



Original Article

## Quantity and quality of the peroneus longus assessed using ultrasonography in leg with chronic ankle instability

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**Abstract.** [Purpose] Muscle quantity (e.g., cross-sectional area) and quality (e.g., muscle adipose tissue), which are muscle strength determinants, can be assessed using ultrasonography. The study aimed to investigate the changes in the quantity and quality of the peroneus longus and evaluate evtor strength in legs with chronic ankle instability (CAI). Furthermore, the associations among cross-sectional area, echogenicity, evtor strength, and frequency of ankle sprain were examined. [Participants and Methods] Nine males with CAI in unilateral legs were the voluntary participants in this study. The cross-sectional area of the peroneus longus, echogenicity, and evtor strength were measured for all the participants on the sides with CAI and that without. [Results] No significant difference in cross-sectional area was observed between the sides. Significant differences in echogenicity (higher on the CAI side) and evtor strength (lower on the CAI side) were observed between the sides. In addition, a moderate correlation was observed between echogenicity and increased sprain frequency on both sides. [Conclusion] Muscle adipose tissue increased, evtor strength decreased, and the cross-sectional area remained unchanged on the CAI side. The study results suggested that muscle adipose tissue increases with increasing frequency of ankle sprain.

**Key words:** Chronic ankle instability, Echogenicity of peroneus longus, Evtor strength

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### INTRODUCTION

Ultrasonography can evaluate muscle quantity and quality with easy accessibility, no invasive procedures and safety. Cross-sectional area (CSA) as an index of muscle quantity can be assessed by ultrasonography. Previously, CSA was evaluated in some clinical studies as they considered CSA assessed by ultrasonography to be reliable<sup>1)</sup> and a determinant of the muscle strength. However, it was revealed that there were changes in muscle quality with atrophy, increasing adipose tissue accumulation within the muscles<sup>2)</sup>, and CSA measurement includes muscle adipose tissue. Thus, it seems that muscle quantity can be overestimated by measuring the CSA alone.

Muscle quality can be also evaluated by measuring the echogenicity by B-mode ultrasonography. Echogenicity is represented by the brightness of ultrasound images. In ultrasound images, the more the adipose tissue in the muscles, the higher is the echogenicity, owing to the difference in the acoustic impedances of muscle fibers and adipose tissue. Previously, echogenicity was reported to be correlated to the percentage of muscle adipose tissue<sup>3)</sup> and muscle strength<sup>4)</sup>. Consequently, it is important to evaluate not only muscle quantity but also muscle quality.

Ankle inversion sprain is one of the most frequent sports injuries<sup>5, 6)</sup>. Many ankle sprains occur when performing a lateral

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cutting or landing and damage the anterior talofibular ligament or calcaneofibular ligament<sup>7</sup>). A high incidence and relapse rate has been reported for these sprains<sup>8</sup>). If one experiences repeated ankle inversion sprains, chronic ankle instability (CAI) may develop<sup>9</sup>). The peroneus longus (PL), which is an evertor muscle, prevents ankle inversion sprain by antagonizing the sudden ankle inversion. Some previous studies have reported that ankle evertor strength decreases in legs with CAI<sup>10, 11</sup>), whereas others suggest that it does not<sup>12, 13</sup>). This is because few studies have examined muscle quantity and quality, which are determinants of muscle strength, with evertor strength.

The first aim of this study was to investigate changes in muscle quantity and quality of the PL and to determine whether evertor strength decreases in legs with CAI. The second aim was to examine the relationship among evertor strength, CSA, and echogenicity. It was hypothesized that evertor strength and CSA would decrease and echogenicity would increase in the leg with CAI.

## PARTICIPANTS AND METHODS

This study was a cross-sectional and comparative study to evaluate the CAI side and non-CAI side. Nine males with CAI in unilateral legs voluntarily participated (Table 1). All participants were allowed to exercise once a week and did not have a history of receiving rehabilitation after ankle inversion sprains. The participants were included in the study if they qualified per the CAI criteria adopted from the study by Gribble et al<sup>14</sup>). This study protocol was approved by the Institutional Review Board of the Graduate School of Health Sciences, Hiroshima University, Japan (approval number: E-957), and informed consent was obtained from all participants.

For muscle quantity and quality measurement, the participants were seated with their hips and knees flexed at 90° and ankles in a neutral position. B-mode ultrasonography (Noblus, Aloka, Japan) was performed using a linear transducer (8–12 MHz)<sup>15</sup>), and transverse ultrasound images of the PL were used for measurements at the proximal 25% point between the fibular head and the lateral malleolus in both legs. The transducer was positioned perpendicular to the transverse plane of the PL with minimum pressure applied to the skin. Three images of the PL were obtained for each participant. Specific settings were maintained for ultrasonography (gain and focus) throughout all experiment. The images of the PL were transferred to a computer (Pavilion Power 580, Hewlett-Packard Inc., USA) and the CSA and echogenicity were measured using Image J (National Institute of Health, USA). The CSA of the PL was calculated along the fascia and with 112 pixels scaled at a distance of 10 mm (Fig. 1). The region of interest for echogenicity was the same as that for the CSA. The echogenicity was determined by 8-bit gray-scale analysis using the histogram function of Image J. The mean echogenicity of the regions was expressed as a value between 0 (black) and 255 (white). The mean CSA and echogenicity were calculated from the three images, and a high echogenicity value indicated a muscle with more adipose tissue. Intra-class correlation coefficients (ICC1.3) were evaluated using the three images to assess test-retest reliabilities. ICC1.3 for the CSA and for echogenicity was 0.99.

The isokinetic evertor peak torque (Nm) was measured to obtain evertor strength using Biodex System 3 (Biodex Medical Systems Inc., Shirley, NY, USA). Isokinetic testing of the evertor peak torque was performed at a velocity of 60°·s<sup>-1</sup> for concentric contraction of both ankles, and the measurements were taken from the non-CAI side to the CAI side<sup>16</sup>). The reason of velocity setting was comparable to velocities used in the previous studies<sup>17, 18</sup>). Participants were seated in the dynamometer chair with one leg elevated with the help of a support arm under the knee, and the other leg put on a leg rest (Fig. 2). The ankle of the elevated leg was placed on the foot plate, and the foot was fixed with two Velcro straps and a rubber heel cup. The subtalar joint was maintained in the neutral position. The trunk was secured with two harnesses to limit compensatory body movement. Before the measurement, a warm-up session was conducted to familiarize the participants with the test protocol. The warm-up session consisted of five non-fatigue and two maximal contractions. A 2-min rest period was given between the warm-up session and test. Participants were instructed to do the best effort. The tests were performed five times for both the CAI side and non-CAI side for all participants, and the isokinetic evertor torque was recorded using the Biodex software. The peak torque value calibrated by body-weight (Nm/kg) was used during data analysis for comparison to revise the body weight.

Statistical analysis was performed using SPSS ver. 20.0 for Windows (IBM Japan Ltd., Japan). Descriptive data were shown as means ± SD. Differences in ankle evertor strength, CSA, and echogenicity between the CAI side and non-CAI side were calculated using a paired t-test. Correlations among CSA, echogenicity, and evertor strength calculated using Pearson's correlation coefficient or Spearman's correlation coefficient. The significance level was set at 0.05.

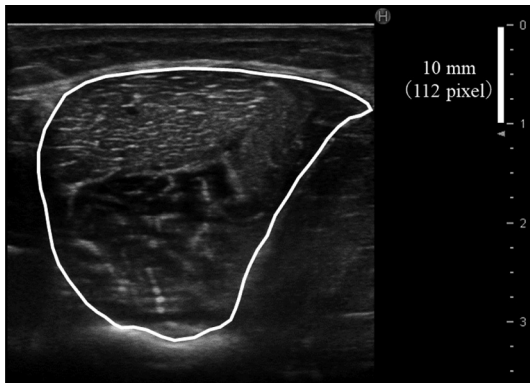
## RESULTS

Table 2 shows the ultrasonography measurement and evertor strength of the participants. No significant difference was observed in the CSA between sides. Echogenicity on the non-CAI side showed a significantly higher value than that on the CAI side. In addition, evertor strength on the CAI side showed a significantly lower value than that on the non-CAI side.

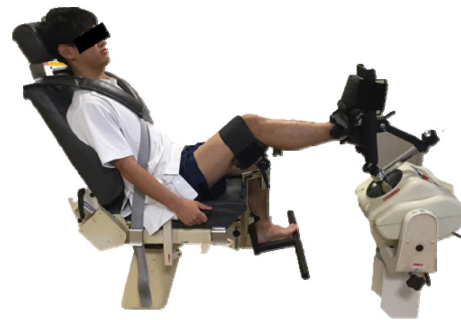
Table 3 shows the correlation coefficients for evertor strength, CSA, and echogenicity of PL of the participants. Evertor strength showed a significantly positive correlation with CSA. CSA had a significant correlation to anything without evertor strength. Echogenicity showed a significantly positive correlation with number of ankle sprains.

**Table 1.** Physical characteristics of the participants

Physical characteristic	Side	Mean $\pm$ SD
Age (years)	-	22.2 $\pm$ 1.4
Height (cm)	-	172.9 $\pm$ 5.8
Body weight (kg)	-	65.1 $\pm$ 8.4
BMI (kg•m <sup>-2</sup> )	-	21.7 $\pm$ 1.7
The number of ankle sprains (times)	CAI side	7.9 $\pm$ 2.8
	non-CAI side	1.3 $\pm$ 2.1

**Fig. 1.** Measurement region for cross-sectional area (CSA) and echogenicity.

The region of interest was calculated along with fascial of PL.  
CSA: 599 (mm<sup>2</sup>), echogenicity: 63.2 (a.u.).

**Fig. 2.** The position of the participants for measuring evetor strength on Biodex System 3.**Table 2.** Evertor strength, CSA and echogenicity (n=9)

	Side		Effect size	95% confidence interval
	CAI	non-CAI		
Evertor strength (Nm/kg)	0.40 $\pm$ 0.13	0.48 $\pm$ 0.13 <sup>††</sup>	0.85	2.72–7.96
CSA (mm <sup>2</sup> )	573.1 $\pm$ 98.4	604.3 $\pm$ 110.0	0.61	–2.08–59.79
Echogenicity (a.u.)	67.7 $\pm$ 8.4	65.0 $\pm$ 9.1 <sup>†</sup>	0.68	0.19–5.24

CAI: chronic ankle instability; CSA: cross-sectional area. <sup>†</sup>p<0.05 (vs. CAI); <sup>††</sup>p<0.01 (vs. CAI).

**Table 3.** Correlation coefficients for evertor strength, CSA, echogenicity, and physical characteristics (n=18)

	Evertor strength	CSA	Echogenicity	Age	Height	Body weight	The number of ankle sprains
Evertor strength	-	0.47 <sup>†</sup>	0.09	–0.17	–0.03	–0.20	–0.07
CSA		-	0.03	–0.10	0.13	0.23	–0.27
Echogenicity			-	–0.04	0.56 <sup>†</sup>	0.53 <sup>†</sup>	0.56 <sup>††</sup>
Age				-	0.42	0.07	–0.07
Height					-	0.85 <sup>††</sup>	0.18
Body weight						-	0.17
The number of ankle sprain							-

CSA: cross-sectional area. <sup>†</sup>p<0.05; <sup>††</sup>p<0.01.

## DISCUSSION

The important findings of this study were as follows: (1) echogenicity on the CAI side was higher than that on the non-CAI side, although there was no difference in the CSA, (2) evtor strength on the CAI side was lower than that on the non-CAI side, and (3) echogenicity positively correlated with the number of ankle sprains. A higher value of echogenicity and a decrease in evtor strength on the CAI side supported our hypothesis; however, the maintained CSA did not.

Although there was no significant difference in the CSA between the CAI and non-CAI sides, echogenicity on the CAI side was higher value than that on the non-CAI side. This result indicated that the muscle quantity of PL was maintained, but the muscle quality declined on the CAI side. This is because the orthopedic condition causes a decline in muscle quality before affecting muscle quantity<sup>19</sup>. In general, the adipose tissue accumulates in the hypodermis or around the viscus. However, adipose tissue can accumulate within the muscle in various diseases<sup>19, 20</sup>. Previous studies have reported that adipose tissue accumulation within the muscle is caused by obesity<sup>21</sup>, muscle atrophy from aging<sup>22</sup>, muscle atrophy from neuropathy<sup>23</sup>, and decreased muscle activity in young individuals<sup>24</sup>. Studies have reported that sprained ankle occurred reflex inhibition<sup>25</sup> and decreased muscle activity in the PL<sup>26</sup>. Reflex inhibition and decreasing muscle activity after ankle sprain could contribute to increase adipose tissue within the muscle. In addition, increasing adipose tissue within muscles has been reported to decrease muscle strength<sup>27</sup>. Uezumi et al. reported that mesenchymal stem cells differentiated into fat cells within the muscles due to decreased inhibitory signals from muscle cells<sup>28</sup>.

More interestingly, echogenicity showed a positive correlation with the number of ankle sprains in our study. This result suggested that muscle adipose tissue increased as the number of ankle sprains increased. Ankle sprains are caused by excessive ankle inversion resulting from an inversion torque from the ground reaction force<sup>7</sup>. The PL contracts to generate an eversion torque and prevent excessive ankle inversion. In this study, the evtor strength on the CAI side was lower than that on the non-CAI side. It was considered that low evtor strength on the CAI side could cause chronic ankle inversion sprains and that increased muscle adipose tissue would contribute to decreasing the evtor strength. To prevent this state, it is important to receive rehabilitation and train evtor muscles after ankle sprain. It has been reported that high-velocity muscle training for patients with hip osteoarthritis improved echogenicity and physical performance<sup>29</sup>. This result implies the possibility that high-velocity muscle training is effective for patient with CAI. We need intervention study for the CAI patient in the future.

There are several limitations to this study. First, this cross-sectional study could not reveal whether the deficit in evtor strength and increase in adipose tissue in the muscle were congenital or postnatal. Second, there was no correlation between evtor strength and the echogenicity of PL in this study. This was because evtor strength includes the strength of some muscles; however, the measurement of echogenicity in this study was at only one point of PL. For future studies, more points along PNM should be measured and the correlation among various parameters should be examined.

In conclusion, although CSA was maintained in legs with CAI, echogenicity increased. Further, evtor strength decreased in legs with CAI. Finally, muscle quality decreased in legs with CAI that experienced ankle sprains; however, the muscle quantity did not decrease.

### *Conflict of interest*

The authors declare no conflicts of interest associated with this manuscript.

## REFERENCES

- 1) Schneebeli A, Egloff M, Giampietro A, et al.: Rehabilitative ultrasound imaging of the supraspinatus muscle: Intra- and interrater reliability of thickness and cross-sectional area. *J Bodyw Mov Ther*, 2014, 18: 266–272. [[Medline](#)] [[CrossRef](#)]
- 2) Goodpaster BH, Kelley DE, Thaete FL, et al.: Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. *J Appl Physiol* 1985, 2000, 89: 104–110. [[Medline](#)] [[CrossRef](#)]
- 3) Pillen S, Tak RO, Zwarts MJ, et al.: Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound Med Biol*, 2009, 35: 443–446. [[Medline](#)] [[CrossRef](#)]
- 4) Fukumoto Y, Ikezoe T, Yamada Y, et al.: Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur J Appl Physiol*, 2012, 112: 1519–1525. [[Medline](#)] [[CrossRef](#)]
- 5) Hootman JM, Dick R, Agel J: Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. *J Athl Train*, 2007, 42: 311–319. [[Medline](#)]
- 6) Woods C, Hawkins R, Hulse M, et al.: The Football Association Medical Research Programme: an audit of injuries in professional football: an analysis of ankle sprains. *Br J Sports Med*, 2003, 37: 233–238. [[Medline](#)] [[CrossRef](#)]
- 7) Wright IC, Neptune RR, van den Bogert AJ, et al.: The influence of foot positioning on ankle sprains. *J Biomech*, 2000, 33: 513–519. [[Medline](#)] [[CrossRef](#)]
- 8) Yeung MS, Chan KM, So CH, et al.: An epidemiological survey on ankle sprain. *Br J Sports Med*, 1994, 28: 112–116. [[Medline](#)] [[CrossRef](#)]
- 9) Freeman MA: Instability of the foot after injuries to the lateral ligament of the ankle. *J Bone Joint Surg Br*, 1965, 47: 669–677. [[Medline](#)] [[CrossRef](#)]
- 10) Willems T, Witvrouw E, Verstuyft J, et al.: Proprioception and muscle strength in subjects with a history of ankle sprains and chronic instability. *J Athl Train*, 2002, 37: 487–493. [[Medline](#)]

- 11) Hartsell HD, Spaulding SJ: Eccentric/concentric ratios at selected velocities for the invertor and evertor muscles of the chronically unstable ankle. *Br J Sports Med*, 1999, 33: 255–258. [[Medline](#)] [[CrossRef](#)]
- 12) Bernier JN, Perrin DH, Rijke A: Effect of unilateral functional instability of the ankle on postural sway and inversion and eversion strength. *J Athl Train*, 1997, 32: 226–232. [[Medline](#)]
- 13) Kaminski TW, Perrin DH, Gansneder BM: Eversion strength analysis of uninjured and functionally unstable ankles. *J Athl Train*, 1999, 34: 239–245. [[Medline](#)]
- 14) Gribble PA, Delahunt E, Bleakley CM, et al.: Selection criteria for patients with chronic ankle instability in controlled research: a position statement of the International Ankle Consortium. *J Athl Train*, 2014, 49: 121–127. [[Medline](#)] [[CrossRef](#)]
- 15) Boonstra AM, van Weerden TW, te Strake L, et al.: Ultrasonography of the peroneal nerve muscle group in normal subjects and patients with peroneal palsy. *J Clin Ultrasound*, 1988, 16: 17–24. [[Medline](#)] [[CrossRef](#)]
- 16) Maeda N, Urabe Y, Tsutsumi S, et al.: Symmetry tensiomyographic neuromuscular response after chronic anterior cruciate ligament (ACL) reconstruction. *Knee Surg Sports Traumatol Arthrosc*, 2018, 26: 411–417. [[Medline](#)] [[CrossRef](#)]
- 17) Aydoğ E, Aydoğ ST, Çakıcı A, et al.: Reliability of isokinetic ankle inversion- and eversion-strength measurement in neutral foot position, using the Biodex dynamometer. *Knee Surg Sports Traumatol Arthrosc*, 2004, 12: 478–481. [[Medline](#)] [[CrossRef](#)]
- 18) Munn J, Beard DJ, Refshauge KM, et al.: Eccentric muscle strength in functional ankle instability. *Med Sci Sports Exerc*, 2003, 35: 245–250. [[Medline](#)] [[CrossRef](#)]
- 19) Liikavainio T, Lyytinen T, Tyrväinen E, et al.: Physical function and properties of quadriceps femoris muscle in men with knee osteoarthritis. *Arch Phys Med Rehabil*, 2008, 89: 2185–2194. [[Medline](#)] [[CrossRef](#)]
- 20) Arts IM, Pillen S, Schelhaas HJ, et al.: Normal values for quantitative muscle ultrasonography in adults. *Muscle Nerve*, 2010, 41: 32–41. [[Medline](#)] [[CrossRef](#)]
- 21) Greco AV, Mingrone G, Giancaterini A, et al.: Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes*, 2002, 51: 144–151. [[Medline](#)] [[CrossRef](#)]
- 22) Kim JY, Kim DH, Choi J, et al.: Changes in lipid distribution during aging and its modulation by calorie restriction. *Age (Dordr)*, 2009, 31: 127–142. [[Medline](#)] [[CrossRef](#)]
- 23) Carpenter S, Karpati G: Cells and structures other than skeletal muscle fibers, in: *pathology of skeletal muscle*. New York: Oxford University Press, 2001, pp 314–369.
- 24) Teramoto K, Ikezaki K, Suda K, et al.: Effects of habitual exercise to skeletal muscle fat content in young adult. *Bull Aichi Univ Edu*, 2016, 65: 39–44.
- 25) McVey ED, Palmieri RM, Docherty CL, et al.: Arthrogenic muscle inhibition in the leg muscles of subjects exhibiting functional ankle instability. *Foot Ankle Int*, 2005, 26: 1055–1061. [[Medline](#)] [[CrossRef](#)]
- 26) Palmieri-Smith RM, Hopkins JT, Brown TN: Peroneal activation deficits in persons with functional ankle instability. *Am J Sports Med*, 2009, 37: 982–988. [[Medline](#)] [[CrossRef](#)]
- 27) Visser M, Goodpaster BH, Kritchevsky SB, et al.: Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *J Gerontol A Biol Sci Med Sci*, 2005, 60: 324–333. [[Medline](#)] [[CrossRef](#)]
- 28) Uezumi A, Fukada S, Yamamoto N, et al.: Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat Cell Biol*, 2010, 12: 143–152. [[Medline](#)] [[CrossRef](#)]
- 29) Fukumoto Y, Tateuchi H, Ikezoe T, et al.: Effects of high-velocity resistance training on muscle function, muscle properties, and physical performance in individuals with hip osteoarthritis: a randomized controlled trial. *Clin Rehabil*, 2014, 28: 48–58. [[Medline](#)] [[CrossRef](#)]