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Original Article

# **Relationship between muscle echo intensity on ultrasound and isokinetic strength of the three superficial quadriceps femoris muscles in healthy young adults**

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**Abstract.** [Purpose] The purpose of this study was to clarify the relationship between muscle echo intensity measured with ultrasound and the isokinetic strength of each of the three superficial quadriceps femoris muscles in healthy young adults. [Participants and Methods] We measured the echo intensity of the three superficial muscles of the quadriceps femoris in 25 healthy adults (10 males and 15 females; mean age, 22.3 years) using ultrasound. Moreover, we obtained the maximum force during isokinetic knee extension at 60°/s using an isokinetic dynamometer. [Results] In males and females, a significant negative correlation between echo intensity and muscle strength was found in the VM (r=−0.65 and r=−0.63, respectively). [Conclusion] In both males and females, only the muscle echo intensity of the vastus medialis was found to have a negative correlation with the maximum force during isokinetic knee extension at 60%. Our data lay the foundation for simplifying and rationally performing the measurement of muscle echo intensity of the quadriceps femoris. And it would therefore be sufficient to only measure the VM to clarify a relationship between EI and maximum isokinetic force in the quadriceps. **Key words:** Echo intensity, Isokinetic strength, Quadriceps femoris muscle

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# **INTRODUCTION**

Muscle echo intensity (EI) is an established method to evaluate muscle quality using diagnostic ultrasound. Muscle EI is quantified using gray-scale ultrasound that entails the analysis of 256 gray levels from 0 to 255 and correlates with the amount of non-contractile tissue in skeletal muscle<sup>[1, 2](#page-3-0))</sup>. This non-invasive and safe method allows to easily assess the proportion of non-contractile intramuscular elements, with an increase in EI being primarily interpreted as increased amounts of fat and connective tissue<sup>1, 3, 4</sup>). A predominantly white appearance suggests that there is a substantial amount of non-contractile tissue in the skeletal muscle, and a black appearance suggests that there is little non-contractile tissue<sup>1</sup>). The validity of ultrasound measurements of muscle fiber mass and non-contractile tissues was proven in studies using magnetic resonance imaging for comparison<sup>[5, 6](#page-3-1)</sup>. Although it has been reported that the EI of the different quadriceps femoris muscles and their isometric peak torque are negatively correlated<sup>7</sup>, a negative correlation between isokinetic strength and the EI has so far only been shown for the rectus femoris  $(RF)^2$  $(RF)^2$ . The relationship between the EI of the different quadriceps muscles and their isokinetic strength is unknown. Previous studies have selected different imaging sites of the quadriceps muscle as a method to target either

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only the  $RF^2$  $RF^2$ ) or using the average EI of the RF and vastus lateralis  $(VL)^8$  $(VL)^8$ ), or the RF, VL, vastus medialis (VM), and vastus intermedius (VI)<sup>[9](#page-3-5))</sup>. However, it should be noted that when all ultrasound settings are held constant, muscles examined at a greater depth may experience greater ultrasound attenuation<sup>[6, 10](#page-3-6)</sup> and subsequently lower muscle EI values (a darker image), which influence the interpretation of the overall quality of the muscle tissue. The EI of the quadriceps femoris muscle was measured with an ultrasound diagnostic device, and the correlation between the muscle EI of the three quadriceps muscles (RF, VL, VM) or the average value of the EI of these muscles and the isokinetic knee extension strength was clarified. Considering these findings, quadriceps muscle EI measured by ultrasound correlates with knee extension strength. However, the quadriceps femoris muscles to be measured have different target muscles based on previous studies. In general, ultrasonic waves are attenuated at a deeper position and the EI is low; however, several previous studies involve the EI of the VI, which is the deep muscle. The significance of this study is that when measuring quadriceps femoris muscle EI, instead of measuring all three superficial quadriceps femoris muscles, if the muscle most strongly associated with knee extensor strength can be clarified, it will be possible to ascertain knee extensor strength only by measuring that muscle, simplifying and rationally performing the procedure.

Therefore, the purpose of this study was to clarify the relationship between muscle EI obtained with ultrasound and the isokinetic strength of each of the three superficial quadriceps femoris muscles individually in healthy young adults.

### **PARTICIPANTS AND METHODS**

This was a cross-sectional study. Twenty-five healthy adults (mean age  $\pm$  SD, 22.3  $\pm$  2.4 years; range, 20–32) who had not been engaged in any regular and systematic training programs in the previous 6 months volunteered for this study. The participants presented themselves following announcements on widely distributed university campus posters. Participants were carefully informed about the design of the study with specific information on the possible risks and any discomfort that could occur related to the procedures. All participants completed and signed an ethical consent form. All study procedures were conducted according to the 1964 Declaration of Helsinki and its later amendments, and the study was approved by the Ethics Committee of Tokyo Metropolitan University Graduate School and Human Health Sciences (No. 20001).

The inclusion criteria were heathy young adult volunteers between the age of 18 and 35. Conversely, the exclusion criteria were if they were unable to walk without assistive devices and reported history of lower limb trauma or surgery, neuromuscular disorder, or acute or chronic diseases that might have impaired their muscle strength and power; any history of metabolic, hormonal, and cardiovascular diseases; and were taking any medication with an influence on hormonal and neuromuscular metabolism. We analyzed 25 of the 26 persons who participated in the measurement, excluding those who met the exclusion criteria.

Transverse ultrasound images of the three superficial quadriceps femoris muscles (RF, VL, and VM) of the right leg were obtained with a B-mode ultrasound imaging device (Aplio i800; Canon Medical Systems Inc., Tokyo, Japan) and a multifrequency linear probe (i18LX5; 18 MHz; width of probe, 64 mm). All ultrasound images were obtained using the following acquisition parameters: gain, 80 dB, and depth, 40 mm. All measurements were performed under the same conditions. Before the measurement, the participants rested in a supine position with their lower limbs relaxed and fully extended for 15 min to allow fluid shifts to stabilize<sup>[11](#page-4-0)</sup>). The probe was positioned perpendicularly to the longitudinal axis of the quadriceps femoris muscles. A water-soluble gel was used to provide acoustic contact, and care was taken not to compress the subcutaneous tissue. The probe was fixed at the position where the femur was the whitest and clearest as depicted on the ultrasound monitor and just below its center. The images of the RF and VL muscles were obtained at the midpoint of a line drawn from the lateral condyle of the femur to the greater trochanter, whereas the VM images were obtained at the distal third of a line<sup>[9\)](#page-3-5)</sup>. One image was obtained for each muscle. Ultrasound images were stored in an ultrasound machine for future analysis. A medical image processing, analysis, and visualization software, version 1.48 (National Institutes of Health, Bethesda, MD, USA), was used to analyze the images on a personal computer. A rectangular region of interest (ROI), including as much muscle as possible but avoiding the bone and surrounding fascia, was determined for the EI calculation for each of the three muscles. The mean EI of each muscle was determined using a standard histogram gray-scale function and expressed as a value between 0 (black) and 255 (white) (RF echo intensity, RFEI; VL echo intensity, VLEI; and VM echo intensity, VMEI). The quadriceps femoris muscle echo intensity (QEI) was calculated as the mean EI of the three individual quadriceps femoris muscles (RFEI + VLEI + VMEI / 3). One investigator performed all the calculations. To assess test-retest reliability, intra-class correlation coefficients (ICC(1.1)) were evaluated in six female participants with a mean age of  $21.5 \pm 0.7$  years. The ICC(1.1) values of EI were 0.89 for the RF, 0.93 for the VL, and 0.85 for the VM. The EI is affected by the distance of the tissue in question from the probe; hence, the distance from the probe surface to the base of the ROI in each muscle was measured.

The maximal isokinetic strength of the knee extensors on the right side was measured using an isokinetic dynamometer (Cybex Norm; CSMi, Computer Sports Medicine Inc., Stoughton, MA, USA). Participants were seated with a hip flexion of  $85^{\circ}$  (0°=anatomic position) and the lateral condyle of the right leg aligned with the axis of rotation of the dynamometer. After two practice trials, isokinetic strength (N) was measured for a once three repetitions, submaximal isokinetic knee extension/ flexion repetitions at a velocity of 60°/s. We then used the maximal value. A maximal value was obtained, and the torque (Nm) was calculated by multiplying the strength (N) with the lever arm (m).

Statistical analysis was performed using SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

All values were reported as mean  $\pm$  standard deviation (SD). The Shapiro-Wilk normality test was applied to verify the distribution of data. If the sample was normally distributed ( $p>0.05$ ), the Pearson product-moment correlation coefficient (r) was used to determine correlations between parametric data. One-way analysis of variance was used to analyze the distance from the probe surface to the base of the ROI in each muscle. Statistical significance was defined as a p-value <0.05. Since males are larger than females, for the maximum force during isokinetic knee extension at 60°/s, all analyses were performed by gender.

# **RESULTS**

The participants were 10 males and 15 females (mean age  $\pm$  SD, 22.3  $\pm$  2.4 years; range, 20–32). Their baseline physical characteristics and the measurement parameters are shown in Table 1. The correlation coefficients between the three individual muscle EIs, the mean quadriceps muscle EI, and the maximum force during isokinetic knee extension 60°/s are shown in Table 2. In males, a significant negative correlation between EI and muscle strength was found for the VM (r=−0.65, p<0.05). Additionally, in females, a significant negative correlation between EI and muscle strength was found for the VM (r=−0.63, p<0.05). In the one-way analysis of variance, there was no statistically significant difference in the distance from the probe surface to the base of the ROI in each muscle between males (F-value  $(2.27)=3.35$ , p=0.18) and females (F-value  $(2,42)=3.21$ , p=0.77)) (Table 3).

**Table 1.** Baseline physical characteristics and quadriceps femoris muscle ultrasound measurements in healthy young adults (n=25)

	Total $(n=25)$	Male $(n=10)$	Female $(n=15)$
Age (years)	$22.3 \pm 2.4$	$23.4 \pm 3.3$	$21.6 \pm 0.8$
Height (cm)	$164.4 \pm 7.5$	$171.0 \pm 4.3$	$160.0 \pm 5.7$
Weight (kg)	$54.0 \pm 6.6$	$59.7 \pm 3.1$	$50.2 \pm 5.6$
Body mass index $(kg/m2)$	$19.9 \pm 1.4$	$20.4 \pm 0.8$	$19.5 \pm 1.7$
Echo intensity (arbitrary unit)			
Rectus femoris	$44.2 \pm 14.3$	$29.9 \pm 9.7$	$53.8 \pm 7.0$
Vastus lateralis	$41.8 \pm 18.7$	$27.7 \pm 8.9$	$51.3 \pm 17.6$
Vastus medialis	$34.9 \pm 15.1$	$25.1 \pm 4.0$	$41.5 \pm 16.2$
Average all three muscles	$40.3 \pm 14.3$	$27.6 \pm 6.5$	$48.8 \pm 11.6$
Maximum torque during isokinetic knee extension at $60\%$ (Nm/kg)	$2.2 \pm 0.4$	$2.6 \pm 0.2$	$2.0 \pm 0.3$

**Table 2.** Correlation coefficient between quadriceps echo intensity and maximum torque during isokinetic knee extension at a velocity of 60°/s



EI: echo intensity; a.u.: arbitrary unit.





ROI: region of interest; RF: rectus femoris; VL: vastus lateralis; VM: vastus medialis; a.u.: arbitrary unit.

### **DISCUSSION**

In the 25 young, healthy adults of this study, the EI of the three superficial muscles of the quadriceps femoris, measured using ultrasound, and the maximum force during isokinetic knee extension  $60^{\circ}/s$ , measured using an isokinetic dynamometer, were moderately negatively correlated only in the VM, both in males and females. There was no significant difference in the position of the ROI base when measuring the muscle EI of each muscle. In this regard, the image obtained with B-mode ultrasound generally has a darker appearance in the deeper parts, showing a low EI value. We conclude that the muscle EI values obtained in this study were not affected by the ROI position. Our results are similar to those of a previous study in elderly people who were independent in daily life, with no exercise habits, although the previous study used the maximum isometric muscle strength<sup>7</sup>. Anatomical and physiological parameters that determine muscle strength are the cross-sectional muscle area, muscle composition (fiber type), and motor units. The only information in this regard that was obtained in our study is that on contractile and non-contractile tissue in the cross-sectional area influencing EI. As described, maximum muscle strength is proportional to the cross-sectional area<sup>12</sup>. In this study, the VM had the lowest EI in both males and females, and we concluded that there is more contractile tissue in the VM than in the other quadriceps muscles. A previous study of the quadriceps femoris in a 37 year-old man showed a physiological cross-sectional area in the RF of 43 cm<sup>2</sup>, VL of 64 cm<sup>2</sup>, and VM of  $67 \text{ cm}^{2}$ ,  $^{13}$ ), and the authors reported that the physiological cross-sectional area of VM is the largest of the three superficial quadriceps femoris muscles. Therefore, in our study, it was considered that the VM, with its large physiological cross-sectional area, had the strongest correlation with the maximum peak torque during isokinetic knee extension at 60°/s. Therefore, it would be sufficient to only measure the VM.

Our study has some limitations. First, it is necessary to note that absolute EI values are not comparable between studies because they vary between different ultrasound devices and setups. This technical issue remains a limiting factor for the comparison of absolute EI values across studies. Second, it is unclear whether our results can be applied to healthy elderly people, frail elderly people, and people with sarcopenia. This is an aspect that we will consider in the future.

In conclusion, we clarified the relationship between the EI of each of the three superficial quadriceps femoris muscles measured with ultrasound and the maximum force during isokinetic knee extension at 60°/s in 25 healthy adults. In both males and females, only the EI of the VM had a negative correlation with the maximum torque during isokinetic knee extension. And it would therefore be sufficient to only measure the VM to clarify a relationship between EI and maximum isokinetic force in the quadriceps. We consider our data as the basis for simplifying and rationally performing the measurement of muscle EI of the quadriceps femoris muscle.

#### *Conflict of interest*

The authors have no conflicts of interest to disclose.

# **Acknowledgment**

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