

Association of Regional Muscle Thickness and Echo Intensity with Muscle Volume, Intramuscular Adipose Tissue, and Strength of the Quadriceps Femoris

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Purpose: This study aimed to investigate the association of muscle thickness (MT) and echo intensity (EI) obtained at different regions along the muscle length with muscle volume (MV), intramuscular adipose tissue (IntraMAT), and muscle strength of the quadriceps femoris (QF).

Patients and Methods: A total of 135 community-dwelling adults (64 men and 71 women) participated in the study. Ultrasound scanning of the rectus femoris (RF) and vastus intermedius (VI) was performed at three locations (from mid- to distal thigh). The MT of the RF and VI and EI of the RF were measured. MRI-derived MV, IntraMAT, and muscle strength of the QF were measured.

Results: The correlation between RF-MT and RF-MV weakened as scanning approached the distal thigh, and the difference between the coefficients for the scanning locations was significant for women. However, the correlation of VI-MT with VI-MV and that of the combined MT of RF and VI with the MV of the whole QF and muscle strength were comparable among the scanning locations for both sexes. The correlation of RF-EI with the IntraMAT of the RF and the whole QF and muscle strength was also comparable among the scanning locations for both sexes.

Conclusion: The results of this study suggest that ultrasound measurements at the distal thigh can predict MV, IntraMAT, and muscle strength of the QF to the same degree as those at the mid-thigh.

Keywords: ultrasound scanning, rectus femoris, thigh, muscle strength, MRI

Introduction

Sarcopenia, characterized by an age-related decline in muscle mass, strength, and physical performance, is a serious health problem in the aging population. Loss of muscle mass with aging is muscle-specific, with greater loss in the quadriceps femoris (QF) than in other muscles.^{1,2} Additionally, the cross-sectional area (CSA) and muscle thickness (MT) of the QF show better correlation with physical functions (such as walking speed) than with the total muscle mass of limbs.^{3,4} Thus, assessing QF mass is essential in older adults for the early detection of muscle-specific sarcopenia and decline in physical performance.

Ultrasound (US) is a widely available, portable, and non-invasive technique that can be used to promptly assess MT in clinical settings or communities. US can also assess echo intensity (EI) as a surrogate measure of muscle quality, including non-contractile parameters such as intramuscular adipose tissues (IntraMAT). The rectus femoris (RF) and underlying vastus intermedius (VI) have been studied using US, and the MT and EI of these muscles are reportedly associated with various

clinical outcomes such as muscle strength,^{5–7} power,⁷ physical^{7–9} and cardiovascular performance,⁶ physical activity,^{8,10} frailty,^{9,11} and nutritional status.¹²

These muscles are generally scanned at the mid-thigh – at a distance 50% between the anterior superior iliac spine and proximal border of the patella – or at the anterior aspect at a distance 50% between the greater trochanter and lateral condyle, as this location is the thickest zone of the RF or QF.¹³ Using the thickest region seems logical for estimating muscle size and contractile force.¹³ However, the mid-thigh is not easy to expose; thus, participants should remove their trousers during scanning. This is a shortcoming when US is used as a screening tool in busy clinical settings or for large-scale medical examinations in communities. Scanning at the distal thigh can overcome this shortcoming as this region can be easily exposed with the participant wearing short pants or wide trousers. The distal QF has occasionally been used to measure MT and EI.¹³ However, it is unclear whether MT or EI at the distal QF can be used to predict muscle volume (MV) and IntraMAT. This study aimed to investigate the association of MT and EI obtained at different regions along the muscle length with the MV, IntraMAT, and muscle strength of the QF.

Materials and Methods

Participants

This study recruited older (>60 years) and younger (20–39 years) adults who lived independently in the community and walked without assistive devices. The older adults were recruited from among those who participated in a physical fitness test conducted in Kyoto, and the young adults were recruited from among the students, graduates, and staff members of Kyoto University.¹⁴ Exclusion criteria were as follows: cognitive impairments, severe neurological or musculoskeletal disorders, and general contraindications for MRI. Between April and September 2019, 92 older adults (42 men and 50 women) with a mean age, height, and body mass of 75.3 ± 6.7 years, 158.5 ± 9.1 cm, and 54.3 ± 9.1 kg, respectively, and 43 young adults (22 men and 21 women) with a mean age, height, and body mass of 26.1 ± 4.7 years, 165.5 ± 6.6 cm, and 57.8 ± 7.7 kg, respectively, participated in this study. Written informed consent was obtained from all participants before examination. The study protocol was approved by the ethics committee of Kyoto University (approval no. R1746).

Data Collection

US images of the QF of the right thigh were obtained using real-time B-mode ultrasonography (LOGIQ e, GE Healthcare UK, Chalfont, Buckinghamshire, UK) with an 8–12 MHz linear-array probe. Participants were placed in a supine and completely relaxed position. Transverse images were obtained using the following equipment settings: frequency of 8 MHz, gain of 58 dB, and time-gain compensation in the neutral position. Three scanning locations were determined along the muscle length: 1) proximal: 50% between the anterior superior iliac spine and the proximal border of the patella; 2) middle: the anterior aspect of 50% between the greater trochanter and lateral condyle; and 3) distal: 75% between the anterior superior iliac spine and proximal border of the patella. The scanning locations are shown in Figure 1. At each location, the probe was placed on the part of the RF with the largest diameter, and the focus depth (one focus point) was adjusted to the center of the RF.¹⁴ On the US image, the subcutaneous fat thickness (SFT) and MT of the RF and VI were measured, and the MT of the QF was calculated by summing the values of the RF and VI. The EI of the RF was evaluated as 256 grey-scale levels with arbitrary units using ImageJ version 1.51 (National Institutes of Health, Bethesda, MD, USA). Details of SFT, MT, and EI measurements have been previously described.⁵

MR images of the right thigh were acquired using a 3.0-T magnetic resonance imaging (MRI) scanner (MAGNETOM Verio; Siemens AG, Germany). Participants were placed in a supine and completely relaxed position, and T1-weighted and two-point Dixon images of the entire thigh were acquired with body matrix and spine coils. T1-weighted images (T1WI) were acquired using the following sequences: TR, 2820 ms; TE, 16 ms; optimized field of view, 320×240 mm; flip angle, 129° ; voxel size, $0.5 \times 0.5 \times 4.0$ mm; and slice thickness, 4 mm. Two-point Dixon images were acquired using the following parameters: TR, 4.33 ms; TE1, 1.31 ms; TE2, 2.54 ms; optimized field of view, 365×286.4 mm; flip angle, 9° ; and slice thickness, 3 mm. Water and fat images were produced from the two-point Dixon sequence based on the water/fat chemical shift difference and, consequently, on their phase difference in signal intensity.

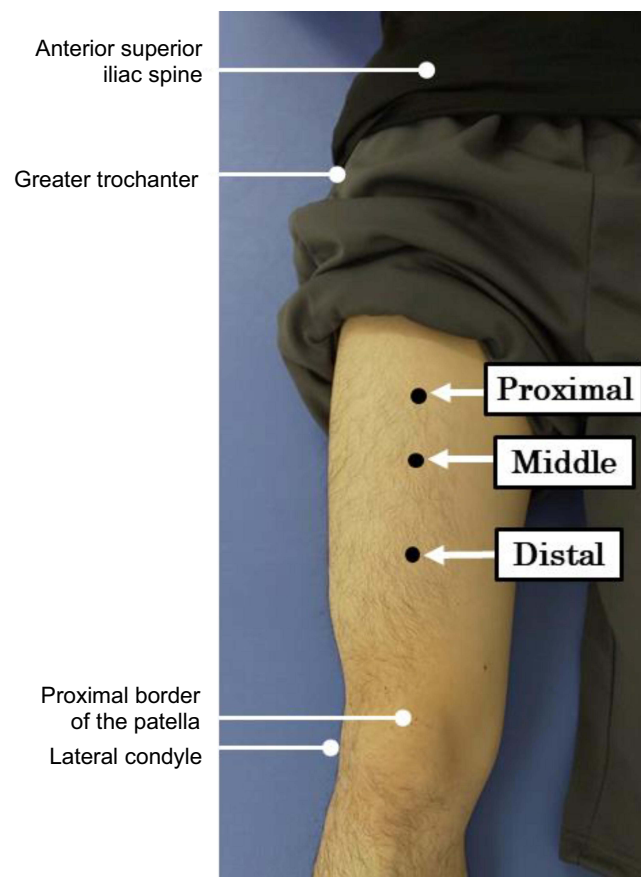


Figure 1 The proximal, middle, and distal locations of US scanning.
Abbreviation: US, ultrasound.

All MR images were analyzed using OsiriX MD (version 134, 11.0; OsiriX, Geneva, Switzerland). MV was estimated on T1WI. Regions of interest in the RF, VI, vastus lateralis (VL), and vastus medialis (VM) were carefully traced on each image slice, and the CSA (cm^2) was calculated. The volume (cm^3) of each slice was calculated by multiplying the CSA by the slice thickness (4 mm), and the entire MV was obtained by summing the volume of each slice throughout the entire muscle length. The volumes of the four muscles were summed to obtain the total QF volume. IntraMAT was estimated using two-point Dixon imaging. Ten consecutive slices of the mid-thigh (with the fifth slice matching the same location as the proximal location on the US scan) were analyzed. Regions of interest in the RF, VI, VL, and VM were carefully traced on each image slice, and IntraMAT (%) was calculated from the signal intensities derived from the water and fat images. The mean IntraMAT of 10 slices was calculated for each muscle. The IntraMAT of the four muscles was averaged to obtain the QF IntraMAT.

Maximal isometric knee extensor strength on the right leg was measured at a 60° knee flexion using an isometric dynamometer (Isoforce GT-330; OG GIKEN Co., Okayama, Japan). Isometric strength was measured twice and presented as torque (Nm), and the maximal value was used for further analysis. The details of knee extensor strength measurement have been described previously.⁵

Information on comorbidities of hypertension, diabetes, and cardiovascular disease, and the number of medications being taken was acquired using a questionnaire. For older adults, handgrip strength (kg), walking speed (m/s), and skeletal muscle mass index (SMI, kg/m^2) were measured. In the walking speed, the time taken to walk 10 m at their comfortable speed was measured and was converted to speed. For calculating SMI, appendicular skeletal muscle mass was estimated using a multi-frequency bioelectrical impedance analysis instrument (Body Composition Analyzer MC-780A-N; TANITA, Tokyo, Japan) and was divided by height squared. Based on the value of handgrip strength, walking speed, and SMI, the presence of sarcopenia was identified according to the criteria of the Asian Working Group for Sarcopenia (AWGS).¹⁵

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 22.0 (SPSS Inc., Tokyo, Japan). Statistical significance was set at $P < 0.05$. We performed statistical analysis separately for each sex, for a combined sample of younger and older adults. This was because an appropriate sample size and a wide range of values in variables was needed to obtain better statistical correlations. We assessed whether the variables were normally distributed based on histogram features, the Kolmogorov–Smirnov test, and Q-Q plots. Since knee extensor strength was positively skewed, log-transformed data were used for statistical analyses. Comparisons of MT and EI among the three measurement locations were made using repeated-measures one-way analyses of variance, followed by Bonferroni's post hoc test. The association of MT and EI at the three measurement locations with MV, IntraMAT, and knee extensor strength was examined using Pearson's correlation coefficient. The difference in the correlation coefficients among the three measurement locations was examined using the method reported by Meng et al¹⁶ (with Bonferroni correction, $p < 0.017$).

Results

Knee extensor strength was not measured in 16 participants (five men and 11 women). Thus, only data from the remaining 119 participants (59 men and 60 women) were analyzed for knee extensor strength. There were no missing data for the US and MRI measurements. For men, mean RF-MV, VI-MV, and QF-MV were 211.5 ± 53.8 , 436.3 ± 99.4 , and $1512.3 \pm 322.7 \text{ cm}^3$, respectively. Mean RF-IntraMAT and QF-IntraMAT were $5.21 \pm 1.50\%$ and $6.76 \pm 1.44\%$, respectively. Mean knee extensor strength was $167.0 \pm 55.3 \text{ Nm}$. For women, mean RF-MV, VI-MV, and QF-MV were 139.6 ± 34.0 , 268.3 ± 68.9 , and $961.6 \pm 227.3 \text{ cm}^3$, respectively. Mean RF-IntraMAT and QF-IntraMAT were $6.32 \pm 2.07\%$ and $7.95 \pm 2.20\%$, respectively. Mean knee extensor strength was $104.5 \pm 29.8 \text{ Nm}$. Characteristics of the participants for each age and sex group are shown in Table 1.

Table 1 Characteristics of the Study Participants

	Men		Women	
	Younger Adults (n = 22)	Older Adults (n = 42)	Younger Adults (n = 21)	Older Adults (n = 50)
Age (years)	27.3± 4.3	76.7± 6.1	24.9± 4.6	74.4± 6.9
Height (cm)	170.4± 4.2	166.1± 5.9	160.4± 4.2	152.3± 5.6
Weight (kg)	63.0± 5.4	60.8± 7.8	52.5± 5.5	48.8± 5.6
BMI (kg/m ²)	21.7± 1.5	22.0± 2.6	20.4± 2.1	21.1± 2.5
Hypertension (n)	0(0.0)	5(11.9)	0(0.0)	10(20.0)
Diabetes (n)	0(0.0)	0(0.0)	0(0.0)	3(6.0)
Cardiovascular disease (n)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Number of medications (n)	0.1± 0.3	2.1± 2.3	0.5± 1.1	1.9± 1.5
Handgrip strength (kg)	–	37.1± 5.9	–	23.8± 3.6
Walking speed (m/s)	–	1.59± 0.23	–	1.55± 0.17
SMI (kg/m ²)	–	7.85± 0.65	–	6.50± 0.59
Sarcopenia (n)	–	1(2.4)	–	1(1.9)
RF-MV (cm ³)	267.5± 35.8	182.1± 35.1	170.1± 28.8	126.8± 27.2
VI-MV (cm ³)	526.6± 95.8	389.0± 61.1	331.4± 63.3	241.8± 51.8
QF-MV (cm ³)	1851.8± 223.1	1334.5± 201.6	1190.6± 184.7	865.4± 166.6

(Continued)

Table 1 (Continued).

	Men		Women	
	Younger Adults (n = 22)	Older Adults (n = 42)	Younger Adults (n = 21)	Older Adults (n = 50)
RF-IntraMAT (%)	4.08± 0.45	5.80± 1.51	4.52± 0.60	7.08± 2.00
QF-IntraMAT (%)	5.80± 0.67	7.26± 1.47	6.20± 0.62	8.69± 2.21
Knee extensor strength (Nm) ^a	207.3± 53.7	149.3± 45.9	121.8± 30.6	99.1± 26.8

Notes: ^an = 18 and 41 for younger and older men, and 15 and 45 for younger and older women for knee extensor strength. Values are expressed as mean ± standard deviations or number (percentage).

Abbreviations: BMI, body mass index; SMI, skeletal muscle mass index; RF, rectus femoris; MV, muscle volume; VI, vastus intermedius; QF, quadriceps femoris; IntraMAT, intramuscular adipose tissue.

Differences in SFT, MT, and EI among the three measurement locations are shown in Table 2. There was a significant main effect of measurement location on the SFT for women and the MT and EI for men and women ($p < 0.001$). The SFT for women at the middle and distal locations was significantly less than that at the proximal location ($p < 0.001$) and that at the distal location was significantly less than that at the middle location ($p < 0.05$). For both sexes, RF-MT, VI-MT, and QF-MT at the middle and distal locations were significantly less than those at the proximal location ($p < 0.001$), and MT at the distal location was significantly less than that at the middle location ($p < 0.001$). RF-EI at the middle and distal locations was significantly higher than that at the proximal location ($p < 0.001$). RF-EI at the distal location was significantly higher than that at the middle location ($p < 0.001$).

The correlation coefficients between MT and EI at each measurement location with MV, IntraMAT, and knee extensor strength are shown in Table 3. MT at all measurement locations was significantly positively correlated with MV and knee extensor strength for both sexes ($p < 0.001$). The correlation coefficient between RF-MT and RF-MV at the proximal and middle locations was significantly higher than that at the distal location for women ($p < 0.001$); however, there was no significant difference between those correlation coefficients in the locations for men. For both sexes, there was no significant difference in correlation coefficients between VI-MT and VI-MV and between QF-MT and QF-MV and knee extensor strength among the locations. RF-EI at all measurement locations was significantly positively correlated with RF-IntraMAT and QF-IntraMAT ($p < 0.001$) and significantly negatively correlated with knee extensor strength for both sexes ($p < 0.05$). There were no significant differences in the correlation coefficients among the locations for both sexes.

Discussion

This study revealed that the correlation between QF-MT (combining RF and VI) and QF-MV was comparable among the three measurement locations. Age-related atrophy of the QF is observed across the entire muscle length, except for the most proximal and distal muscle-tendon insertions.^{1,17} Therefore, MT in every region along the muscle length may predict the MV of the entire QF. However, when the correlation between MT and MV was examined separately for the RF, the coefficient gradually weakened as scanning approached the distal thigh, and the difference in the coefficient was significant for women. Thus, RF-MT should be measured at the thickest region to predict RF-MV accurately. For VI, the correlation between MT and MV was comparable among the locations. However, the correlation of VI was weaker than that of QF or RF, except for RF at the distal location for women. VI forms a unique morphology in the transverse plane surrounding the femoral bone. It is speculated that VI-MT – the distance between the superficial fascia of the VI and the upper margin of the femoral bone at the anterior thigh – is less sensitive as an index of VI-MV. In contrast, RF-MT can be measured as the diameter of the ellipse. It is also speculated that combining the MT of some of the QF improves its accuracy as an index of QF-MV, possibly due to averaging the variability of the individual muscle shapes along the length of the QF.

The RF-EI increased toward the distal thigh. This finding supports that of previous reports.^{18,19} SFT influences EI since US waves attenuate during propagation. However, the influence of SFT on the difference in EI may not have been large in this study since the difference in SFT among measurement locations was small. An increase in EI may be

Table 2 SFT, MT, and EI at Each Measurement Location

	Men (n =64)					Women (n =71)				
	Proximal	Middle	Distal	F value	P value	Proximal	Middle	Distal	F value	P value
SFT (cm)	0.49 ± 0.20	0.43 ± 0.18	0.45 ± 0.19	3.9	0.053	1.00 ± 0.42	0.93 ± 0.40 ^a	0.88 ± 0.37 ^{a,b}	420.4	<0.001
RF MT (cm)	2.15 ± 0.41	1.78 ± 0.44 ^a	1.22 ± 0.33 ^{a,c}	926.9	<0.001	1.85 ± 0.34	1.53 ± 0.35 ^a	1.03 ± 0.29 ^{a,c}	707.1	<0.001
VI MT (cm)	1.71 ± 0.49	1.41 ± 0.39 ^a	1.15 ± 0.34 ^{a,c}	284.6	<0.001	1.46 ± 0.46	1.19 ± 0.39 ^a	0.92 ± 0.28 ^{a,c}	309.2	<0.001
QF MT (cm)	3.86 ± 0.79	3.19 ± 0.75 ^a	2.38 ± 0.61 ^{a,c}	1029.8	<0.001	3.30 ± 0.71	2.72 ± 0.68 ^a	1.95 ± 0.53 ^{a,c}	1140.6	<0.001
RF EI (a.u.)	73.4 ± 14.8	77.6 ± 13.0 ^a	83.3 ± 17.4 ^{a,c}	48.8	<0.001	85.0 ± 13.2	90.2 ± 14.4 ^a	96.8 ± 17.7 ^{a,c}	70.2	<0.001

Notes: ^ap <0.001, significant difference compared with the proximal. ^bp <0.05 and ^cp <0.001, significant difference compared with the middle.

Abbreviations: SFT, subcutaneous fat thickness; MT, muscle thickness; EI, echo intensity; RF, rectus femoris; QF, quadriceps femoris; VI, vastus intermedius; a.u., arbitrary unit.

Table 3 Correlation Between MT and EI per Measurement Location with MV, IntraMAT, and Knee Extensor Strength

US Variables	MRI and Muscle Strength Variables	Men (n =64)			Women (n =71)		
		Proximal	Middle	Distal	Proximal	Middle	Distal
RF MT	RF MV	0.806	0.757	0.734	0.659	0.640	0.436 ^b
VI MT	VI MV	0.657	0.673	0.693	0.547	0.559	0.554
QF MT	QF MV	0.788	0.782	0.809	0.606	0.626	0.571
	Knee extensor strength ^a	0.576	0.598	0.570	0.581	0.591	0.561
RF EI	RF IntraMAT	0.557	0.514	0.565	0.579	0.585	0.636
	QF IntraMAT	0.518	0.486	0.488	0.522	0.557	0.598
	Knee extensor strength ^a	- 0.282	- 0.314	- 0.321	- 0.501	- 0.576	- 0.575

Notes: ^an = 59 for men and 60 for women for knee extensor strength. ^bp <0.001, significant difference compared with the proximal and middle. **Abbreviations:** MT, muscle thickness; EI, echo intensity; MV, muscle volume; IntraMAT, intramuscular adipose tissue; US, ultrasound; RF, rectus femoris; VI, vastus intermedius; QF, quadriceps femoris; MRI, magnetic resonance imaging.

associated with the collagen content within a muscle, which tends to increase toward the muscle-tendon junctions.²⁰ Regardless of the difference in EI, the correlations between EI-RF with RF-IntraMAT and QF-IntraMAT were comparable among the measurement locations. Thus, US scanning of the distal thigh is useful for estimating QF-IntraMAT.

Identifying the US measurement location that is most closely related to muscle function is clinically important. In this study, the correlation between QF-MT, RF-EI, and knee extensor strength showed no significant differences among the measurement locations. Notably, these results suggest that QF-MT and RF-EI measured in the distal thigh are useful for predicting the quantity, quality, and strength of the QF.

The QF is reported to be more susceptible to atrophy with aging compared to other muscles. In this study, only one participant each among the older men and women had sarcopenia based on the AWGS criteria. However, when low QF-MV was defined as two standard deviations below the values of the younger participants, 27 (64.3%) and 18 (36.0%) of the older men and women, respectively, were classified as having low QF-MV in this study. Thus, a certain number among community-dwelling older adults may have muscle-specific loss of the QF even if they are not diagnosed with sarcopenia. The muscle-specific loss of the QF is also observed in some diseases, such as stroke. Stroke survivors in the acute phase experience loss of QF-MT in both the paretic and non-paretic limbs at two weeks after admission,²¹ while they do not show loss of SMI at the same period.²² For early detection of muscle-specific loss of the QF in communities or clinical settings, US measurements of the distal thigh are preferable as the distal thigh can be easily exposed and contributes to quick scanning compared to the mid-thigh.

The present study demonstrates the usefulness of scanning the distal QF. However, when a clinician or researcher wants to assess individual muscles of the QF, it is recommended that they scan the thickest region of each muscle. For example, VM should be assessed in individuals with knee osteoarthritis because it is reportedly the most affected of the QF muscles.²³ Additionally, muscle hypertrophy in response to resistance training differs along the muscle length.²⁴ Therefore, when US measurements of the distal thigh are applied as an outcome of resistance training, the results should be interpreted cautiously.

A limitation of this study is that IntraMAT was measured from only 10 slices of MR images at the proximal location. This was because previous studies reported that the distribution of IntraMAT is constant throughout the length of QF¹⁷ and that the IntraMAT in the largest region of the thigh is representative of that of the entire thigh.² However, we cannot exclude the possibility that our results were influenced by the limited IntraMAT measurements.

Conclusions

In conclusion, distal thigh US-derived MT and EI of the QF are useful for predicting MV, IntraMAT, and strength of the QF. The QF is the muscle most susceptible to atrophy with aging^{1,2} and is more closely associated with physical

functions than total muscle measurements.^{3,4} Since the distal thigh can be easily exposed for scanning compared with the mid-thigh, US measurements at the distal thigh could be a quick screening tool for sarcopenia of the QF in busy clinical settings or for large-scale medical examinations in communities.

Abbreviations

AWGS, Asian Working Group for Sarcopenia, CSA, cross-sectional area, EI, echo intensity, IntraMAT, intramuscular adipose tissues, MRI, magnetic resonance imaging, MT, muscle thickness, MV, muscle volume, QF, quadriceps femoris, RF, rectus femoris, SFT, subcutaneous fat thickness, SMI, skeletal muscle mass index, T1WI, T1-weighted images, VI, vastus intermedius, VL, vastus lateralis, VM, vastus medialis, US, ultrasound.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available due to ethical restrictions but are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

Written informed consent was obtained from all participants before examination. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1964 and its later amendments. The study protocol was approved by the ethics committee of Kyoto University (approval no. R1746).

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Disclosure

The authors declare that they have no competing interests in this work.

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