results, although the knee joint angle differed among patients during testing, indicating an increase in vastus lateralis muscle stiffness at various angles in KOA patients when measured with Myoton PRO.

In conclusion, KOA is a common disease in elderly adults. Previous studies have mainly focused on pathological changes, such as synovial and cartilage alterations. With the deepening of understanding, more attention should be paid to the changes in muscle quality. Echo intensity, shear modulus, and Myoton PRO are all potential and convenient tools for evaluating muscle properties. In addition to measuring muscle morphology, echo intensity, and shear modulus also expand the scope of ultrasound assessment of muscles by quantifying muscle composition and stiffness, thereby providing more information about muscles. Our study used ultrasound to assess the muscle echo intensity and shear modulus and employed Myoton PRO to evaluate the tone and stiffness in patients with knee osteoarthritis (KOA) on both affected and unaffected sides. Additionally, we assessed these parameters in age-matched healthy older individuals on their dominant sides. We also examined the correlation between these muscle parameters and clinical severity scores (VAS, WOMAC, K-L grade) among KOA patients.

The differences in ultrasound and muscle properties of the superficial quadriceps muscle between elderly patients with KOA and healthy elderly individuals were preliminarily investigated. Furthermore, a weak to moderate correlation was found between muscle echo intensity and K-L grade, as well as between tone and pain score in knee osteoarthritis. This indicates that future prevention and treatment strategies for knee osteoarthritis in clinical settings should take into account

differences in muscle parameters. The correlation between muscle parameters and clinical severity observed in this study should be viewed with caution, and further studies with larger sample sizes are needed. However, we believe that our study provides a reference for clinicians to use the objective tools and methods to evaluate muscle quality around the knee in patients with KOA. In the future, it is expected that the muscle differences in patients with KOA will attract more attention from researchers. Additionally, we hope for wider utilization of scientific and objective assessment tools and methods in the clinical evaluation of muscle quality to assist clinicians in developing appropriate intervention strategies.

## Limitations

Our article also has limitations. Firstly, the assessment of muscle differences only selects a limited number of aspects and does not comprehensively evaluate the quality of thigh muscles in KOA patients from multiple dimensions, such as strength and function. However, the preliminary results have shown differences, which can provide a reference for future research. Second, the extensor muscles of the knee joint were selected as the focus of observation, while the flexor muscles were not included. Since flexors are crucial in maintaining knee function, future studies should also analyze and evaluate the differences in the flexor muscle group around the knee. Thirdly, we investigated the differences in muscle parameters at relax status. Various angles and changes in contraction force may impact muscle parameters. More factors should be considered and controlled to minimize the influences on muscle evaluation. Finally, our study only observed the difference in muscle among older individuals of the same age. In future studies, a young group should be included to compare the impact of age on muscle further.

## Conclusion

The parameters of the quadriceps femoris muscle exhibit asymmetry between the affected and unaffected sides in patients with unilateral KOA and a difference between the dominant side of healthy older individuals and the affected side of KOA. The echo intensity and muscle tone are relevant variables for the clinical grade and score of KOA, thus deserving consideration in prevention and rehabilitation strategies.

## Data Sharing Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

## Ethics Statement

This study adhered to the principles outlined in the Declaration of Helsinki and was reviewed by the ethics committee of Guangdong Provincial Second Hospital of Traditional Chinese Medicine. All participants provided written informed consent before being included.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Barnett R. Osteoarthritis. *Lancet*. 2018;391(10134):1985. doi:10.1016/s0140-6736(18)31064-x
2. Goff AJ, Elkins MR. Knee osteoarthritis. *J Physiother*. 2021;67(4):240–241. doi:10.1016/j.jphys.2021.08.009
3. Lee SY, Chang HS, Song JB, Jee H, Lee S-C, Jeong HS. Proprioceptive Training and Outcomes of Patients With Knee Osteoarthritis: a Meta-Analysis of Randomized Controlled Trials. *J Athl Train*. 2019;54(4):418–428. doi:10.4085/1062-6050-329-17
4. Yao Q, Wu X, Tao C, et al. Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct Target Ther*. 2023;8(1):56. doi:10.1111/sms.12143
5. Battistelli M, Favero M, Burini D, et al. Morphological and ultrastructural analysis of normal, injured and osteoarthritic human knee menisci. *Eur J Histochem*. 2019;63(1):2998. doi:10.4081/ejh.2019.2998
6. Belluzzi E, Macchi V, Fontanella CG, et al. Infrapatellar Fat Pad Gene Expression and Protein Production in Patients with and without Osteoarthritis. *Int J Mol Sci*. 2020;21(17):6016. doi:10.3390/ijms21176016
7. Murphy LB, Cisternas MG, Pasta DJ, Helmick CG, Yelin EH. Medical Expenditures and Earnings Losses Among US Adults With Arthritis in 2013. *Arthritis Care Res*. 2018;70(6):869–876. doi:10.1002/acr.23425
8. Katz JN, Arant KR, Loeser RF. Diagnosis and Treatment of Hip and Knee Osteoarthritis: a Review. *JAMA*. 2021;325(6):568–578. doi:10.1001/jama.2020.22171
9. Sánchez Romero EA, Meléndez Oliva E, Alonso Pérez JL, et al. Relationship between the Gut Microbiome and Osteoarthritis Pain: review of the Literature. *Nutrients*. 2021;13(3):716. doi:10.3390/nu13030716
10. Vina ER, Kwok CK. Epidemiology of osteoarthritis: literature update. *Curr Opin Rheumatol*. 2018;30(2):160–167. doi:10.1097/BOR.0000000000000479
11. Takagi S, Omori G, Koga H, et al. Quadriceps muscle weakness is related to increased risk of radiographic knee OA but not its progression in both women and men: the Matsudai Knee Osteoarthritis Survey. *Knee Surg Sports Traumatol Arthrosc*. 2018;26(9):2607–2614. doi:10.1007/s00167-017-4551-5
12. Georgiev T, Angelov AK. Modifiable risk factors in knee osteoarthritis: treatment implications. *Rheumatol Int*. 2019;39(7):1145–1157. doi:10.1007/s00296-019-04290-z
13. Tayfur B, Charuphongsa C, Morrissey D, Miller SC. Neuromuscular joint function in knee osteoarthritis: a systematic review and meta-analysis. *Ann Phys Rehabil Med*. 2023;66(2):101662. doi:10.1016/j.rehab.2022.101662
14. Muñoz-Fernández AC, Barragán-Carballar C, Villafañe JH, et al. A new ultrasound-guided percutaneous electrolysis and exercise treatment in patellar tendinopathy: three case reports. *Front Biosci*. 2021;26(11):1166–1175. doi:10.52586/5017
15. Hoang P, Saboisky JP, Gandevia SC, Herbert RD. Passive mechanical properties of gastrocnemius in people with multiple sclerosis. *Clin Biomech*. 2009;24(3):291–298. doi:10.1016/j.clinbiomech.2008.12.008
16. Sobolewski EJ, Wein LD, Crow JM, Carpenter KM. Intra-rater and inter-rater reliability of the process of obtaining cross-sectional area and echo intensity measurements of muscles from ultrasound images. *J Ultrason*. 2021;21(84):e7–e11. doi:10.15557/JoU.2021.0002
17. Giuliani HK, Laffan MR, Trivisonno AJ, et al. Measuring muscle quality: associations between echo intensity and normalized strength and power. *Physiol Meas*. 2021;42(3):03NT01. doi:10.1088/1361-6579/abea24
18. Stock MS, Thompson BJ. Echo intensity as an indicator of skeletal muscle quality: applications, methodology, and future directions. *Eur J Appl Physiol*. 2021;121(2):369–380. doi:10.1007/s00421-020-04556-6
19. Akazawa N, Kishi M, Hino T, Tsuji R, Tamura K, Moriyama H. Increased intramuscular adipose tissue of the quadriceps is more strongly related to declines in ADL than is loss of muscle mass in older inpatients. *Clin Nutr*. 2021;40(3):1381–1387. doi:10.1016/j.clnu.2020.08.029
20. Fukumoto Y, Yamada Y, Ikezoe T, et al. Association of physical activity with age-related changes in muscle echo intensity in older adults: a 4-year longitudinal study. *J Appl Physiol*. 2018;125(5):1468–1474. doi:10.1152/jappphysiol.00317.2018
21. Otsuka S, Shan X, Kawakami Y. Dependence of muscle and deep fascia stiffness on the contraction levels of the quadriceps: an in vivo supersonic shear-imaging study. *J Electromyogr Kinesiol*. 2019;45:33–40. doi:10.1016/j.jelekin.2019.02.003
22. Xu J, Hug F, Fu SN. Stiffness of individual quadriceps muscle assessed using ultrasound shear wave elastography during passive stretching. *J Sport Health Sci*. 2018;7(2):245–249. doi:10.1016/j.jshs.2016.07.001
23. Wang L, Xiang X, Zhu B-H, Qiu L. Determination of reference ranges for normal upper trapezius elasticity during different shoulder abduction using shear wave elastography: a preliminary study. *Sci Rep*. 2020;10(1):17104. doi:10.1038/s41598-020-74307-2
24. Mendes B, Firmino T, Oliveira R, et al. Hamstring stiffness pattern during contraction in healthy individuals: analysis by ultrasound-based shear wave elastography. *Eur J Appl Physiol*. 2018;118(11):2403–2415. doi:10.1007/s00421-018-3967-z
25. Wu Z, Ye X, Ye Z, et al. Asymmetric Biomechanical Properties of the Paravertebral Muscle in Elderly Patients With Unilateral Chronic Low Back Pain: a Preliminary Study. *Front Bioeng Biotechnol*. 2022;10. doi:10.3389/fbioe.2022.814099
26. Aird L, Samuel D, Stokes M. Quadriceps muscle tone, elasticity and stiffness in older males: reliability and symmetry using the MyotonPRO. *Arch Gerontol Geriatr*. 2012;55(2):e31–e39. doi:10.1016/j.archger.2012.03.005
27. Chang -T-T, Zhu Y-C, Li Z, et al. Modulation in the Stiffness of Specific Muscles of the Quadriceps in Patients With Knee Osteoarthritis and Their Relationship With Functional Ability. Original Research. *Front Bioeng Biotechnol*. 2022;9. doi:10.3389/fbioe.2021.781672
28. Chen G, Wu J, Chen G, et al. Reliability of a portable device for quantifying tone and stiffness of quadriceps femoris and patellar tendon at different knee flexion angles. *PLoS One*. 2019;14(7):e0220521. doi:10.1371/journal.pone.0220521

29. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis Rheum.* 1986;29(8):1039–1049. doi:10.1002/art.1780290816
30. Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. *Ann Rheum Dis.* 1957;16(4):494. doi:10.1136/ard.16.4.494
31. Lenskjold A, Kongsgaard M, Larsen JO, et al. The influence of physical activity during youth on structural and functional properties of the Achilles tendon. *Scand J Med Sci Sports.* 2015;25(1):25–31. doi:10.1111/sms.12143
32. Chen W, Li C, Wang Y, et al. Comparison of the asymmetries in muscle mass, biomechanical property and muscle activation asymmetry of quadriceps femoris between patients with unilateral and bilateral knee osteoarthritis. *Orig Res Front Physio.* 2023;14,1126116. doi:10.3389/fphys.2023.1126116
33. Chopp-Hurley JN, Wiebenga EG, Bulbrook BD, Keir PJ, Maly MR. Evaluating the relationship between quadriceps muscle quality captured using ultrasound with clinical severity in women with knee osteoarthritis. *Clin Biomech.* 2020;80. doi:10.1016/j.clinbiomech.2020.105165
34. Agyapong-Badu S, Warner M, Samuel D, Stokes M. Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device. *Arch Gerontol Geriatr.* 2016;62:59–67. doi:10.1016/j.archger.2015.09.011
35. Ackerman I. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). *Aust J Physiother.* 2009;55(3):213. doi:10.1016/s0004-9514(09)70088-1
36. Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An K-N. Validation of shear wave elastography in skeletal muscle. *J Biomech.* 2013;46(14):2381–2387. doi:10.1016/j.jbiomech.2013.07.033
37. Roos EM, Herzog W, Block JA, Bennell KL. Muscle weakness, afferent sensory dysfunction and exercise in knee osteoarthritis. *Nat Rev Rheumatol.* 2011;7(1):57–63. doi:10.1038/nrrheum.2010.195
38. Culvenor AG, Ruhdorfer A, Juhl C, Eckstein F, Øiestad BE. Knee Extensor Strength and Risk of Structural, Symptomatic, and Functional Decline in Knee Osteoarthritis: a Systematic Review and Meta-Analysis. *Arthritis Care Res.* 2017;69(5):649–658. doi:10.1002/acr.23005
39. Segal NA, Glass NA. Is Quadriceps Muscle Weakness a Risk Factor for Incident or Progressive Knee Osteoarthritis? *Phys Sportsmed.* 2011;39(4):44–50. doi:10.3810/psm.2011.11.1938
40. Kemnitz J, Wirth W, Eckstein F, Ruhdorfer A, Culvenor AG. Longitudinal change in thigh muscle strength prior to and concurrent with symptomatic and radiographic knee osteoarthritis progression: data from the Osteoarthritis Initiative. *Osteoarthritis Cartilage.* 2017;25(10):1633–1640. doi:10.1016/j.joca.2017.07.003
41. Berger MJ, Kean CO, Goela A, Doherty TJ. Disease severity and knee extensor force in knee osteoarthritis: data from the Osteoarthritis Initiative. *Arthritis Care Res.* 2012;64(5):729–734. doi:10.1002/acr.21608
42. Ruhdorfer A, Wirth W, Eckstein F. Association of knee pain with a reduction in thigh muscle strength – a cross-sectional analysis including 4553 osteoarthritis initiative participants. *Osteoarthritis Cartilage.* 2017;25(5):658–666. doi:10.1016/j.joca.2016.10.026
43. Luc-Harkey BA, Safran-Norton CE, Mandl LA, Katz JN, Losina E. Associations among knee muscle strength, structural damage, and pain and mobility in individuals with osteoarthritis and symptomatic meniscal tear. *BMC Musculoskl Disord.* 2018;19(1):258. doi:10.1186/s12891-018-2182-8
44. Øiestad BE, Juhl CB, Eitzen I, Thorlund JB. Knee extensor muscle weakness is a risk factor for development of knee osteoarthritis. A systematic review and meta-analysis. *Osteoarthritis Cartilage.* 2015;23(2):171–177. doi:10.1016/j.joca.2014.10.008
45. Knoop J, Steultjens MPM, Roorda LD, et al. Improvement in upper leg muscle strength underlies beneficial effects of exercise therapy in knee osteoarthritis: secondary analysis from a randomised controlled trial. *Physiotherapy.* 2015;101(2):171–177. doi:10.1016/j.physio.2014.06.002
46. Takacs J, Krowchuk NM, Garland SJ, Carpenter MG, Hunt MA. Dynamic Balance Training Improves Physical Function in Individuals With Knee Osteoarthritis: a Pilot Randomized Controlled Trial. *Arch Phys Med Rehabil.* 2017;98(8):1586–1593. doi:10.1016/j.apmr.2017.01.029
47. Kus G, Yeldan I. Strengthening the quadriceps femoris muscle versus other knee training programs for the treatment of knee osteoarthritis. *Rheumatol Int.* 2019;39(2):203–218. doi:10.1007/s00296-018-4199-6
48. García-Bernal MI, González-García P, Madeleine P, Casuso-Holgado MJ, Heredia-Rizo AM. Characterization of the Structural and Mechanical Changes of the Biceps Brachii and Gastrocnemius Muscles in the Subacute and Chronic Stage after Stroke. *Int J Environ Res Public Health.* 2023;20(2):1405. doi:10.3390/ijerph20021405
49. Newcom DW, Baas TJ, Lampe JF. Prediction of intramuscular fat percentage in live swine using real-time ultrasound. *J Anim Sci.* 2002;80(12):3046–3052. doi:10.2527/2002.80123046x
50. Tait RG. Ultrasound Use for Body Composition and Carcass Quality Assessment in Cattle and Lambs. *Vet Clin North Am Food Anim Pract.* 2016;32(1):207–218. doi:10.1016/j.cvfa.2015.09.007
51. Young H-J, Jenkins NT, Zhao Q, McCully KK. Measurement of intramuscular fat by muscle echo intensity. *Muscle Nerve.* 2015;52(6):963–971. doi:10.1002/mus.24656
52. Young H-J, Southern WM, McCully KK. Comparisons of ultrasound-estimated intramuscular fat with fitness and health indicators. *Muscle Nerve.* 2016;54(4):743–749. doi:10.1002/mus.25105
53. Scott D, Trbojevic T, Skinner E, et al. Associations of calf inter- and intra-muscular adipose tissue with cardiometabolic health and physical function in community-dwelling older adults. *J Musculoskl Neurol Intera.* 2015;15(4):350–357.
54. Karapınar M, Ayyıldız VA, Unal M, Fırat T. Effect of intramuscular fat in the thigh muscles on muscle architecture and physical performance in the middle-aged women with knee osteoarthritis. *J Orthop Sci.* 2022. doi:10.1016/j.jos.2022.11.011
55. Rech A, Radaelli R, Goltz FR, da Rosa LHT, Schneider CD, Pinto RS. Echo intensity is negatively associated with functional capacity in older women. *Age.* 2014;36(5). doi:10.1007/s11357-014-9708-2
56. Fukumoto Y, Ikezoe T, Yamada Y, et al. Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur J Appl Physiol.* 2012;112(4):1519–1525. doi:10.1007/s00421-011-2099-5
57. Akima H, Yoshiko A, Tomita A, et al. Relationship between quadriceps echo intensity and functional and morphological characteristics in older men and women. *Arch Gerontol Geriatr.* 2017;70:105–111. doi:10.1016/j.archger.2017.01.014
58. Osawa Y, Arai Y, Oguma Y, et al. Relationships of Muscle Echo Intensity with Walking Ability and Physical Activity in the Very Old Population. *J Aging Phys Act.* 2017;25(2):189–195. doi:10.1123/japa.2015-0203
59. Boon AJ, Wijntjes J, O'Brien TG, Sorenson EJ, Cazares Gonzalez ML, van Alfen N. Diagnostic accuracy of gray scale muscle ultrasound screening for pediatric neuromuscular disease. *Muscle Nerve.* 2021;64(1):50–58. doi:10.1002/mus.27211

60. van Alfen N, Gijssbertse K, de Korte CL. How useful is muscle ultrasound in the diagnostic workup of neuromuscular diseases? *Curr Opin Neurol.* 2018;31(5):568–574. doi:10.1097/WCO.0000000000000589
61. Sánchez Romero EA, Pollet J, Martín Pérez S, et al. Lower Limb Tendinopathy Tissue Changes Assessed through Ultrasound: a Narrative Review. *Medicina.* 2020;56(8):378. doi:10.3390/medicina56080378
62. Sánchez-Romero EA, Pecos-Martín D, Calvo-Lobo C, et al. Clinical features and myofascial pain syndrome in older adults with knee osteoarthritis by sex and age distribution: a cross-sectional study. *Knee.* 2019;26(1):165–173. doi:10.1016/j.knee.2018.09.011
63. Sánchez Romero EA, Fernández Carnero J, Villafañe JH, et al. Prevalence of Myofascial Trigger Points in Patients with Mild to Moderate Painful Knee Osteoarthritis: a Secondary Analysis. *J Clin Med.* 2020;9(8):2561. doi:10.3390/jcm9082561
64. Makoto K, Keigo T, Tomoyuki S, Masaki K. Estimation of quadriceps femoris muscle dysfunction in the early period after surgery of the knee joint using shear-wave elastography. *BMJ Open Sport Exerc Med.* 2018;4(1):e000381. doi:10.1136/bmjsem-2018-000381
65. Lin M, Deng W, Liang H, Yu S, Xu Q, Liu C. Effects of Knee Joint Angle and Contraction Intensity on the Triceps Surae Stiffness. Original Research. *Front Bioeng Biotechnol.* 2022;10. doi:10.3389/fbioe.2022.913423
66. Deng W, Lin M, Yu S, et al. Effects of Hip Joint Angle on Quadriceps Recruitment Pattern During Knee Extension in Healthy Individuals: analysis by Ultrasound-Based Shear-Wave Elastography. *Orig Res Front Physio.* 2022;13:836435. doi:10.3389/fphys.2022.836435
67. Van Deun B, Hobbelen JSM, Cagnie B, Van Eetvelde B, Van Den Noortgate N, Cambier D. Reproducible Measurements of Muscle Characteristics Using the MyotonPRO Device: comparison Between Individuals With and Without Paratonia. *J Geriatr Phys Ther.* 2018;41(4):194–203. doi:10.1519/JPT.0000000000000119
68. Wu Z, Wang Y, Ye Z, et al. Effects of Age and Sex on Properties of Lumbar Erector Spinae in Healthy People: preliminary Results From a Pilot Study. Original Research. *Front Physiol.* 2021;12:718068. doi:10.3389/fphys.2021.718068
69. Gafin Ericson M, Rhodri M, Lisa W, Owen P, Keith M. Objective assessment of stiffness in Achilles tendinopathy: a novel approach using the MyotonPRO. *BMJ Open Sport Exerc Med.* 2018;4(1):e000446. doi:10.1136/bmjsem-2018-000446
70. Javier Núñez F, Carlos Martínez J, Overberg J-A, Torreno N, Suarez-Arrones L. Hamstring muscle architecture and myotonometer measurements in elite professional football players with a prior strained hamstring. *Biol Sport.* 2023;40(1):93–99. doi:10.5114/biolSport.2023.112092
71. Stefaniak W, Marusiak J, Bączkiewicz D. Heightened tone and stiffness with concurrent lowered elasticity of peroneus longus and tibialis anterior muscles in athletes with chronic ankle instability as measured by myotonometry. *J Biomech.* 2022;144:111339. doi:10.1016/j.jbiomech.2022.111339

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