

Research article

Acute Effects of Selective Strength Exercise on the Peroneus Longus and Brevis

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Abstract

The peroneus muscles are muscles that mainly act in ankle eversion and can be divided into PL and PB, which have different but important roles in foot and ankle functions. Therefore, PL and PB dysfunction can lead to foot and ankle issues, making selective strength exercise necessary. This study aimed to identify the effect of two different exercise techniques on PL and PB morphologies. Two interventions were performed on separate days: the PL intervention, in which a Thera-Band® was placed on the ball of the foot and pushed out from the contact point, and the PB intervention, in which the Thera-Band® was pulled from the base of the fifth metatarsal. Cross-sectional area (CSA) and thickness of the peroneus muscles at 25% (showing the PL morphology) and 75% (showing the PB morphology) proximal to the line connecting the fibular head and lateral malleolus, as well as ankle strength was measured before and immediately after the interventions and at 10, 20, and 30 min later. A repeated-measures two-way analysis of variance was conducted to identify differences in the effects of the interventions on the PL and PB. Main and interaction effects on CSA, thickness, and ankle strength, with a significant increase in CSA and thickness in the proximal 25% in the PL intervention and the distal 75% in the PB intervention immediately after implementation, were observed ($p < 0.05$). The transient increase in muscle volume due to edema immediately after exercise indicates the acute effect of exercise. The CSA and thickness of the proximal 25% in the PL intervention and the distal 75% in the PB intervention increased immediately after the intervention, indicating that these interventions can be used to selectively exercise the PL and PB.

Key words: Peroneus muscles, ultrasound system, ankle, sports injury, muscle strength exercise.

Introduction

Peroneus muscles are representative muscles that mainly act on ankle eversion (Seo and Lee, 2022). Its main roles include providing mediolateral stability of the ankle during movements such as walking (Louwerens et al., 1995), and preventing sudden and involuntary ankle inversion (Konradsen et al., 1998; Sutherland, 2001). Peroneus muscles can be divided mainly into the peroneus longus (PL) and peroneus brevis (PB). PL and PB insert into the first and fifth metatarsals, respectively. Therefore, PL acts on ankle plantar flexion from the first ray of the foot and ankle eversion, and PB acts on a total of 63% of the ankle eversion power (Koutsogiannis et al., 2022). Additionally, it is interesting to note that PL and PB have different actions and roles in the same eversion when examined in detail. While there are differences in the actions of PL and PB, dysfunction

of each muscle can cause various foot and ankle problems. For example, PL stabilizes and supports the medial longitudinal arch by pulling the base of the first metatarsal bone downward (Kirby., 2017). Accordingly, previous studies have reported that low PL activity is associated with flat feet (Murley et al., 2009). In addition, PB contributes to the stability of the fifth ray due to its insertion into the fifth metatarsal (Jarrett et al., 2020). When it fails in function, the stability of the fifth ray is reduced, leading to the fracture of the fifth metatarsal bone and hypermobility (Jarrett et al., 2020). Furthermore, instability of the fifth ray associated with PB dysfunction can indirectly be a major cause of the occurrence of the lateral ankle sprain that frequently occur in sports activities (Jarrett et al., 2020). In this way, it is necessary to consider the functions and roles of the PL and PB separately, rather than lumping them together as peroneus muscles, not only for the treatment of foot and ankle injuries but also for the improvement of muscle function and injury prevention in healthy individuals.

Muscle strength exercise is one way to improve muscle function (Bavdek et al., 2018). It is desirable to understand the differences in the roles of PL and PB and perform muscle strength exercises for each muscle. However, no reports have been found to date examining selective muscle strength exercises for PL and PB. In this context, previous studies have reported foot and ankle movements that can emphasize PL and PB activities. Schunk et al. performed eight different bridge exercises and investigated the PL electrical muscle activity during these exercises (Schunk, 1982). The results showed that the values of the PL muscle activity were higher in the unilateral bridge exercise with ankle plantar flexion (Schunk, 1982). This was because, in this type of bridge exercise, the load is concentrated on the ball of the foot, and the weight is maintained at the first ray (the point where the PL attaches), which is considered to increase the PL activity (Schunk, 1982). Therefore, it is implied that the ball of the foot loading leads to an increase in PL activity. Otis et al. used fresh-frozen cadaver foot-ankle specimens to simulate the early heel rise phase when the PL and PB are most active during the gait cycle and confirmed the differences in external rotation, and valgus angle of the subtalar joint when the PL and PB were loaded independently (Otis et al., 2004). As a result, it was reported that the subtalar joint showed more external rotation and valgus when the load was applied to the PB alone than to the PL (Otis et al., 2004). In brief, muscle strength exercises that emphasize the subtalar joint's external rotation and valgus are likely to lead to an

increase in the activity of the PB alone. Furthermore, considering the difference between PL and PB in terms of the moment arm, a previous study has demonstrated that the moment arm of PL is maximized during foot eversion, while the moment arm of PB is maximized during ankle external rotation (McCullough et al., 2011). It can be inferred that the ball of the foot loading is a movement that emphasizes more foot eversion, and therefore, the load is more likely to be applied to the PL, while the movement coordinated with ankle external rotation is a movement that easily applies a load to the PB. Therefore, it would be possible to selectively exercise the PL and PB by performing exercises that emphasize the ball of the foot loading in the PL and those that emphasize the ankle external rotation and valgus in the PB.

Methods using ultrasound imaging systems have recently been reported to confirm the effects of muscle strength exercises. A previous study pointed out that transient muscle hypertrophy due to edema caused by muscle injury occurs immediately after muscle strength exercise, which may indicate an immediate effect of the exercise (Damas et al., 2016). Another report measured transient muscle hypertrophy in terms of muscle cross-sectional area (CSA) using an ultrasound imaging system (Damas et al., 2016). In this study, we would like to examine the effects of selective exercise on PL and PB. Previously, we measured the CSA of PL and PB separately using an ultrasound imaging system in participants with a history of lateral ankle sprain and reported the results with reliability (Arima et al., 2022). The measurement position was set at the proximal 25% of the line connecting the fibular head and lateral malleolus, where the majority of the CSA of the PL is located, and the distal 75%, where most of the CSA of the PB is located, considering reports of anatomic location in previous studies (Lee et al., 2011). Measuring the transient muscle hypertrophy of the PL and PB after muscle strength exercises separately with an ultrasound imaging system would be a valid means of confirming the effect of the exercises on muscle hypertrophy. This measurement method could also be a valid and convenient method of evaluation in clinical practice.

This study aimed to identify the acute effects of two different strength exercise methods, namely, exercises with an emphasis on the ball of the foot loading and exercises with an emphasis on ankle external rotation and valgus, on the muscle morphologies of the PL, PB, and ankle strength, and to determine the validity of selective strength exercise methods for the peroneus muscles. We hypothesize that, with respect to CSA and thickness, the proximal 25% of the peroneus muscles would increase after the implementation of exercises with an awareness of the ball of the foot loading and that the distal 75% would increase after exercises with emphasis on ankle external rotation and valgus. With regard to ankle strength, we expected a decrease in ankle strength measured by the same ankle movements as the interventions that emphasized the ball of the foot loading and ankle external rotation and valgus, immediately after the interventions.

Methods

Participants

Twenty-two healthy adults (men, $n = 11$; women, $n = 11$) participated in this study. The general characteristics of the participants were as follows: age, 22.4 ± 1.4 years; height, 164.4 ± 7.6 cm; body weight, 59.2 ± 10.0 kg; body mass index, 21.7 ± 2.1 kg/m²; no LAS experienced and Cumberland Ankle Instability Tool score, 28.9 ± 1.4 . The exclusion criteria were as follows: (1) a history of a LAS or other orthopedic conditions and orthopedic surgery on the lower extremity; (2) acute musculoskeletal injuries, such as a lower extremity sprain or fracture within the past 3 months; and (3) a Cumberland Ankle Instability Tool score < 27 points (Forsyth et al., 2022). In accordance with the Declaration of Helsinki, the purpose and content of this study were fully explained to the participants, and their written consent was obtained. The study protocol complied with the tenets of the Declaration of Helsinki, and this study was approved by the Ethical Committee for Epidemiology of Hiroshima University (approval number: E-2659).

Experimental procedures

All participants performed two types of ankle movements using Thera-Band® (strength, level +3 [black]; Sakai Medical Co., Ltd.) on the right leg as an intervention for the PL and PB. Figure 1 shows the flowchart of the methodology used in this study. Two interventions on separate days, 48 hours apart: PL intervention, in which a Thera-Band® was placed on the ball of the foot and pushed out from the contact point in the long sitting position, and PB intervention with Thera-Band® pulled out from the base of the fifth metatarsal, emphasizing external rotation and valgus of the ankle in the lateral position. In the lateral position, the upper side lower extremity was instructed to have 90° hip and knee joint flexion, whereas the lower side lower extremity was instructed to have 0° hip and knee joint flexion-extension. The number of intervention movements performed was two sets of 100 times at a speed of 1 time/2 s. The order in which the two interventions were performed for each participant was assigned randomly. The length of the Thera-Band® was designed to unify the amount of load applied during the intervention movements. In the PL intervention, the Thera-Band® was placed around the plantar foot in the mid-ankle position with no tension applied so that both ends of the Thera-Band® were half the length from the fibular head to the plantar foot. The interventions were performed with both ends of the Thera-Band® pulled up to the knee joint space. In the PB intervention, the length of the Thera-Band® was set to 50 cm, the same as the height of the bed used in the measurements, with no tension applied to the Thera-Band®, which was joined at both ends. A new Thera-Band® was used for each participant so that the resistance due to the elongation of the Thera-Band® would not change from participant to participant. CSA and thickness of the PL and PB at 25% and 75% were measured by the ultrasound imaging system, and PL and PB strength were measured using a handheld dynamometer before (pre-intervention) and immediately after the intervention (post-intervention) and at 10, 20, and 30 min after each intervention.

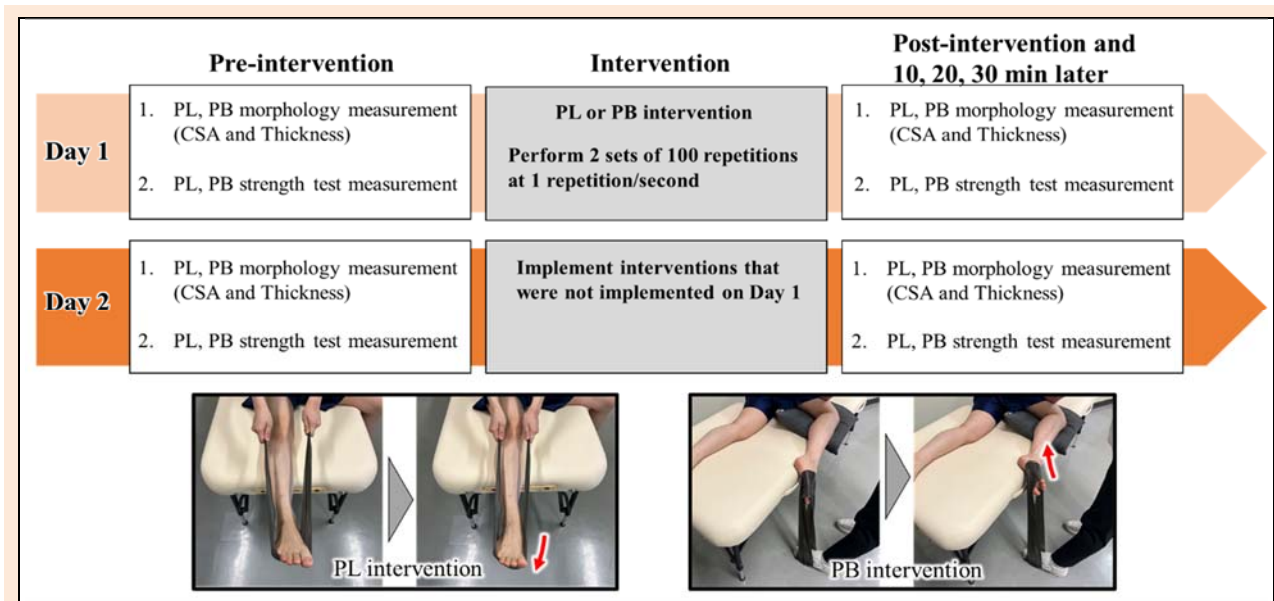


Figure 1. Flowchart of the methodology of this study. Two tasks were performed on separate days: PL intervention in which a Thera-Band was placed on the ball of the foot and pushed out from the contact point, and PB intervention with Thera-Band® pulled out from the base of the fifth metatarsal, emphasizing external rotation and valgus of the ankle. The CSA and thickness of the PL and PB at proximal 25% (indicates the morphology of PL) and distal 75% (indicates the morphology of PB), and PL and PB strength tests were conducted before (pre-intervention) and immediately after the intervention (post-intervention) and at 10, 20, and 30 min later. PL: peroneus longus, PB: peroneus brevis, CSA: cross-sectional area.

Ultrasound measurement of peroneus longus and peroneus brevis morphologies

A B-mode ultrasound imaging system (Teleded, ArtUs EXT - 1H) and probe (Teleded, LF11 - 5H60-A3 [frequency, 5 - 11 MHz; length, 60 mm]) were used to measure the CSA and thickness of the PL and PB. One examiner with at least 3 years of experience in operating the ultrasound imaging system was assigned. The measurement position was placed in a lateral position on the bed. The ultrasound system was used to measure the proximal 25% and distal 75% of the straight line connecting the fibular head and lateral malleolus (Arima et al., 2022). Considering the anatomical location, the PL occupies most of the CSA in the proximal 25% of the measurement points, and the PB

occupies most of the CSA in the distal 75% of the measurement points (Lee et al., 2011). Figure 2 shows the measurement method of the peroneus muscles' CSA and thickness. In the measurement of CSA, the probe was placed perpendicular to the line connecting the fibular head and lateral malleolus. For that of thickness, the probe was rotated 90° from the position at which the CSA was measured. Markings were placed on the measurement points to reproduce the probe position. The probe was coated with sufficient gel and applied to the measurement points with minimal force to capture the images of the CSA and thickness thrice at each measurement point. All measurements were taken thrice per intervention, pre- and post-intervention, and 10, 20, and 30 min after each intervention.

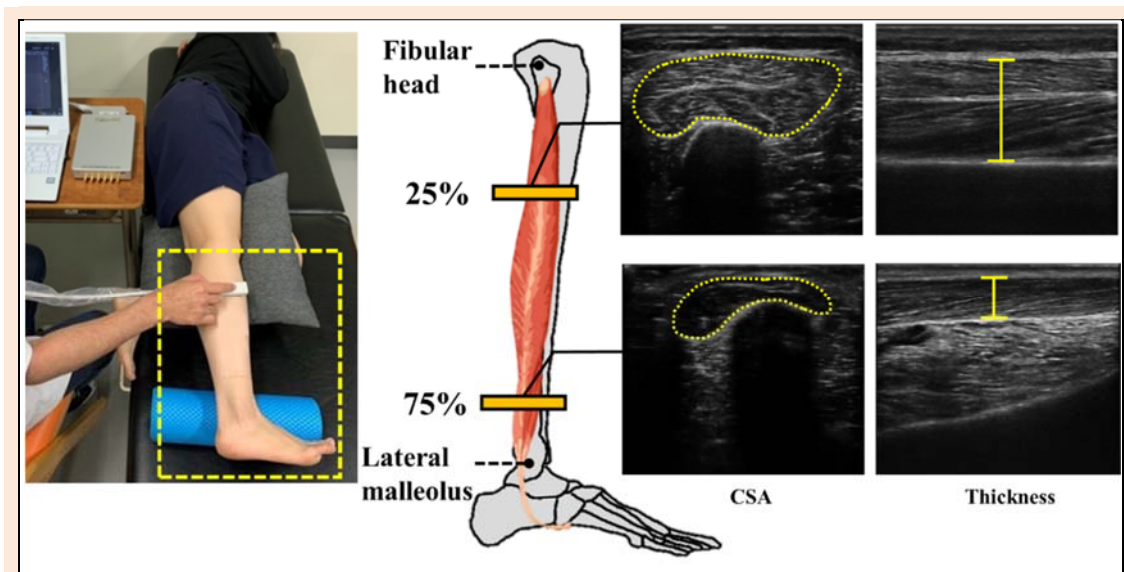


Figure 2. Peroneus muscles CSA and thickness of the proximal 25% (indicates the morphology of PL) and distal 75% (indicates the morphology of PB) in a straight line connecting the fibular head and the lateral malleolus were measured using an ultrasound imaging system. PL: peroneus longus, PB: peroneus brevis, CSA: cross-sectional area.

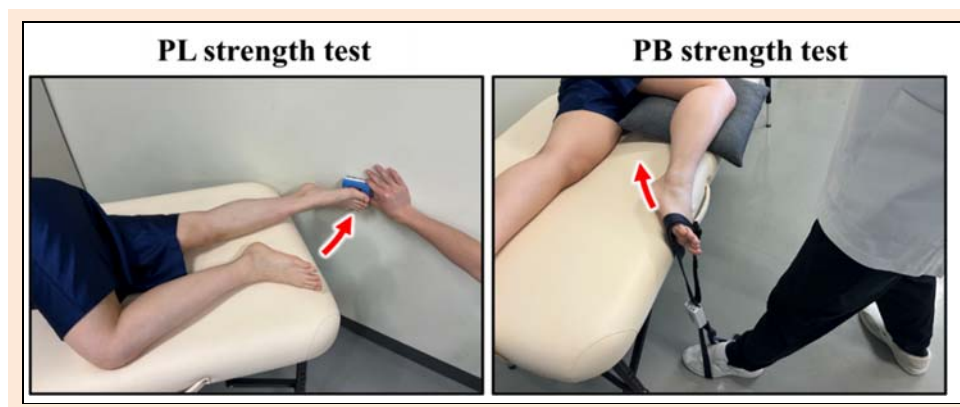


Figure 3. Ankle strength test measurement method, PL strength test: Push out from the ball of the foot toward the handheld dynamometer from the maximum ankle plantar flexed position, PB strength test: In maximum ankle plantar flexion, pull the belt of handheld dynamometer out from the base of the fifth metatarsal. PL: peroneus longus, PB: peroneus brevis

Measurement of ankle strength

Figure 3 shows the method used to measure PL and PB strength tests. Two different types of ankle strength tests were conducted using a handheld dynamometer (Mobie; Sakai Medical Co., Ltd., Tokyo, Japan). The first measured PL strength test, which is the ankle strength test exerted by pushing isometrically with maximal effort for 5 s against the ball of the foot from maximum plantar flexion of the ankle joint in the lateral position. Second, the PB strength test, which is the ankle strength test when the belt of the handheld dynamometer is placed on the base of the fifth metatarsal, was pulled isometrically for 5 s at maximum effort from the maximum plantar flexion position of the ankle joint in the lateral position (Ahn et al., 2020). The strength to which this study refers is a quantified value of the force exerted in one direction, obtained by using a hand-held dynamometer. Measurements were taken pre-intervention and post-intervention, and at 10, 20, and 30 min after each intervention, taking care to limit the number of measurements to three so as not to affect muscle morphology as much as possible. The measurements were taken in a random order each time. The values obtained with the handheld dynamometer were divided by body weight to obtain the ankle strength per body weight (N/kg).

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 28.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The normality of measurements was confirmed using the Shapiro-Wilk test for CSA and thickness at the proximal 25% and distal 75% and PL and PB muscle strengths. When normality was found, homoscedasticity was assessed using Levene's test. If the sphericity assumption was violated, a Greenhouse-Geisser adjustment was performed.

Intra-rater reliability of CSA and thickness at the proximal 25% and distal 75%, and PL and PB strength tests were calculated by intra-class correlation coefficient (ICC) (1,3) based on values obtained from three measurements. The standard error of measurement and minimum detectable change were determined to estimate measurement error.

All measurements were evaluated using a paired *t*-test to ensure that the values measured in the pre-interven-

tion were not different between the PL and PB interventions.

A two-way repeated-measures analysis of variance (ANOVA) was conducted to identify differences in the effects of interventions on the PL and PB. In this analysis, the within-group variable was time (pre-intervention, post-intervention, 10, 20, and 30 min after each intervention), and the between-group variable was the type of intervention (PL vs. PB intervention). The absolute change from pre-intervention (Δ) for all measurements was used as the value in this analysis. The main and interaction effects were assessed for within- and between-group variables. Multiple comparisons with Bonferroni correction were performed as post-hoc comparisons when significant interaction was found to determine the point where there was a difference compared with pre-intervention in the within-group variables. The significance level was set at 5%. Partial eta squared (η^2) was used to determine the index of effect size. Small, medium, and large effect sizes were set at 0.01, 0.06, and 0.14, respectively (Richardson, 2011).

Post-hoc power analysis was performed using G power 3.1.9.2 (Kiel University, Germany). The main consideration of this study was to assess whether CSA or thickness in the PL and PB is selectively hypertrophied by different interventions in the interaction of the within-group (time) and between-group (intervention) variables. Using $\eta^2 = 0.311$ for the proximal 25% thickness value, which was the smallest value among the CSA or thickness interaction results in this study, the effect size *f* was calculated to be 0.67 by G power. The input parameters were as follows: effect size *f* = 0.67; α err prob = 0.05; total sample size = 22; number of groups = 2; number of measurements = 5; Corr among rep measures = 0.5; nonsphericity correction $\epsilon = 1$; and power ($1 - \beta$ err prob) = 1.0, confirming sufficient power.

Results

The ICC (1,3) results for CSA and thickness at the proximal 25% and distal 75% and PL and PB strength tests are shown in Table 1. The ICCs (1,3) of all measurements corresponded almost perfectly (Kappa statistic = 0.81-1.00) to those classified by Landis et al. (Landis and Koch, 1977). A paired *t*-test to verify that pre-intervention measurement

values did not differ from intervention to intervention confirmed that there was no difference in pre-intervention measurements between the PL and PB interventions for all measures.

The results of the two-way repeated-measures ANOVA are presented in Table 2. Significant time and intervention main effects were found in the proximal 25% and distal 75% of CSA, thickness, and PL and PB strength tests ($p < 0.05$). There was also a significant interaction between time and intervention in the proximal 25% and distal 75% of CSA, thickness, and PL and PB strength tests.

The results of the post-hoc comparison are shown in Figure 4. For the proximal 25% CSA, only the PL intervention showed significantly higher CSA at post-intervention, 10 and 20 min compared to pre-intervention. ($p < 0.05$; pre-intervention vs. post-intervention, $53.2 \pm 30.7 \text{ mm}^2$; 10 min, $28.3 \pm 20.3 \text{ mm}^2$; 20 min, $13.6 \pm 17.8 \text{ mm}^2$), but no significant difference was observed 30 min after the PL intervention (pre-intervention vs. 30 min, $4.0 \pm 9.4 \text{ mm}^2$). Regarding the distal 75% CSA, only PB intervention resulted in significantly higher CSA at post-intervention, 10 and 20 min compared with pre-intervention ($p < 0.05$; pre-intervention vs. post-intervention, $33.6 \pm 16.0 \text{ mm}^2$; 10 min, $16.4 \pm 9.2 \text{ mm}^2$; 20 min, $8.3 \pm 8.1 \text{ mm}^2$), but no significant difference was observed 30 min after the PB intervention (pre-intervention vs. 30 min, $1.8 \pm 3.2 \text{ mm}^2$). For the proximal 25% thickness, only PL intervention resulted in significantly higher thickness at post-intervention compared with pre-intervention ($p < 0.05$; pre-intervention vs. post-intervention, $0.79 \pm 0.65 \text{ mm}$), with no significant difference at 10, 20, and 30 min after the PL intervention (pre-intervention vs. 10 min, $0.24 \pm 0.42 \text{ mm}$; 20 min, $0.08 \pm 0.38 \text{ mm}$; 30 min, $-0.03 \pm 0.32 \text{ mm}$). For the distal 75% thickness, only PB intervention resulted in significantly higher thickness at post-intervention, 10 and 20 min compared with pre-intervention ($p < 0.05$; pre-intervention vs. post-intervention, $0.88 \pm 0.89 \text{ mm}$; 10 min, 0.55 ± 0.59

mm; 20 min, $0.37 \pm 0.49 \text{ mm}$), with no significant difference at 30 min after the PB intervention (pre-intervention vs. 30 min, $0.18 \pm 0.42 \text{ mm}$). Regarding PL strength test, only PL intervention resulted in significantly lower strength at post-intervention and 10 min compared with pre-intervention ($p < 0.05$; pre-intervention vs. post-intervention, $-0.14 \pm 0.05 \text{ N/kg}$; 10 min, $-0.08 \pm 0.03 \text{ N/kg}$), and no significant difference was found at 20 and 30 min after PL intervention (pre-intervention vs. 20 min, $-0.02 \pm 0.05 \text{ N/kg}$; 30 min, $0.00 \pm 0.02 \text{ mm}^2$). Regarding PB strength test, only PB intervention resulted in significantly lower strength at post-intervention, 10 and 20 min compared with pre-intervention ($p < 0.05$; pre-intervention vs. post-intervention, $-0.31 \pm 0.15 \text{ N/kg}$; 10 min, $-0.17 \pm 0.10 \text{ N/kg}$; 20 min, $-0.07 \pm 0.06 \text{ N/kg}$), but no significant difference was found at 30 min after the PB intervention (pre-intervention vs. 30 min, $-0.01 \pm 0.02 \text{ N/kg}$).

Discussion

This study aimed to examine the acute effects of two different muscle strength exercise methods on the morphologies and strength of PB and PL, and to examine the selective PL and PB strength exercise methods.

The most remarkable result of this study was the significant interaction between time and intervention in CSA and thickness in the proximal 25% and distal 75%, indicating that PL and PB interventions result in different changes in PL and PB morphologies. Post-hoc comparisons showed that CSA and thickness increased in the proximal 25% only during PL intervention and in the distal 75% only during PB intervention immediately after the intervention, followed by a decrease in values and a trend toward pre-intervention. Transient muscle volume increases due to edema immediately after strength exercise indicates an acute effect of strength exercise (Vieira et al., 2018).

Table 1. ICC (1,3) for the cross-sectional area and thickness at the proximal 25% and distal 75%, and PL and PB strength tests.

		ICC _{1,3}	95% CI	SEM	MDC	p-value
Cross-sectional area (mm ²)	Proximal 25%	0.93	0.86 - 0.97	16.37	45.37	<0.001
	Distal 75%	0.97	0.96 - 0.99	9.07	25.15	<0.001
Thickness (mm)	Proximal 25%	0.92	0.85 - 0.96	0.58	1.60	<0.001
	Distal 75%	0.95	0.91 - 0.98	0.38	1.06	<0.001
Ankle strength (N/kg)	PL strength test	0.92	0.84 - 0.96	0.06	0.16	<0.001
	PB strength test	0.94	0.88 - 0.97	0.12	0.34	<0.001

ICC: intraclass correlation coefficients, ICC (95% CI: confidence interval), SEM: standard error of measurement, MDC: minimal detectable change, PL: peroneus longus, PB: peroneus brevis.

Table 2. Two-way repeated-measures ANOVA results of peroneus muscles cross-sectional area and thickness at proximal 25% and distal 75%, and PL and PB strength tests

		Main effect (Time)			Main effect (Intervention)			Interaction (Time*Intervention)		
		F	p-Value	η^2	F	p-Value	η^2	F	p-Value	η^2
Cross-sectional area (mm ²)	Proximal 25%	56.00	<0.001	0.727	44.02	<0.001	0.677	58.70	<0.001	0.737
	Distal 75%	77.15	<0.001	0.786	84.81	<0.001	0.802	82.72	<0.001	0.798
Thickness (mm)	Proximal 25%	8.07	<0.001	0.278	6.20	0.021	0.228	9.49	<0.001	0.311
	Distal 75%	15.13	<0.001	0.419	11.04	0.003	0.345	9.67	<0.001	0.315
Ankle strength (N/Kg)	PL strength test	66.71	<0.001	0.761	88.32	<0.001	0.808	70.86	<0.001	0.771
	PB strength test	55.48	<0.001	0.725	84.19	<0.001	0.800	70.99	<0.001	0.772

Mean \pm SD, p-Value < 0.05, PL: Peroneus longus, PB: Peroneus brevis, F: F-Value, η^2 : Partial eta-squared. The within-group variable was the time (pre-intervention, post-intervention, 10 min, 20 min, 30 min) and the between-group variable was intervention (PL intervention vs PB intervention).

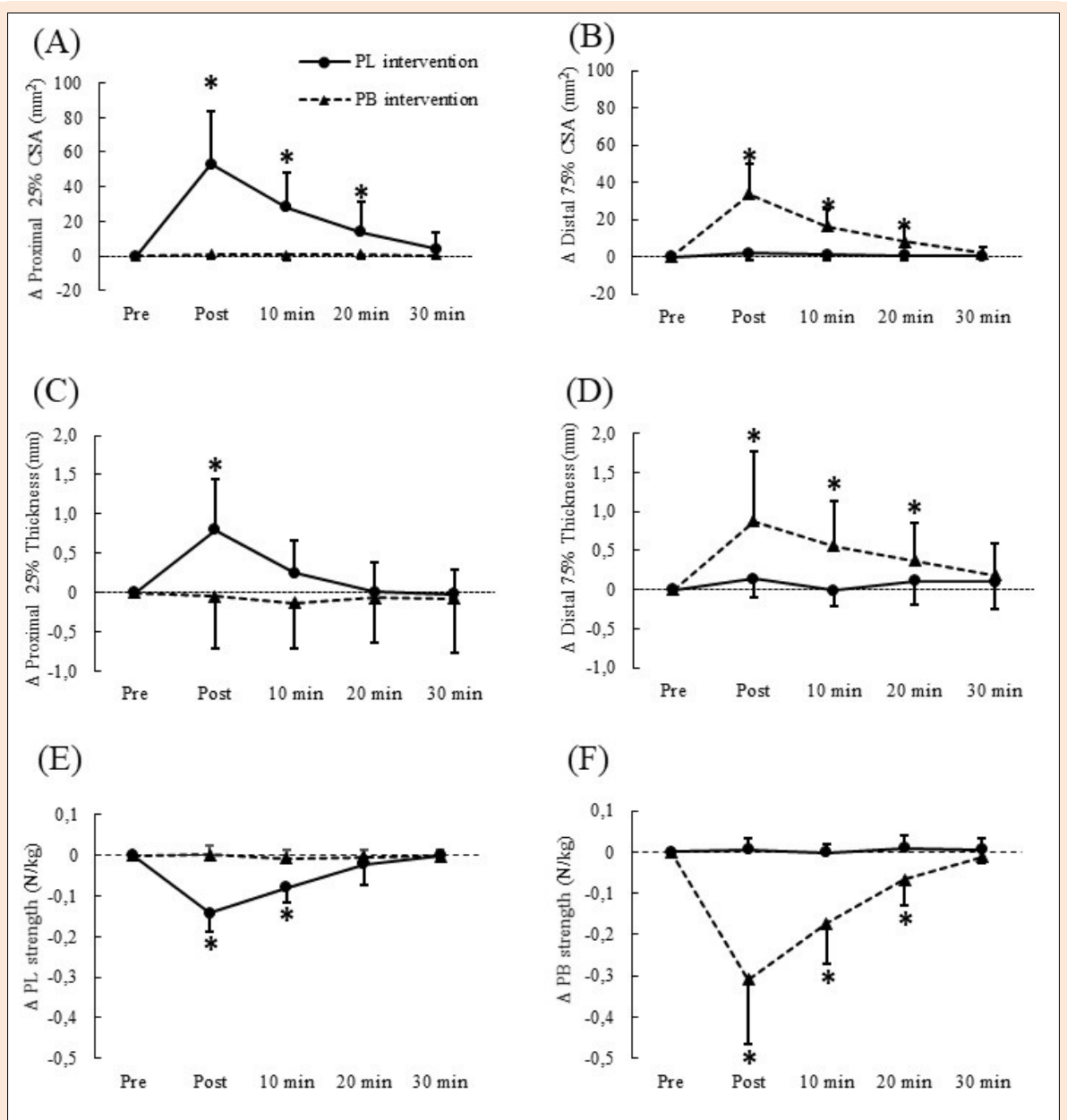


Figure 4. Absolute change results for (A) proximal 25% CSA, (B) distal 75% CSA, (C) proximal 25% thickness, (D) distal 75% thickness, (E) PL strength test, and (F) PB strength test immediately after intervention (post-intervention), 10, 20, and 30 min after the intervention. $p < 0.05$: vs pre-intervention; All comparisons were made with pre-intervention within the group. CSA: Cross-sectional area, PL: Peroneus longus, PB: Peroneus brevis, Δ : Absolute change from pre-intervention.

The increase in CSA and thickness immediately after PL and PB interventions observed in this study, as in previous studies, may also be due to the transient increase in muscle volume immediately after strength exercise. Therefore, our results suggest that PL intervention can selectively strengthen the proximal 25%, where the PL makes up most of the CSA, and PB intervention can selectively strengthen the distal 75%, where the PB makes up most of the CSA. In detail, PL and PB have different roles in the foot and ankle (Davda et al., 2017). For example, in the case of lateral ankle sprain, we have previously reported that the morphology and function of the PL and PB are different in individuals with a history of lateral ankle sprain compared to healthy individuals (Arima et al., 2022). This suggests that PL and PB have different actions and roles within the same peroneus muscles, but that the morphology and function of

the PL and PB may be altered separately in certain foot and ankle injuries. Therefore, in clinical practice, the morphological and functional changes of the PL and PB should be evaluated separately, rather than lumping them together as peroneus muscles. Then, strength exercises should be performed selectively on the PL and PB or both muscles, not only to treat foot and ankle injuries but also to improve muscle function and prevent injuries in healthy individuals.

In this study, two different PL and PB strength tests were measured using the handheld dynamometer to examine not only changes in muscle morphology before and after the intervention by the ultrasound imaging system but also changes in power output during ankle movements, such as the PL and PB interventions. The results showed a significant interaction between time and intervention for both tests. Post-hoc test results showed that the values of

