

## Use of B-mode ultrasonography for measuring femoral muscle thickness in dogs

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**ABSTRACT.** Assessment of muscle mass is important for evaluating muscle function and rehabilitation outcomes. Ultrasound has recently been successfully used to estimate muscle mass in humans by measuring muscle thickness. This study attempted to standardize procedures for measuring femoral muscle thickness ultrasonographically, as well as quantify the reliability and validity of ultrasound evaluations of muscle thickness compared to measurements made by magnetic resonance imaging (MRI) in dogs. We evaluated the quadriceps femoris (QF), biceps femoris (BF), semitendinosus (ST) and semimembranosus (SM) muscles of 10 clinically healthy Beagle dogs. Scans were taken in 5 different sections divided equally between the greater trochanter and proximal patella. MRI was performed, followed by T1-weighted and contrast-enhanced T1-weighted imaging. Muscle cross-sectional area (CSA) was measured with MRI, and muscle thickness was measured with MRI and ultrasonography. The thickness of the QF, BF and ST muscles as measured by ultrasound at slices 1–3 (from the proximal end to the middle of the femur), 2–4 (middle of the femur) and 2 (more proximal than the middle of the femur), respectively, was correlated with muscle thickness and CSA as measured by MRI. These sites showed a flat interface between muscle and transducer and were situated over belly muscle. No correlation between measurement types was seen in SM muscle. We must confirm this assessment method for various breeds, sizes, ages and muscle pathologies in dogs, thereby confirming that muscle thickness as measured ultrasonographically can reflect muscle function.

**KEY WORDS:** canine, magnetic resonance imaging, muscular atrophy, quadriceps muscle, rehabilitation

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Central and peripheral neurological disorders, orthopedic disorders and surgical treatments of injuries induce neurogenic or disuse muscular atrophy [1, 13]. Rehabilitation and physical therapy have been developed to maintain and improve muscle mass, function and strength [11, 17].

Assessment of muscle mass is important for evaluating disuse muscular atrophy and rehabilitation outcomes [17]. An ideal assessment instrument for determining muscle mass should be objective, easy to apply, inexpensive and noninvasive. Practical methods of muscle assessment in dogs include the measurement of limb circumference [3] and radiographic examination. However, clinical information regarding the appropriate regions for measurement or use of soft tissue landmarks is scant. Measurements can thus be inaccurate and inconsistent [17] or fail to distinguish muscle from body fat and bone. Quantitative evaluation of individual muscles is also difficult. Computed tomography (CT) [5, 14] and magnetic resonance imaging (MRI) [15, 21] have been used to assess muscle mass in humans. However, the repeated iodination radiation exposure, high costs, limited access to equipment and long scan times associated with CT and MRI often limit the use of these techniques in

humans [23, 25]. In veterinary medicine, much less data are available describing morphological changes to muscle under different conditions using these imaging techniques, with the exception of canine muscular dystrophy [8, 12, 26] and experimental canine models of human pathologies [22]. In addition to the problems reported in humans, anesthesia is needed to perform CT or MRI in dogs.

Ultrasound has recently been successfully applied to measure muscle size [10, 21, 23, 24] and thickness [7, 18, 19, 25, 28] in humans. Changes in muscle size are used as indices of muscle weakening and atrophy or, conversely, of strengthening and hypertrophy in human medicine [7, 10]. Ultrasonography using a real-time brightness mode (B-mode) has the same advantages as CT or MRI in visualizing fat and muscle tissue in humans [9]. Ultrasonography is noninvasive and applicable in the clinical field, and the method allows for repeated measurements without anesthesia. Human studies have revealed that muscle strength correlates with muscle mass [10, 25], muscle cross-sectional area (CSA) [2, 5, 6, 16, 23, 24] and muscle thickness [25]. We hypothesized that the assessment of skeletal muscle thickness under ultrasound would be very useful as an index of muscle atrophy or hypertrophy and strengthening in veterinary medicine. However, ultrasonography has not yet been widely used to assess musculature in veterinary medicine, and whether the measurement of muscle thickness is useful for evaluating muscle mass in dogs remains unclear.

The purpose of the present study was to standardize procedures for measuring the thickness of femoral muscles using ultrasonography in dogs. We quantified the reliability and validity of the evaluation of femoral muscle thickness

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using ultrasound in comparison to that of measurements using MRI. We targeted the quadriceps femoris (QF), biceps femoris (BF), semitendinosus (ST) and semimembranosus (SM) muscles, which are important in hind limb function.

## MATERIALS AND METHODS

**Animals:** The left hind limbs of 10 (male, n=8; female, n=2) clinically healthy Beagle dogs (age, 1–9 years; weight, 8.4–14.2 kg; and body condition scores were 3 out of 5) were assessed. All dogs were free of hematological abnormalities and orthopedic or neurological disease. The Institute of Experimental Animal Sciences of Tokyo University of Agriculture and Technology approved the study protocol (Permit number; 26–69), and all animals were maintained in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

**Study protocol:** Ultrasonography and MRI were conducted on different days, but within 3 days. Images were taken in 5 different sections, dividing the femur equally into 6 parts between the greater trochanter of the femur and the proximal point of the patella. Slice numbers were designated as 1–5 from proximal to distal (Fig. 1). In these slices, QF, BF, ST and SM muscles were evaluated for muscle CSA and thickness by MRI and for muscle thickness by ultrasound.

**Images from MRI:** MRI was performed under anesthesia. All dogs were subcutaneously injected with 40  $\mu\text{g}/\text{kg}$  atropine sulfate (Atropine Sulfate Injection 0.5 mg; Mitsubishi Tanabe Pharma, Osaka, Japan). Anesthesia was induced by intravenous bolus administration of 6 mg/kg propofol (Rapinovet; Intervet, Tokyo, Japan), and a tracheal tube was inserted. Anesthesia was maintained by isoflurane inhalation (Forane; Abbott Japan, Tokyo, Japan) mixed with oxygen during MRI.

Transverse images of the left femoral muscle were obtained by MRI using a knee coil (AIRIS- $\alpha$  comfort 0.3T; HITACHI Medical, Tokyo, Japan). All images were scanned in a supine position in the coil with both knees extended to 135°. We imaged a femur and tibia and made a template in 135° by cardboard. We confirmed the angle of the knee using this template. MRI markers (sunflower oil packaged food; Kobayashi Pharmaceutical, Osaka, Japan) were set on the body surface at the left greater trochanter of the femur and the proximal point of the patella. The left femoral muscle was imaged in the sagittal plane with a T1-weighted image sequence (spin-echo sequence: repetition time (TR), 400 ms; echo time (TE), 25 ms; flip angle, 90°; number of excitations (NEX), 4; slice thickness, 2.0 mm; interslice gap, 6.0 mm; number of slices, 10; field of view (FOV), 200 mm; and matrix, 320  $\times$  224) as a positioning imaging (Fig. 1). Transverse images were taken from 5 different sections dividing the length between the 2 markers into 6 equal parts, with a T1-weighted image sequence (spin-echo sequence: TR, 400 ms; TE, 25 ms; flip angle, 90°; NEX, 4; slice thickness, 4 mm and 5 mm; interslice gap, 15.5–16.5 mm; FOV, 200 mm; and matrix, 320  $\times$  224) and a contrast-enhanced T1-weighted image sequence (spin-echo sequence: TR, 400 ms; TE, 25

ms; flip angle, 90°; NEX, 4; slice thickness, 4 mm; interslice gap, 15.5–16.5 mm; FOV, 200 mm; and matrix, 320  $\times$  224). Gadodiamide was used as the contrast medium with intravenous administration of 0.2 mg/kg meglumine gadopentetate (Magnevist; Bayer, Osaka, Japan) without a pressure injector.

**Ultrasound imaging:** Muscle thickness was determined using a B-mode ultrasound system (ProSound F75 Premier; Hitachi-Aloka Medical, Tokyo, Japan) with a 10-MHz linear transducer (UST-5415; Hitachi-Aloka Medical). Scans were taken in conscious dogs, but sedation with a subcutaneous injection of 0.1 mg/kg butorphanol tartrate (Vetorphale; Meiji Seika Pharma, Tokyo, Japan) was applied if necessary. Each dog was placed in the right lateral recumbent position with the left knee extended to 135°. We confirmed the angle of the knee using the same template for MRI. The hair on the left femoral external side was clipped. The transducer was placed perpendicular to the long axis of the femur or muscle. To avoid muscle compression, a generous amount of gel was used under the transducer. The cross-sectional planes of each muscle were scanned in the same lines as for MRI (Fig. 1). Prior to the study, 2 sonographers measured the muscle thickness of the measuring sites (20 sites) of 1 dog to confirm that muscle thickness could be measured precisely using ultrasound. The intraclass correlation coefficient (ICC (2,1)) was then calculated.

**Measurement and calculation by MRI and ultrasound:** From each transverse image of the left femoral muscle by MRI, an outline of each muscle was traced, and the CSA was calculated. Among the T1-weighted or contrast-enhanced T1-weighted images, we selected images in which it was easy to distinguish the outline of muscles. From each transverse image by MRI and each cross-sectional image by ultrasound, muscle thickness was measured at the maximal vertical thickness in each muscle. QF muscle thickness was measured passing through the center of the rectus femoris muscle. The trace and thickness were digitized using software (OsiriX; Newton Graphics, Sapporo, Japan).

**Statistics:** The results are expressed as mean  $\pm$  standard deviation (SD). The correlation between muscle CSA and muscle thickness as measured by MRI was analyzed using Pearson's product-moment correlation coefficient in each slice. The correlation between muscle thickness as measured by MRI and muscle thickness as measured by ultrasonography was then analyzed in those slices showing a correlation between muscle CSA and muscle thickness as measured by MRI. We compared the differences of both measurement procedures with muscle thickness using the Bland-Altman comparison. Finally, the correlation between muscle CSA as calculated by MRI and muscle thickness as measured by ultrasonography was analyzed in those slices showing a correlation in the second step. All data were statistically analyzed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, U.S.A.). Values of  $P < 0.05$  were considered significant. The intraclass correlation coefficient for muscle thickness as measured using ultrasound was statistically analyzed using IBM SPSS Statistics 20.0 (IBM SPSS Statistics Server).

Table 1. Muscle cross-sectional areas and thickness as measured by MRI and ultrasonography

		Slice 1	Slice 2	Slice 3	Slice 4	Slice 5
QF	CSA (cm <sup>2</sup> )	10.5 ± 2.0	11.9 ± 2.1	10.8 ± 1.8	7.5 ± 1.8	3.6 ± 1.2
	MT <sub>MR</sub> (mm)	29.2 ± 3.3	27.2 ± 4.1	23.2 ± 4.3	16.7 ± 3.6	11.0 ± 2.2
	MT <sub>US</sub> (mm)	34.0 ± 3.5	31.3 ± 3.3	28.0 ± 2.7	19.9 ± 4.1	12.4 ± 2.0
BF	CSA (cm <sup>2</sup> )	5.4 ± 1.2	6.4 ± 1.3	7.4 ± 1.5	7.9 ± 1.5	5.8 ± 1.7
	MT <sub>MR</sub> (mm)	14.3 ± 2.0	15.8 ± 2.5	17.8 ± 3.5	18.2 ± 2.9	12.6 ± 3.4
	MT <sub>US</sub> (mm)	12.6 ± 2.0	14.6 ± 2.2	17.6 ± 3.1	16.6 ± 2.5	13.0 ± 1.4
ST	CSA (cm <sup>2</sup> )	3.7 ± 1.2	4.7 ± 0.9	4.5 ± 0.7	4.5 ± 0.9	3.8 ± 1.0
	MT <sub>MR</sub> (mm)	15.5 ± 2.0	15.5 ± 1.1	16.1 ± 1.7	17.2 ± 2.1	16.5 ± 3.6
	MT <sub>US</sub> (mm)	14.2 ± 1.6	14.4 ± 2.0	14.0 ± 2.5	15.0 ± 1.8	14.5 ± 2.3
SM	CSA (cm <sup>2</sup> )	1.8 ± 0.5	6.0 ± 0.8	6.5 ± 0.9	4.9 ± 1.1	3.2 ± 1.0
	MT <sub>MR</sub> (mm)	11.3 ± 1.2	22.0 ± 2.7	24.3 ± 2.8	21.8 ± 4.1	16.9 ± 2.1
	MT <sub>US</sub> (mm)	15.0 ± 1.9	19.1 ± 2.4	21.2 ± 2.1	19.7 ± 1.4	17.7 ± 1.8

Data are expressed as mean ± SD. CSA, muscle cross-sectional area; MT<sub>MR</sub>, muscle thickness as measured by MRI; MT<sub>US</sub>, muscle thickness as measured by ultrasonography; QF, quadriceps femoris muscle; BF, biceps femoris muscle; ST, semitendinosus muscle; SM, semimembranosus muscle.

## RESULTS

The intraclass correlation coefficient (ICC (2.1)) was 0.948 based on the measurements of 2 sonographers for the muscle thickness of 20 sites of 1 dog.

Values of muscle CSA as measured by MRI, as well as muscle thickness as measured by MRI and ultrasound, are shown in Table 1. T1-weighted images with a slice thickness of 4 mm were most suitable for measurement, because the muscle outlines were more clearly depicted (Fig. 2). However, identification of the vastus medialis, vastus intermedius and vastus lateralis muscles was difficult in the QF muscle group. In the SM muscle, identification of muscle outlines was also difficult, because little connective tissue was present among the ST and adductor muscles in slices 2–4. Muscle contrast was increased in the T1-weighted images with a slice thickness of 5 mm. In the contrast-enhanced T1-weighted images, the muscle signal became higher, and the outlines of the muscle became clearer. Therefore, when we could not identify the outlines of the SM or ST muscles, we used T1-weighted images with a slice thickness of 5 mm or contrast-enhanced T1-weighted images (Fig. 3). The mean amount of time required for MRI was 29.3 min (range, 22–47 min).

During ultrasonography, 3 dogs that could not maintain a right lateral recumbent position required sedation. Ultrasonographic images of the QF muscle in slices 1–5 are shown in Fig. 4. Muscle fibers appeared hypoechoic, and perimysia and fascia appeared hyperechoic. The QF and BF muscles were easy to scan in all slices, because they were closest to the skin. The ST and SM muscles in the proximal slices of the femur were difficult to scan, because the interface between the body and transducer was not flat. The SM muscle in the distal slices was also difficult to scan, because the muscle finished medial to the tibia and the echo was attenuated. The SM muscle was imaged caudal to the BF muscle. The ST muscle was imaged caudal to the SM in slices 1 and 2 and between the BF and SM muscles at a deep site in slices 3–5.

The mean amount of time required for ultrasonography was 16 min (range, 11–30 min).

Correlation coefficients between muscle CSA and muscle thickness as measured by MRI are shown in Table 2. Correlations were obtained in slices 1–4 in the QF and BF muscles, slices 1–5 in the ST muscle and slices 1, 3 and 5 in the SM muscle. Correlation coefficients between muscle thickness as measured by MRI and ultrasound in the slices for which a correlation was obtained between muscle CSA and muscle thickness as measured by MRI are shown in Table 3. Correlations were obtained in slices 1–3 in the QF muscle, slices 2–4 in the BF muscle and slices 2 and 3 in the ST muscle. No correlation was seen in the SM muscle. Bland-Altman plots presenting differences between the 2 measurement procedures and muscle thickness for these slices are given in Fig. 5. These comparisons indicated that the thickness of the QF muscle measured by ultrasound was overestimated compared with that measured by MRI, as well as that the thicknesses of the BF and ST muscles measured by ultrasound were underestimated compared with those measured by MRI. However, except for 1 measurement in slice 3 of the QF muscle and 1 measurement in slice 4 of the BF muscle, the values remained within the 95% confidence intervals. Correlation coefficients between muscle CSA as measured by MRI and muscle thickness as measured by ultrasonography are shown in Table 4. Correlations were obtained in slices 1–3 in the QF muscle, slices 2–4 in the BF muscle and slice 2 in the ST muscle.

## DISCUSSION

This study was performed to validate the use of ultrasonography as a clinical tool for measuring muscle thickness in dogs. Muscle thickness as measured by ultrasound was found to be accurate and correlated well with measurements from MRI. Furthermore, the thicknesses of the QF, BF and ST muscles as measured by ultrasonography at slices 1–3 (from the proximal end to the middle of the femur), 2–4 (middle of



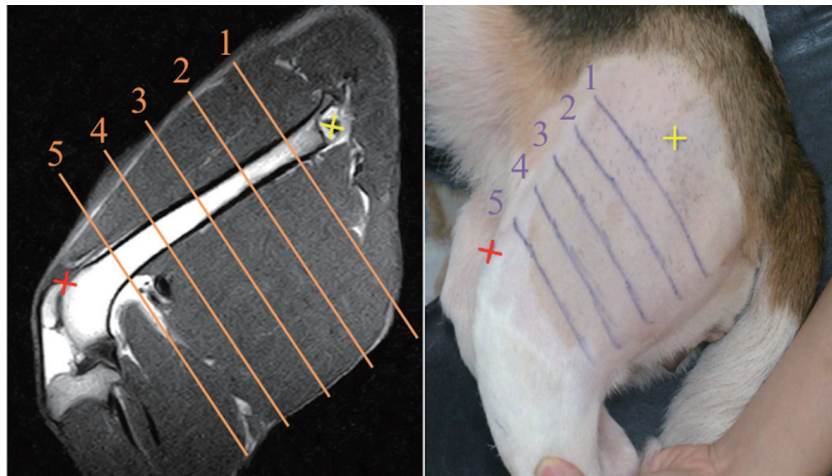


Fig. 1. Slice number and scan location for measurement of muscle cross-sectional area and thickness by MRI (left image) and ultrasonography (right picture). Scans were taken from 5 different sections dividing the limb equally into 6 parts between the greater trochanter of the femur (indicated by a yellow X) and the proximal point of the patella (indicated by a red X). Slice numbers were designated as 1–5 from proximal to distal.

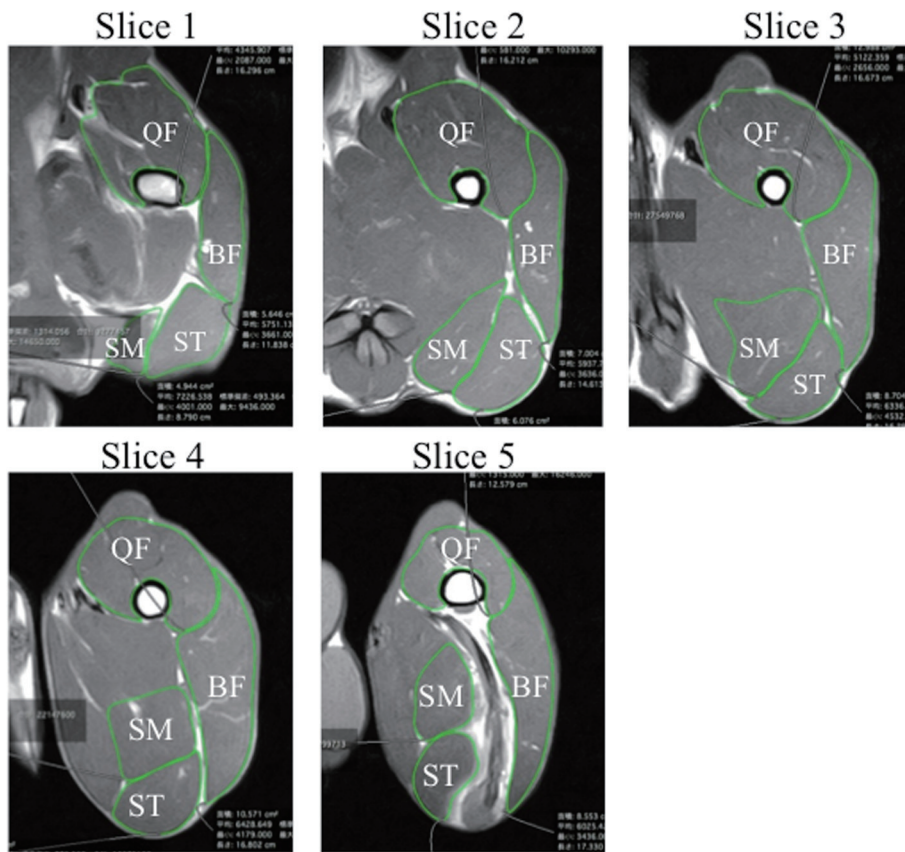


Fig. 2. Transverse images of femoral muscles to measure muscle cross-sectional area by MRI. Green lines show the outlines of each femoral muscle: quadriceps femoris (QF) muscle; biceps femoris (BF) muscle; semitendinosus (ST) muscle; and semimembranosus (SM) muscle. T1-weighted imaging with a slice thickness of 4 mm was most suitable for measurement, because the muscle outlines were clearly depicted.

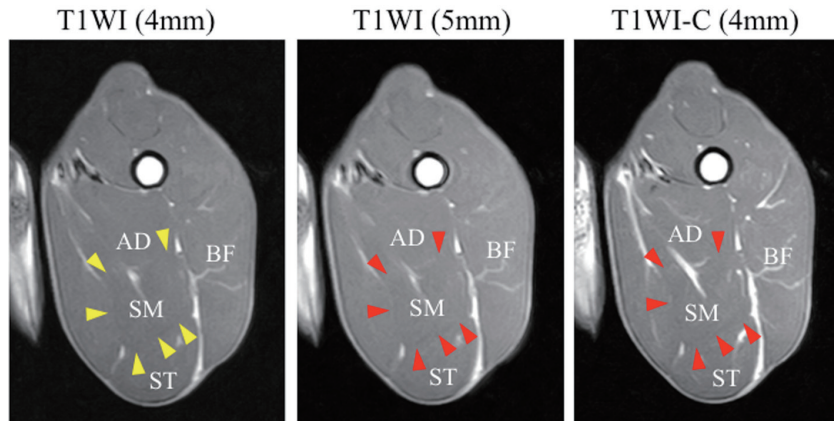


Fig. 3. Transverse images of semimembranosus (SM) muscles in slice 4 by MRI. In the T1-weighted images (T1WI) with a slice thickness of 4 mm, identification of the muscle outline of SM from the semitendinosus (ST) muscle and adductor (AD) muscle was difficult (yellow arrowhead). In the T1-weighted images with a slice thickness of 5 mm, muscle contrast was increased (red arrowhead). In the contrast-enhanced T1-weighted images (T1WI-C), the muscle signal became higher (red arrowhead).

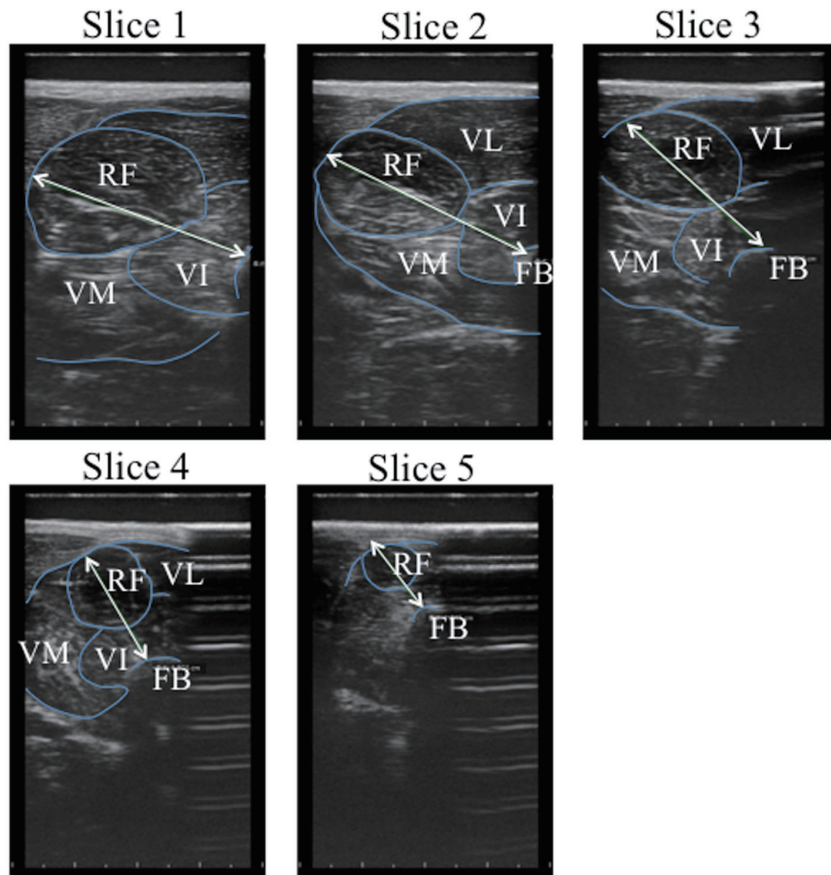


Fig. 4. Ultrasound images of the quadriceps femoris (QF) muscle. The white double-headed arrow shows the muscle thickness. QF muscle thickness was measured passing through the center of the rectus femoris (RF) muscle from the muscle surface to the femoral bone (FB). Horizontal stripes in slices 4 and 5 represent artifacts due to failure to maintain contact between the body and transducer. VL, vastus lateralis muscle; VM, vastus medialis muscle; VI, vastus intermedius muscle.

Table 2. Correlation coefficient between muscle cross-sectional area and diameter as measured by MRI

	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5
QF	0.757 <sup>a)</sup>	0.780 <sup>b)</sup>	0.667 <sup>a)</sup>	0.774 <sup>a)</sup>	0.521
BF	0.763 <sup>a)</sup>	0.690 <sup>a)</sup>	0.776 <sup>b)</sup>	0.884 <sup>c)</sup>	0.405
ST	0.834 <sup>b)</sup>	0.632 <sup>a)</sup>	0.644 <sup>a)</sup>	0.825 <sup>b)</sup>	0.887 <sup>c)</sup>
SM	0.636 <sup>a)</sup>	0.571	0.663 <sup>a)</sup>	0.630	0.860 <sup>b)</sup>

a)  $P < 0.05$ , b)  $P < 0.01$ , c)  $P < 0.001$ . QF, quadriceps femoris muscle; BF, biceps femoris muscle; ST, semitendinosus muscle; SM, semi-membranosus muscle.

Table 3. Correlation coefficient between muscle thickness as measured by MRI and muscle thickness as measured by ultrasonography

	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5
QF	0.701 <sup>a)</sup>	0.857 <sup>b)</sup>	0.751 <sup>a)</sup>	0.486	
BF	0.385	0.934 <sup>c)</sup>	0.970 <sup>c)</sup>	0.638 <sup>a)</sup>	
ST	-0.140	0.790 <sup>b)</sup>	0.653 <sup>a)</sup>	0.277	0.229
SM	0.008		0.543		0.126

a)  $P < 0.05$ , b)  $P < 0.01$ , c)  $P < 0.001$ . QF, quadriceps femoris muscle; BF, biceps femoris muscle; ST, semitendinosus muscle; SM, semi-membranosus muscle.

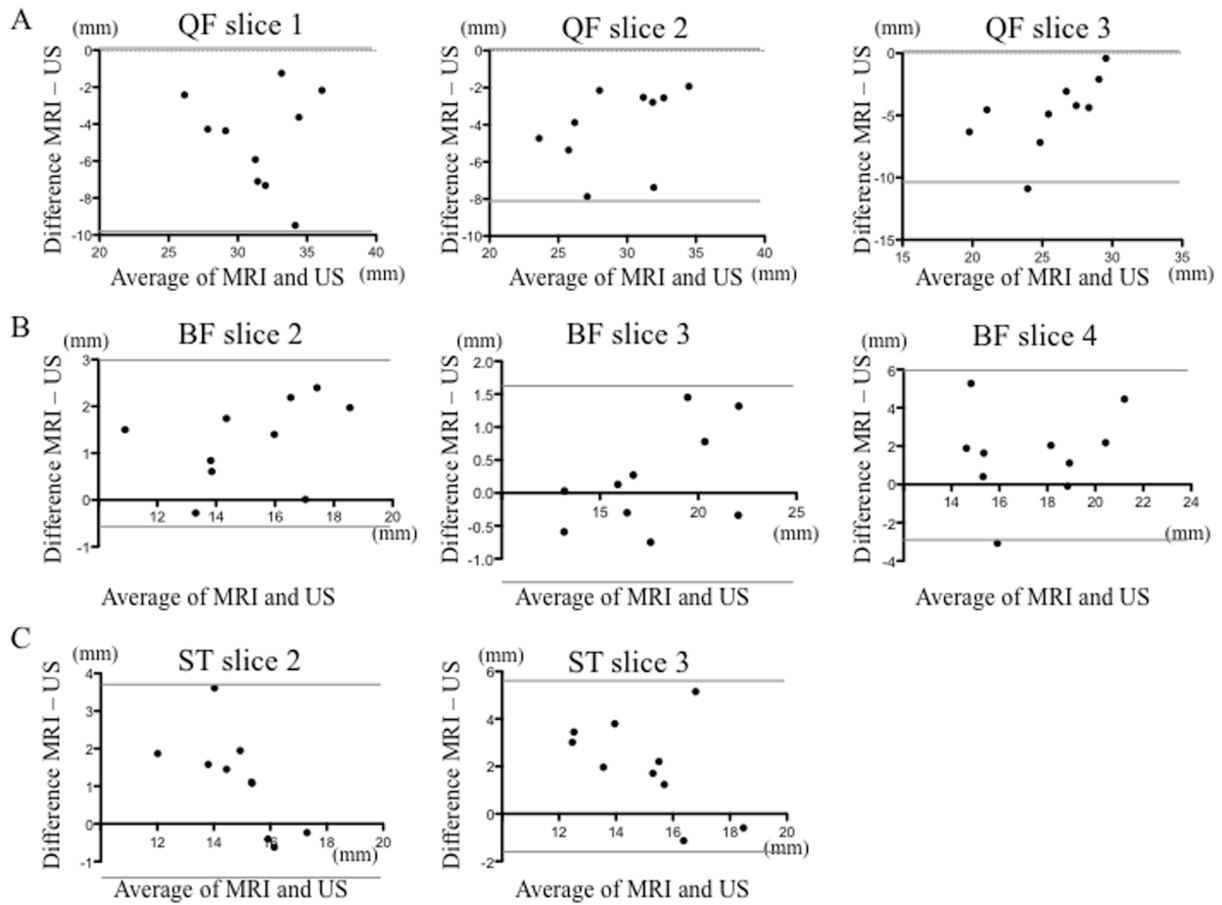


Fig. 5. Bland-Altman comparisons. Differences in muscle thickness measurements between MRI and ultrasonography for: A) quadriceps femoris (QF) at slices 1–3; B) biceps femoris (BF) at slices 2–4; and C) semitendinosus (ST) at slices 2 and 3. Gray lines represent 95% confidence intervals. Solid circles indicate values from 1 dog.

the femur) and 2 (more proximal than the middle of the femur), respectively, correlated with muscle CSA as measured by MRI. These sites were easy to scan, because the interface between the body and transducer was flat, and measurements at these sites were stable. Because these sites represented the belly muscle, where muscle CSA and muscle thickness were large, the changes of these parameters might have been smaller than those of other sites. In humans, the CSA of the

QF muscle has been measured by ultrasonography at a point halfway between the greater trochanter and lateral joint line of the knee [29] and at 30%–50% of the distance from the superior border of the patella to the medial aspect of the anterior superior iliac spine [20]. Measurements of the QF muscle thickness by ultrasonography have also been performed at the halfway point between the epicondylus lateralis and trochanter major of the femur [24, 25], mid-thigh [19],



Table 4. Correlation coefficient between muscle cross-sectional area as measured by MRI and muscle thickness as measured by ultrasonography

	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5
QF	0.691 <sup>a)</sup>	0.878 <sup>c)</sup>	0.737 <sup>a)</sup>		
BF		0.651 <sup>a)</sup>	0.774 <sup>b)</sup>	0.671 <sup>a)</sup>	
ST		0.635 <sup>a)</sup>	0.359		
SM					

a)  $P < 0.05$ , b)  $P < 0.01$ , c)  $P < 0.001$ . QF, quadriceps femoris muscle; BF, biceps femoris muscle; ST, semitendinosus muscle; SM, semimembranosus muscle.

and 45% of the distance between the popliteal crease and the greater trochanter [27] in humans. The present canine study showed that the measurement of muscle thickness for the QF muscle is suitable at 16.7%–50% of the distance from the greater trochanter to the proximal point of the patella, but the results also revealed that sites near the belly muscle are most suitable for ultrasonographic measurements.

Conversely, muscles in which CSA and muscle shape were irregular showed no correlation between muscle CSA and thickness according to MRI, or between muscle thickness as measured by MRI and ultrasonography. Moreover, the proximal and distal sites of the femur correspond approximately to the muscle-tendon junctions, which could have influenced measurements. In humans, muscle CSA in the lower limbs varies with changes in the distribution of tissue fluid in the supine and standing positions [4]. Positional differences between MRI and ultrasonography could thus be related to differences in the measured values. According to the Bland-Altman plots, the QF muscle diameter at slices 1–3 was overestimated by ultrasonography to a greater extent than by MRI. We scanned the QF muscle at these slices from the lateral side of the femur. When we scanned immediately above the rectus femoris muscle, the measurement was complicated by the deep location of the femur. The angle between the direction of the ultrasonic scan and the measurement line was increased, causing measurement error. In addition, ultrasonography underestimated ST muscle diameter at slices 2 and 3 compared with MRI. The scan site at these slices was located at a transition from the lateral side to the caudal femur. The interface between the body and transducer was not flat at this point, and the muscle might therefore have been compressed. We did not find that general anesthesia or sedation influenced muscle thickness.

A compound scanning technique is used when the entire muscle cross-section cannot be encompassed [21, 24]. However, this technique is relatively complicated. Weiss criticized the assessment of muscle CSA by compound scanning, because distortions of the muscle tissue are inevitable [27]. If muscle function can be estimated from muscle thickness as measured by ultrasonography in dogs, this technique would thus be useful in the clinical field.

The amount of time required for the assessment of muscle thickness by ultrasonography was relatively long when measuring all 20 sites examined in the present study. However, we have identified measuring sites where evaluation by ul-

trasonography is convenient. Therefore, the thicknesses of the sites and muscles indicated by the present study can be measured relatively quickly.

It is important to confirm the precision and reliability of muscle thickness measurements using ultrasound. We thus assessed the interobserver variability for measuring femoral muscle thickness using ultrasonography. The intraclass correlation coefficient (ICC (2.1)) was 0.948, indicating high reliability.

This study revealed that ultrasonography offers a useful tool for measuring muscle thickness in the femoral region, particularly that of the QF, BF and ST muscles. We determined that we could perform accurate measurements even in thin muscles. However, we must confirm the applicability of this assessment method to dogs of various breeds, sizes, ages and muscle pathologies. Further studies are also needed to determine whether ultrasonography can be applied to assess muscle function by measuring muscle thickness.

## REFERENCES

- Appell, H. J. 1990. Muscular atrophy following immobilisation. A review. *Sports Med.* **10**: 42–58. [Medline] [CrossRef]
- Arangio, G. A., Chen, C., Kalady, M. and Reed, J. F. 3rd. 1997. Thigh muscle size and strength after anterior cruciate ligament reconstruction and rehabilitation. *J. Orthop. Sports Phys. Ther.* **26**: 238–243. [Medline] [CrossRef]
- Baker, S. G., Roush, J. K., Unis, M. D. and Wodiske, T. 2010. Comparison of four commercial devices to measure limb circumference in dogs. *Vet. Comp. Orthop. Traumatol.* **23**: 406–410. [Medline] [CrossRef]
- Berg, H. E., Tedner, B. and Tesch, P. A. 1993. Changes in lower limb muscle cross-sectional area and tissue fluid volume after transition from standing to supine. *Acta Physiol. Scand.* **148**: 379–385. [Medline] [CrossRef]
- Bernard, S., LeBlanc, P., Whittom, F., Carrier, G., Jobin, J., Belleau, R. and Maltais, F. 1998. Peripheral muscle weakness in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **158**: 629–634. [Medline] [CrossRef]
- Calmels, P., Berthouze, S., Barral, F. G., Domenach, M. and Minaire, P. 1992. A comparative study of the muscle strength and mass of the arm flexors and extensors in paraplegic and in non paraplegic basketball players. *Paraplegia* **30**: 509–516. [Medline] [CrossRef]
- Dupont, A. C., Sauerbrei, E. E., Fenton, P. V., Shragge, P. C., Loeb, G. E. and Richmond, F. J. 2001. Real-time sonography to estimate muscle thickness: comparison with MRI and CT. *J. Clin. Ultrasound* **29**: 230–236. [Medline] [CrossRef]
- Fan, Z., Wang, J., Ahn, M., Shiloh-Malawsky, Y., Chahin, N., Elmore, S., Bagnell, C. R. Jr., Wilber, K., An, H., Lin, W., Zhu, H., Styner, M. and Kornegay, J. N. 2014. Characteristics of magnetic resonance imaging biomarkers in a natural history study of golden retriever muscular dystrophy. *Neuromuscul. Disord.* **24**: 178–191. [Medline] [CrossRef]
- Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., Kimura, M. and Ichihashi, N. 2012. Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur. J. Appl. Physiol.* **112**: 1519–1525. [Medline] [CrossRef]
- Fukunaga, T., Miyatani, M., Tachi, M., Kouzaki, M., Kawakami, Y. and Kanehisa, H. 2001. Muscle volume is a major determinant

- of joint torque in humans. *Acta Physiol. Scand.* **172**: 249–255. [Medline] [CrossRef]
11. Henderson, A. L., Latimer, C. and Millis, D. L. 2015. Rehabilitation and physical therapy for selected orthopedic conditions in veterinary patients. *Vet. Clin. North Am. Small Anim. Pract.* **45**: 91–121. [Medline] [CrossRef]
  12. Kobayashi, M., Nakamura, A., Hasegawa, D., Fujita, M., Orima, H. and Takeda, S. 2009. Evaluation of dystrophic dog pathology by fat-suppressed T2-weighted imaging. *Muscle Nerve* **40**: 815–826. [Medline] [CrossRef]
  13. Lieber, R. L., Fridén, J. O., Hargens, A. R., Danzig, L. A. and Gershuni, D. H. 1988. Differential response of the dog quadriceps muscle to external skeletal fixation of the knee. *Muscle Nerve* **11**: 193–201. [Medline] [CrossRef]
  14. Marquis, K., Debigaré, R., Lacasse, Y., LeBlanc, P., Jobin, J., Carrier, G. and Maltais, F. 2002. Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **166**: 809–813. [Medline] [CrossRef]
  15. Mathur, S., Takai, K. P., Macintyre, D. L. and Reid, D. 2008. Estimation of thigh muscle mass with magnetic resonance imaging in older adults and people with chronic obstructive pulmonary disease. *Phys. Ther.* **88**: 219–230. [Medline] [CrossRef]
  16. Maughan, R. J., Watson, J. S. and Weir, J. 1983. Strength and cross-sectional area of human skeletal muscle. *J. Physiol.* **338**: 37–49. [Medline] [CrossRef]
  17. Millis, D. L. and Ciuperca, I. A. 2015. Evidence for canine rehabilitation and physical therapy. *Vet. Clin. North Am. Small Anim. Pract.* **45**: 1–27. [Medline] [CrossRef]
  18. Miyatani, M., Kanehisa, H. and Fukunaga, T. 2000. Validity of bioelectrical impedance and ultrasonographic methods for estimating the muscle volume of the upper arm. *Eur. J. Appl. Physiol.* **82**: 391–396. [Medline] [CrossRef]
  19. Miyatani, M., Kanehisa, H., Kuno, S., Nishijima, T. and Fukunaga, T. 2002. Validity of ultrasonograph muscle thickness measurements for estimating muscle volume of knee extensors in humans. *Eur. J. Appl. Physiol.* **86**: 203–208. [Medline] [CrossRef]
  20. Noorkoiv, M., Nosaka, K. and Blazevich, A. J. 2010. Assessment of quadriceps muscle cross-sectional area by ultrasound extended-field-of-view imaging. *Eur. J. Appl. Physiol.* **109**: 631–639. [Medline] [CrossRef]
  21. Reeves, N. D., Maganaris, C. N. and Narici, M. V. 2004. Ultrasonographic assessment of human skeletal muscle size. *Eur. J. Appl. Physiol.* **91**: 116–118. [Medline] [CrossRef]
  22. Safran, O., Derwin, K. A., Powell, K. and Iannotti, J. P. 2005. Changes in rotator cuff muscle volume, fat content, and passive mechanics after chronic detachment in a canine model. *J. Bone Joint Surg. Am.* **87**: 2662–2670. [Medline] [CrossRef]
  23. Seymour, J. M., Ward, K., Sidhu, P. S., Puthuchery, Z., Steier, J., Jolley, C. J., Rafferty, G., Polkey, M. I. and Moxham, J. 2009. Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. *Thorax* **64**: 418–423. [Medline] [CrossRef]
  24. Sipilä, S. and Suominen, H. 1991. Ultrasound imaging of the quadriceps muscle in elderly athletes and untrained men. *Muscle Nerve* **14**: 527–533. [Medline] [CrossRef]
  25. Thomaes, T., Thomis, M., Onkelinx, S., Coudyzer, W., Cornelissen, V. and Vanhees, L. 2012. Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients. *BMC Med. Imaging* **12**: 7–12. [Medline] [CrossRef]
  26. Wang, J., Fan, Z., Vandeborne, K., Walter, G., Shiloh-Malawsky, Y., An, H., Kornegay, J. N. and Styner, M. A. 2013. A computerized MRI biomarker quantification scheme for a canine model of Duchenne muscular dystrophy. *Int. J. CARS* **8**: 763–774. [Medline] [CrossRef]
  27. Weiss, L. W. 1984. The Use of 8-mode Ultrasound for Measuring the Thickness of Skeletal Muscle at Two Upper Leg Sites. *J. Orthop. Sports Phys. Ther.* **6**: 163–167. [CrossRef]
  28. Yi, T. I., Han, I. S., Kim, J. S., Jin, J. R. and Han, J. S. 2012. Reliability of the supraspinatus muscle thickness measurement by ultrasonography. *Ann. Rehabil. Med.* **36**: 488–495. [Medline] [CrossRef]
  29. Young, A., Hughes, I., Russell, P., Parkers, M. J. and Nichols, P. J. 1980. Measurement of quadriceps muscle wasting by ultrasonography. *Rheumatol. Rehabil.* **19**: 141–148. [Medline] [CrossRef]