Received: 10 February 2021 | Revised: 28 May 2021 | Accepted: 18 June 2021
DOI: 10.1111/joa.13505

ORIGINAL PAPER

Journal of Anatomy ANATOMICAL SOCIETY WILEY

Investigation of the association between human fascia lata thickness and its neighboring tissues' morphology and function using B-mode ultrasonography

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Funding information
JSPS KAKENHI, Grant/Award Number: 16H01870 and 20K23300; Yamaha Motor Foundation for Sports 2019; TOBE MAKI Scholarship foundation 2020

Abstract
The fascia lata is a membrane tissue which envelopes all thigh muscles and connects with the subcutaneous adipose tissues through loose connective tissues. It is presumable that the morphology of the fascia lata is strongly affected by the unique properties of underlying thigh muscles and subcutaneous adipose tissues. We aimed to investigate the relationships between characteristics of the fascia lata and adjoining adipose tissues and underlying muscles. Twenty healthy people were recruited (25 ± 3 years, 167.1 ± 8.5 cm, 62.5 ± 13.2 kg). The thickness of the skeletal muscles (rectus femoris, vastus lateralis, biceps femoris, and semitendinosus), and their overlying fascia lata and subcutaneous adipose tissues were measured by B-mode ultrasonography. Isometric knee extension and flexion torque during maximal voluntary contraction were also tested. The fascia lata thickness demonstrated site-dependent differences (vastus lateralis: 0.91 ± 0.20 mm > rectus femoris, biceps femoris, and semitendinosus: 0.56–0.69 mm, $p < 0.01$). Furthermore, there were large individual variations in the fascia lata thickness even in the same region of the thigh. The fascia lata showed positive simple correlations with height (rectus femoris: $r = 0.39$ $p = 0.01$, semitendinosus: $r = 0.37$ $p < 0.05$), body mass (rectus femoris: $r = 0.59$, $p < 0.01$, vastus lateralis: $r = 0.47$, $p < 0.01$, semitendinosus: $r = 0.55$, $p < 0.01$), corresponding muscle thickness (rectus femoris: $r = 0.39$, $p < 0.05$, semitendinosus: $r = 0.74$, $p < 0.01$) and knee extension (rectus femoris: $r = 0.52$, $p < 0.01$, vastus lateralis: $r = 0.40$, $p < 0.01$) and flexion (semitendinosus: $r = 0.41$, $p < 0.01$) torques. After adjusting for the influence of height and/or body mass, the fascia lata thickness showed a partial correlation only with the skeletal muscle thickness at the semitendinosus ($r = 0.61$, $p < 0.01$). The present study revealed that the fascia lata has site-specific differences of the thickness, which positively correlates with the underlying muscle thickness and corresponding joint torque. Furthermore, the fascia lata over the semitendinosus is associated with the underlying muscle characteristics independent of the physical constitution. It is assumed that the

fascia lata has the plasticity and changes its thickness, which likely corresponds to the morphology of the neighboring tissues and underlying muscle function.

KEYWORDS
deep fascia thickness, isometric muscle contraction, muscle thickness, site-dependence

1 | INTRODUCTION

In the human body, the thigh segment is comprised of large muscles such as the quadriceps femoris and hamstrings, and the architectural properties of these muscles including the pennation angles, fascicle lengths, and volumes are different from each other (Cutts & Seedhom, 1993; Ema et al., 2016; Maruyama et al., 1991). The subcutaneous adipose tissues and muscles at thigh region also change their shape and volume inhomogeneously depending on age and exercise habits, suggesting the remarkable plasticity of these tissues (Abe et al., 2011; Ema et al., 2013). The deep fascia is a membrane tissue which envelopes all muscles of the human body and connects with the subcutaneous adipose tissues through loose connective tissues (Benjamin et al., 2009; Kumka & Bonar, 2012; Stecco et al., 2008). In particular, the skeletal muscles tightly connect with the deep fascia through epimysium (Stecco et al., 2008). These structures allow the forces produced by muscle contractions to be transmitted to the neighboring tissues which is known as the epimuscular myofascial force transmission (Maas & Sandercock, 2010; Yucesoy et al., 2008). In addition, animal experiment has demonstrated that this force transmission is also observed between agonist and antagonist muscles (Huijing et al., 2007). Considering these connections, it is presumable that the morphology of the fascia lata can be strongly affected by the unique properties of its underlying thigh muscles and overlying subcutaneous adipose tissues.

Indeed, Marshall (2001) noted that the thickness and fibers' orientation of the fascial tissues mirror the forces generated by muscular action. Several cadaveric studies additionally proved that the thickness, fiber directions, and stiffness of the fascia lata showed site-specificity even in the same segment of the human body (Otsuka et al., 2018; Stecco, 2014). Nevertheless, few studies investigated the morphological and mechanical properties of the deep fascia *in vivo*. A recent study showed that the thicknesses of the deep fascia at lumber, abdominal regions, and lower limb were correlated to the body composition and joint flexibility (Wilke et al., 2018). It is assumed that the morphological properties of the deep fascia reflect the morphologies and functions of the neighboring tissues (e.g., subcutaneous adipose tissues and muscles) due to their tight connection in between and myofascial force transmission. However, the individual differences of the characteristics of the fascia lata and its association with the neighboring tissues' properties have not been examined.

Hence, the purpose of this study was to investigate the relationships between characteristics of the fascia lata and adjoining subcutaneous adipose tissues and underlying muscles. We hypothesized that the thickness of the deep fascia would reflect its neighboring muscle size and adipose thickness and associate with muscle strength.

2 | MATERIALS AND METHODS

2.1 | Participants

Twenty healthy people were recruited (10 males: age; 25 ± 2 years, height; 173.6 ± 4.7 cm, body mass; 73.0 ± 10.6 kg, %fat; 19.6 ± 5.9%, 10 females: age; 26 ± 3 years, height; 160.5 ± 6.0 cm, body mass; 52.0 ± 3.4 kg, %fat; 23.9 ± 6.3%, means±SDs). Participants who had the cardiovascular, metabolic, and immunologic disorders, as well as orthopedic abnormality were excluded from this study. The present study was approved by the local ethical committee, and each participant gave their written informed consent.

2.2 | Ultrasound measurements

The thigh length was measured in advance as the distance from the midpoint of greater trochanter to the articular cleft between the femur and tibia condyles in the anatomical neutral standing position. The site of 50% of the thigh length was located using a steel measure and marked on the skin using a non-toxic pen. Participants lay relaxed in supine or prone position with the hip and knee fully extended and ankle in neutral position during measurement. Images of the anterior, lateral and posterior

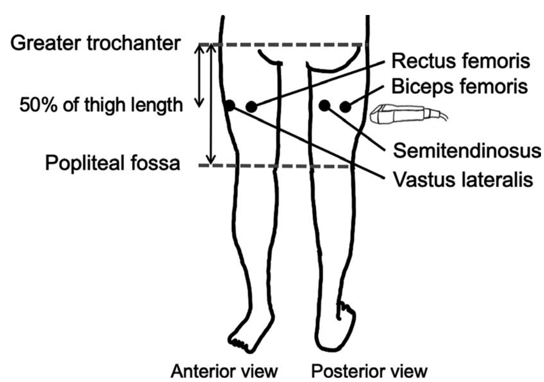

FIGURE 1 Schematic representation of ultrasound probe locations. Ultrasound probe was transversely located on the center of muscle belly of rectus femoris, vastus lateralis, biceps femoris, and semitendinosus at 50% of the thigh length

thigh were taken using a brightness-mode (B-mode) ultrasound device (ARIETTA Prologue) with a 18–5 MHz linear array ultrasound transducer. The transducer was placed transversely at the center of the muscle width. To ensure minimum stress between transducer and skin, the transducer was placed on the skin surface with the ultrasound gel. The ultrasound images of the rectus femoris (RF), vastus lateralis (VL), biceps femoris (BF), semitendinosus (ST) bellies and their neighboring fascia lata were taken according to the marker at 50% of the thigh length (Figure 1). Ultrasound measurement was conducted for both right and left sides of the thigh. A single experienced examiner operated the ultrasound device.

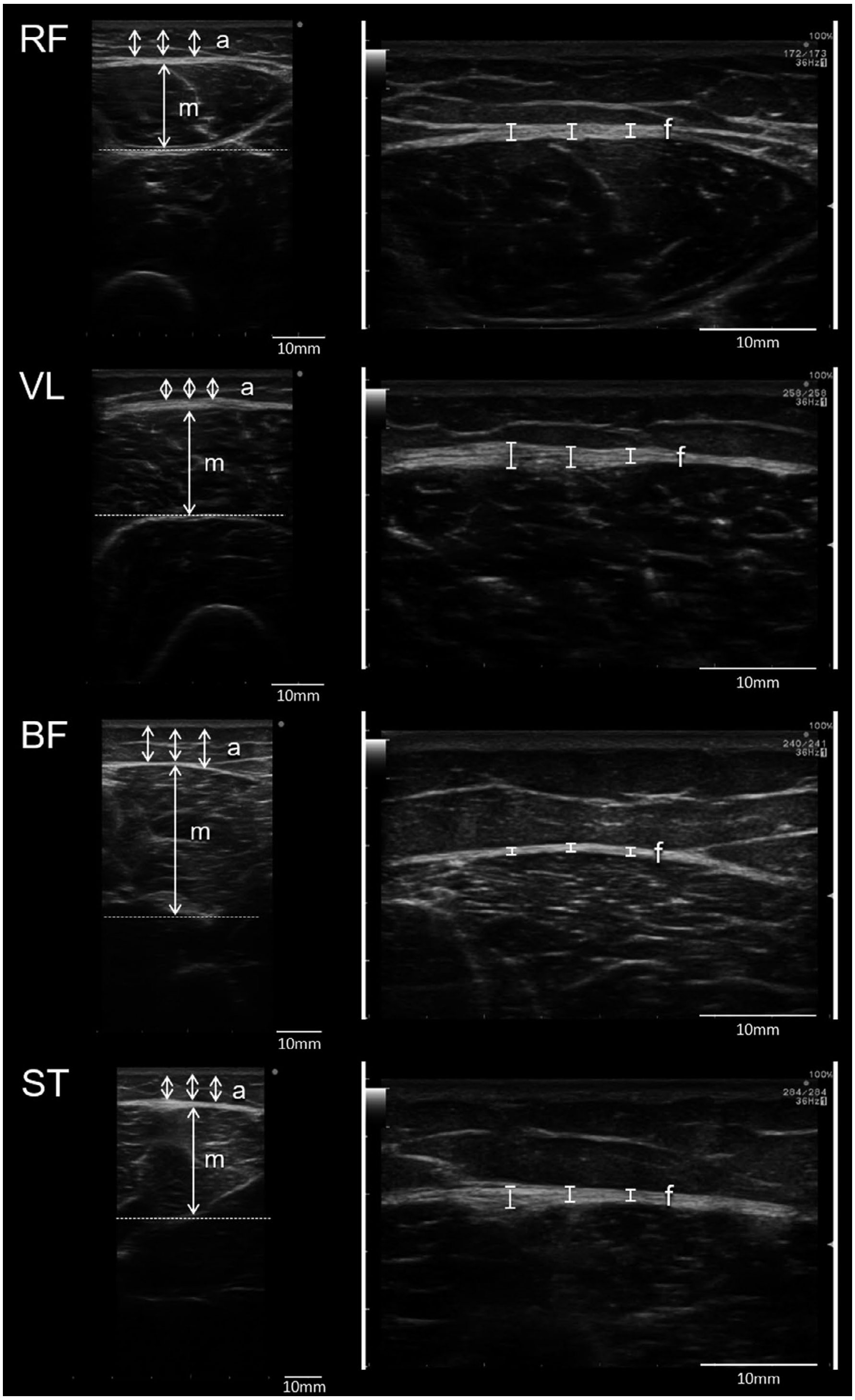

FIGURE 2 The typical images of the B-mode ultrasonography. The arrows show the measurement parts of the subcutaneous adipose tissue, fascia lata, and skeletal muscle thickness at each site. a, f, and m mean the subcutaneous adipose tissue, fascia lata, and skeletal muscle respectively

The thickness of the tissues was calculated using a software (Image J 1.52k, National Institutes of Health) in a personal computer. In this study, the length of the tissue between the deepest part of the skin and top of the deep fascia was determined as the subcutaneous adipose tissue thickness (Figure 2). The fascia lata thickness was determined as the length in between adipose tissues and skeletal muscles (Figure 2). The largest distance between the lower part of the deep fascia and the upper part of the bone or the aponeurosis of the deeper muscle was determined as the muscle thickness (Figure 2). To eliminate the influence of the possible variation of the thickness, three different points per image were analyzed for the deep fascia and adipose tissues (Wilke et al., 2018). The deep fascia thickness was analyzed twice to confirm the intra-observer agreement, and the average was used as a representative value.

2.3 | Strength measurement

The maximal voluntary isometric contraction (MVC) of the knee extension and flexion was performed with an isokinetic dynamometer (CON-TREX, CMV AG). The participants seated on the device with the hip joint at 80° (anatomical position = 0°). The knee angle was kept at 70° and the ankle angle was at neutral position. The trunk of the participant was fixed to the backrest of the dynamometer to avoid any upper body movement. The pad on the actuator arm of the dynamometer was securely fastened to the participant's leg just above the lateral malleolus. All measurements were performed on both right and left legs. After 3–5 times sub-maximal contractions as a warm-up, each subject performed voluntary isometric contraction for 3 s with maximal effort. To avoid any fatigue effect, at least 1 min of rest was taken between trials. More than two trials were performed if peak torques were substantially different (>10%). The torque data were recorded through an analogue-digital converter (Power Lab, AD Instruments) and transferred to a personal computer at 1000 Hz sampling frequency with a 10 Hz low-pass filter. The highest value out of those measurements was adopted as a representative value for each trial.

2.4 | Statistics

Results were presented as means and SDs unless otherwise stated. All analyses were performed with a statistical software (IBM SPSS Statistics 23, IBM). For all analyses, the values of right and left sides were combined. To examine the reliability of the deep fascia thickness analysis, an intraclass correlation coefficient (ICC) of two different measurements was calculated. A one-way analysis of variance (ANOVA) with repeated measures was used to examine differences of the thickness of the fascia lata among measurement sites. The ANOVA was followed by post-hoc tests with a Bonferroni correction. Simple correlations between the thickness of the deep fascia at each site and corresponding parameters (age, height, body mass, %fat, and isometric torque) were tested by the Pearson product-moment correlation. Likewise, simple correlations between the thickness of the deep fascia and those of subcutaneous adipose tissue and skeletal muscle for each site and total sample were tested by the Pearson product-moment correlation. Where a significant correlation was found between physical constitution and the fascia lata thickness, partial correlations were also performed to examine the association between the fascia lata thickness and corresponding muscle thickness and strength by adjusting for the influence of body mass (in the case of VL) or both height and body mass (in the case of RF and ST) on those associations. The significance level was set at $p < 0.05$.

3 | RESULTS

The mean values and SDs of the age, height, body mass, and %fat were shown in Table 1. The ICC of the fascia lata thickness at each site (RF: 0.97, VL: 0.95, ST: 0.96, BF: 0.93) showed that the thickness was "almost perfectly" kept constant between the two analyses (Landis & Koch, 1977). There was a significant main effect of the fascia lata thickness between sites. The thickness of the fascia lata over the VL (0.91 ± 0.20 mm; range: 0.46–1.41 mm) was thicker compared with those of other sites (RF: 0.56 ± 0.12 mm; range: 0.35–0.94 mm, BF: 0.62 ± 0.10 mm; range: 0.38–0.88 mm, ST: 0.69 ± 0.14 mm;

TABLE 1 Means and SDs of the age, height, body mass, %fat, and knee joint torques of the subjects and simple correlations between fascia lata thickness and corresponding parameters

			RF		VL		BF		ST	
Subject Characteristics	Mean	SD	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
Age (years)	25.4	2.6	−0.25	0.13	0.11	0.51	0.03	0.87	−0.31	0.05
Height (cm)	167.1	8.5	**0.39**	**0.01**	0.13	0.43	−0.05	0.76	**0.37**	**0.02**
Boddy mass (kg)	62.5	13.2	**0.59**	**<0.01**	**0.47**	**<0.01**	0.13	0.43	**0.55**	**<0.01**
%fat (%)	21.7	6.4	0.07	0.66	0.30	0.07	0.31	0.06	−0.10	0.53
Knee extension torque (Nm)	171.9	56.7	**0.52**	**<0.01**	**0.40**	**0.01**	–	–	–	–
Knee flexion torque (Nm)	75.3	26.6	–	–	–	–	0.23	0.16	**0.41**	**<0.01**

Notes: RF, VL, BF, and ST present rectus femoris, vastus lateralis, biceps femoris, and semitendinosus respectively. Statistically significant simple correlations are presented with bold values.

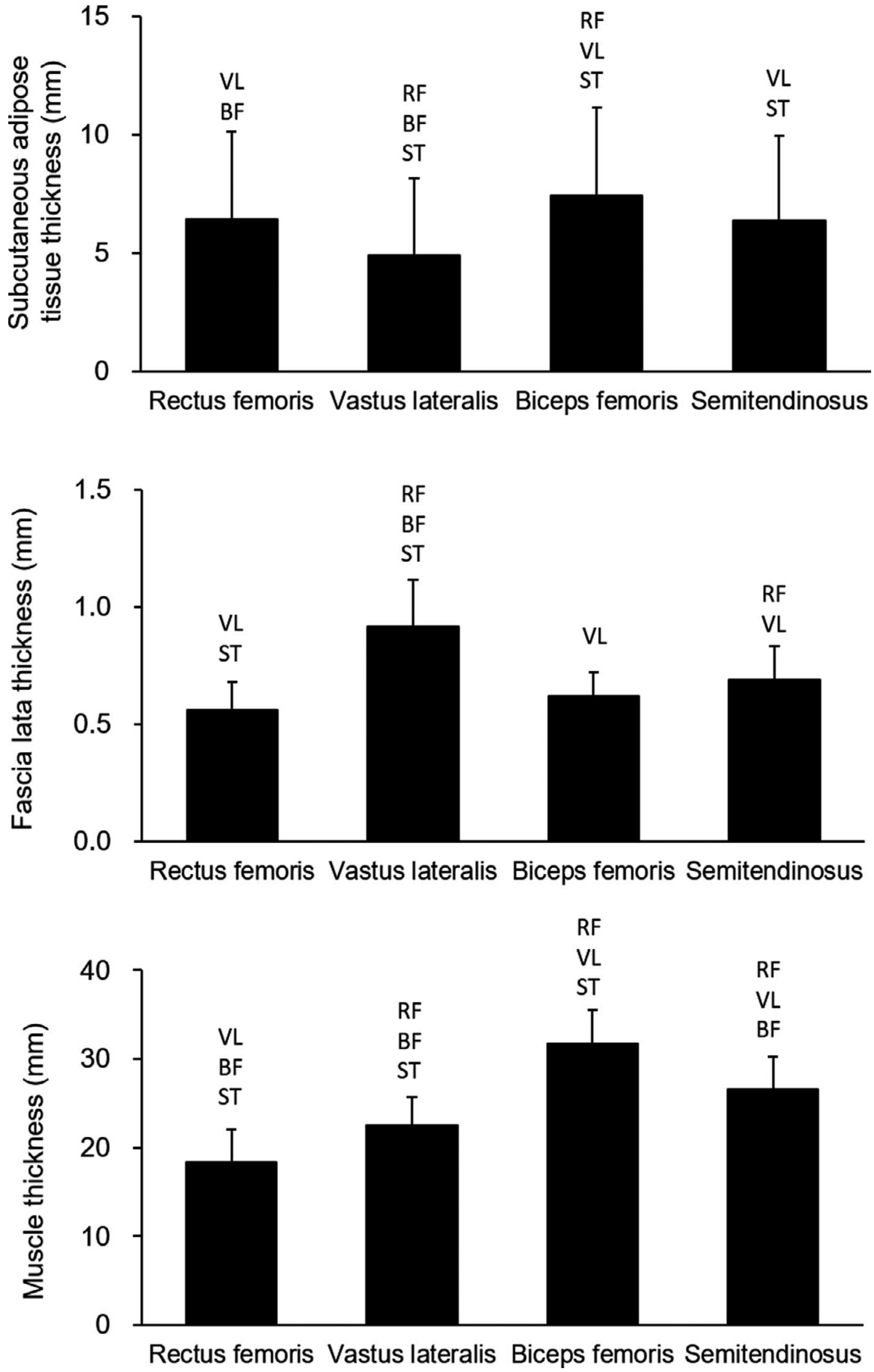

FIGURE 3 The thickness of the adipose, fascia lata, and skeletal muscle thickness at each site. Values are presented as means ± SDs. RF, VL, BF, and ST indicate significant differences compared with the value at Rectus femoris, Vastus lateralis, Biceps femoris, and Semitendinosus, respectively, at $p < 0.05$

range: 0.44–1.05 mm, $p < 0.01$) (Figure 3). Moreover, the fascia lata over the ST was thicker than that of RF ($p < 0.01$, Figure 3).

The fascia lata thickness at each site showed no significant correlation between age and %fat. The thicknesses of the fascia lata over the RF ($r = 0.39$, $p = 0.01$) and ST ($r = 0.37$, $p < 0.05$) showed positive correlations with height. The thicknesses of the fascia lata over the RF ($r = 0.59$, $p < 0.01$), VL ($r = 0.47$, $p < 0.01$), and ST ($r = 0.55$, $p < 0.01$) were positively correlated with the body mass (Table 1).

The fascia lata thickness in the total sample was not significantly correlated with the adipose tissue and muscle thicknesses. A positive correlation between the thickness of the fascia lata and that of the adipose tissue was shown only in the BF ($r = 0.44$, $p < 0.01$) (Figure 4). The thickness of the fascia lata was positively correlated with those of the underlying muscle at the RF ($r = 0.39$, $p < 0.05$) and ST ($r = 0.74$, $p < 0.01$) (Figure 4). Significant positive correlations were shown between the thicknesses of the fascia lata over

TABLE 2 Partial correlations between fascia lata thickness and morphological and functional properties of underlying muscle

		RF		VL		BF		ST	
		r	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
Skeletal muscle thickness (mm)	RF	−0.06	0.72	–	–	–	–	–	–
	VL	–	–	–	–	–	–	–	–
	BF	–	–	–	–	–	–	–	–
	ST	–	–	–	–	–	–	**0.61**	**< 0.01**
Knee extension torque (Nm)		0.18	0.27	0.25	0.13	–	–	–	–
Knee flexion torque (Nm)		–	–	–	–	–	–	0.14	0.42

Notes: Adjusted for height and/or body mass. RF, VL, BF, and ST present rectus femoris, vastus lateralis, biceps femoris, and semitendinosus respectively. Statistically significant partial correlations are presented with bold values.

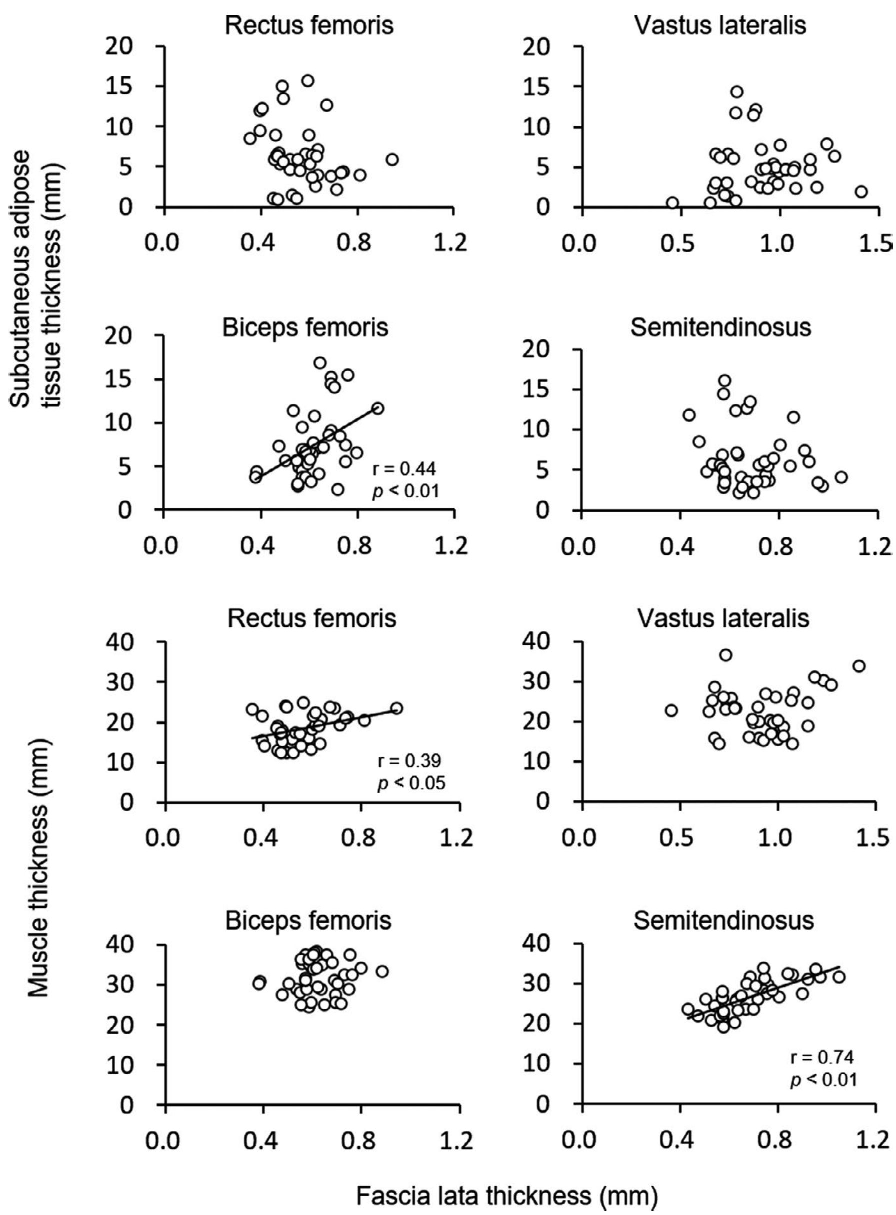

FIGURE 4 Relationships between fascia lata thickness at each site and corresponding subcutaneous adipose tissue and muscle thickness

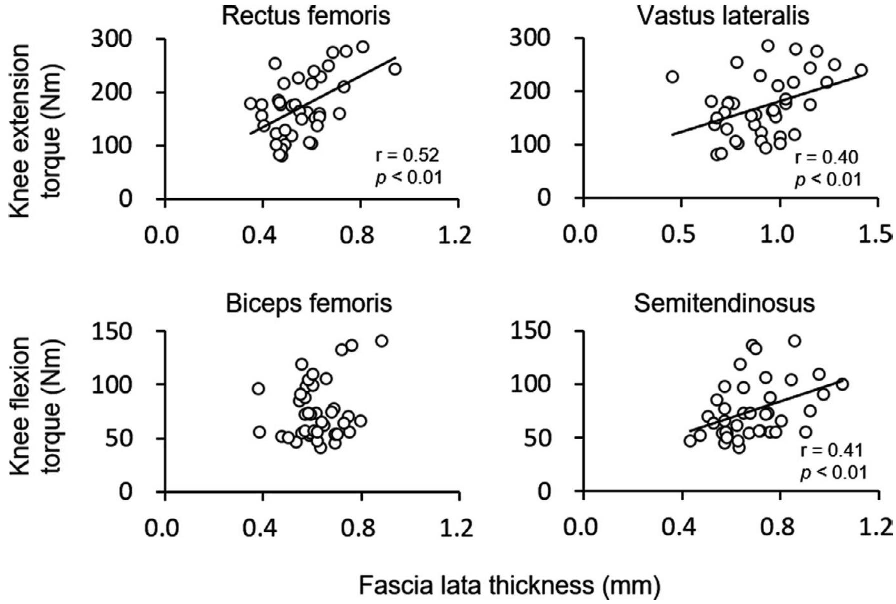

FIGURE 5 Relationships between fascia lata thickness at each site and corresponding muscle strength

the RF ($r = 0.52$, $p < 0.01$) and VL ($r = 0.40$, $p < 0.01$) and knee extension torque (Figure 5). The fascia lata thickness over the ST ($r = 0.41$ $p < 0.01$) was positively correlated with the knee flexion torque. A significant partial correlation adjusted for height and body mass was shown between the fascia lata and muscle thicknesses at the ST ($r = 0.61$, $p < 0.01$) (Table 2).

4 | DISCUSSION

The fascia lata thickness demonstrated site-dependent differences in the current study. Furthermore, as we hypothesized, there were large individual variations in the fascia lata thickness even in the same region of the thigh, partly showing the association with the neighboring tissues' characteristics and functions. To our knowledge, this is the first study which revealed the association between the deep fascia thickness and the morphological and functional properties of its neighboring tissues.

The association between the deep fascia thickness and body constitution was reported in a recent experiment (Wilke et al., 2018). Likewise, the thickness of the fascia lata showed positive simple correlations with the height (RF, ST) and body mass (RF, VL, and ST) in the present study. On the other hand, the fascia lata thickness was not correlated with the %fat and subcutaneous adipose tissue thickness at most sites of the thigh. While it is fairly certain that the deep fascia changes its properties depending on the physical constitution and neighboring tissues' characteristics, the muscles rather than the subcutaneous adipose tissues would mainly affect the individual characteristics of the deep fascia, especially at the lower limbs where the muscles are more influenced by the gravity compared to the upper limbs and trunk (Abe et al., 1997; Akima et al., 1997; Hides et al., 2007). In the present study, the BF was the sole part which showed the positive correlation between the fascia lata and subcutaneous adipose tissue thicknesses. Previous study noted that the degree of the alternation of the subcutaneous adipose tissue by the physical training was more significant at the posterior thigh than the anterior thigh (Ishida et al., 1987). The subcutaneous adipose tissue at thigh region has individual variations of its thickness, which therefore would lead to an association with the underlying fascia lata thickness, in particular at the BF.

The positive simple correlations were observed between the fascia lata thickness and the underlying muscle thickness at the RF and ST. The deep fascia is mainly composed of collagen fibers which shows site-dependent differences of the fiber direction in accordance with underlying muscle characteristics (Otsuka et al., 2018; Stecco et al., 2008). An anatomical observation showed that muscles adhere to the deep fascia via epimysium and/or direct muscle fiber insertion (Stecco, Pavan et al., 2014), which allows the forces generated by the muscle fibers to be transmitted to the neighboring deep fascia (Rijkelijkhuizen et al., 2007). The deep fascia would have the plasticity which changes its thickness according to the degree of mechanical stress produced by the underlying muscle. The present result is in line with the recent report showing that the fascia lata was thicker in younger participants who may have larger muscle size than older counterparts (Wilke et al., 2018). Despite the significant simple correlations at each site, there was no significant correlation between fascia lata and muscle thickness in the total sample. In addition, the lateral site of the fascia lata was thicker compared to other sites, presenting similar result of our cadaveric experiment (Otsuka et al., 2018). The lateral thick part of the fascia lata which called iliotibial band reinforces lateral side of the thigh (Evans, 1979; Terry et al., 1986). It is possible that the present result of the correlation statistics in the total sample may have been influenced by this unique ligament-like structure of the fascia lata at the lateral site regardless of the physical constitution.

The positive simple correlation was found between fascia lata thicknesses over the RF, VL, and ST and knee extension or flexion torque. It is well-known that the muscle size associates with the corresponding joint strength. The present findings suggest that not only the skeletal muscle but also the deep fascia morphologies are the important factors to define the muscle strength. As described above, the force produced by the muscle contraction transmits to the overlayered deep fascia (Huijing et al., 2007; Maas & Sandercock, 2010; Rijkelijkhuizen et al., 2007). Greater torque exertion would lead greater mechanical stress to the deep fascia and make this collagen rich membrane tissue thicker. Several studies reported that the deep fascia showed anisotropic characteristic which was stiff in the longitudinal direction and compliant in the radial direction (Otsuka et al., 2018, 2019; Stecco, 2014). It can be speculated that the individuals who have the superior physical constitution with greater joint torque, have thicker and stiffer deep fascia in the direction of muscle contraction.

Despite several simple correlations between the fascia lata thickness and underlying muscle thickness (RF and ST) and strength (RF, VL, and ST), a significant partial correlation between the fascia lata thickness and muscle thickness or strength was not observed at most of the sites after adjusting for the influence of physical constitution. These results suggest that the physical constitution exerts greater influence on the fascia lata morphologies. On the other hand, the fascia lata thickness over the ST showed the positive partial correlation with the underlying muscle thickness. Anatomical studies reported that the distal tendons of the ST and gracilis muscles had direct insertions to the deep fascia which is called accessory bands (Candal-Couto & Deehan, 2003; Rizvi et al., 2018). Those accessory bands might contribute to transmitting the mechanical stress produced by the ST to the overlying fascia lata, which could explain the strong simple and partial correlations between the fascia lata and muscle thicknesses at this site. Morphology of the fascia lata over the ST, therefore, can be affected by the property of its underlying muscle to a greater extent regardless of the physical constitutions.

Apart from the positive effect on the joint torque, the thicker and stiffer deep fascia could bring about a negative impact on the joint flexibility depending on the body sites. Wilke et al., (2018) showed that the thickness of the posterior thigh connective tissue was not correlated with the flexibility of the hamstrings in young participants but negatively correlated with that of the elderly. In contrast, another study demonstrated that the stiffness of the deep fascia over the gastrocnemius muscle showed a positive correlation with the ankle flexibility both in young and older adults (Hirata et al., 2020). Therefore, the deep fascia might change its thickness and stiffness according to aging, and/or exercise habit, and its plasticity can have either negative or positive impact on the human body movements. Further longitudinal observations focusing on how the deep fascia morphology and property change in response to short and/or long-term exercise intervention, growth, and aging would support our understanding of the deep fascia plasticity and its association with the joint flexibility and exercise performance.

5 | CONCLUSIONS

Our study has revealed that the fascia lata thickness partly shows positive correlations with the subcutaneous adipose tissue and underlying muscle thickness and corresponding joint torque. Especially, the fascia lata over the ST reflects the underlying muscle characteristics regardless of the physical constitution. Due to its plasticity, the fascia lata would change its thickness according to the morphology of the neighboring tissues and underlying muscle function.

ACKNOWLEDGMENTS
This work was supported by JSPS KAKENHI Grant Number 16H01870 (Grant-in-Aid for Scientific Research (A)) and 20K23300 (Grant-in-Aid for Research Activity Start-up). This work was also funded by Yamaha Motor Foundation for Sports 2019 and TOBE MAKI Scholarship foundation 2020.

CONFLICTS OF INTERESTS
The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS
Contributions to concept/design: SOt, XS, MN, YK, acquisition of data: SOt, XS, KK, SOm, data analysis/interpretation: SOt, XS, TY, MN, YK, drafting of the manuscript: SOt, and critical version of the manuscript and approval of the article: SOt, XS, KK, SOm, TY, MN, YK.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Otsuka, S., Shan, X., Kurumisawa, K., Omura, S., Yamagishi, T., Naito, M.&et al (2021) Investigation of the association between human fascia lata thickness and its neighboring tissues' morphology and function using B-mode ultrasonography. *Journal of Anatomy*, 239, 1114–1122. https://doi.org/10.1111/joa.13505