



OPEN Differences in ankle stabilizing function between the upper and lower fiber bundles of the anterior talofibular ligament: an anatomical study

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This study aimed to clarify the differences in the stabilizing functions of the upper and lower fiber bundles of the anterior talofibular ligament (ATFL). Five Thiel-fixed cadavers (10 ft) were divided into an upper fiber bundle cut group (upper-cut group, 5 ft) and a lower fiber bundle cut group (lower-cut group, 5 ft). The angular conditions were set as 0-degree ankle dorsiflexion, 15-degree plantar flexion, and 30-degree plantar flexion. Anterior drawer and inversion stresses were applied using a Telos stress device at 120 N. Measurements were obtained when the upper and lower fiber bundles of the ATFL were intact (intact condition) and when either the upper or lower fiber bundle was severed (cut condition). For each group, the distance between the lateral malleolus and talus was measured using an ultrasound diagnostic device under both the intact and cut conditions and compared. Regarding anterior drawer stress, the displacement rate (%) significantly increased in both the upper- and lower-cut groups under the cut condition at 30-degree plantar flexion. Meanwhile, for the inversion stress, only the upper-cut group demonstrated a significantly increased displacement rate (%) under the cut condition at 0-degree dorsiflexion. No significant difference in either anterior drawer stress or inversion stress at any angle was observed between the two groups. Both the upper and lower fiber bundles of the ATFL may be involved in stabilizing ankle function during anterior drawer stress at 30-degree plantar flexion. In addition, the lower fiber bundle may play a role in stabilizing the ankle during inversion stress at 0-degree dorsiflexion.

Keywords Anterior talofibular ligament, Lateral collateral ligament injury, Ultrasound, Joint, Instability

Lateral collateral ligament injury of the ankle accounts for approximately 77% of all ankle injuries¹ and is one of the most common types of trauma in sports and daily life^{2,3}. Among lateral collateral ligament injuries, isolated damage to the anterior talofibular ligament (ATFL) is the most frequent, accounting for 66%–85%^{4,5}, with isolated injury of the upper fiber bundles representing approximately 91%⁶. Additionally, approximately 20%–40% of cases involve a combined injury of the ATFL and calcaneofibular ligament (CFL), whereas isolated CFL and posterior talofibular ligament (PTFL) injuries are very rare⁷. The morphological characteristics of the lateral ankle ligaments may play a significant role in these injuries, and numerous anatomical studies have been reported^{8–22}.

The lateral collateral ligaments of the ankle comprise the ATFL, CFL, and PTFL⁸. When observed from the superficial layer, in all cases, the ATFL and CFL are continuous at the fibula (100%)^{9,10}. Moreover, the arciform fiber connecting the ATFL and CFL is present in the superficial layer of all ankles (100%)^{11,12}, and the lateral talocalcaneal ligament has been identified in 42%–58% of cases^{13,14}. In addition, when the fibula was removed and inverted, the ATFL, CFL, and PTFL were interconnected at the anteroinferior border of the lateral malleolus, forming an anatomically very close relationship^{10,15,22}.

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Regarding the ATFL, anatomical studies with large sample sizes have reported individual variations including the presence of one-, two-, and three-fiber bundle types, with the two-fiber bundle type being the most common^{9–11}. The two-fiber bundle comprises upper and lower fiber bundles, whereas the three-fiber bundle consists of upper, middle, and lower fiber bundles, with the upper fiber bundle being longer and wider than the others⁹. A study analyzing the mechanical function of the ATFL using fresh cadavers¹⁶ has reported that when the ATFL was considered a single ligament, tension occurred at 20 degrees of ankle plantar flexion, with increasing tension as the plantar flexion angle increased, and the greatest tension occurred during ankle external rotation. Additionally, the internal rotation of the talus is mainly controlled by the ATFL, with only a minimal contribution from the CFL and PTFL¹⁷. More recently, studies have examined the function of each fiber bundle of the ATFL, suggesting that in two- and three-fiber bundle types, the upper and lower fiber bundles have different functions, with implications for their relationship with partial tears of the ATFL^{18–20}. However, these studies are limited to discussions based on anatomical findings or simulations.

Moreover, isolated damage to each fiber bundle could cause micro-instability or chronic anterolateral ankle pain, which reportedly occurs in 30%–40% of patients after an ankle sprain^{20,23,24}. Therefore, the differences in the function of each fiber bundle in the ATFL need to be clarified.

Dalmau-Pasto et al.²¹ examined the effects of severing only the upper fiber bundle of the ATFL and both fiber bundles on the talocrural joint stability. They have reported that when only the upper fiber bundle was severed, joint stability decreased in the plantar flexion position, and when both fiber bundles were severed, joint stability decreased in both the plantar flexion and dorsiflexion positions. However, this previous study only investigated the effects of isolated severance of the upper fiber bundle of the ATFL; the effects of isolated severance of the lower fiber bundle on talocrural joint stability remain unclear. Considering the morphological characteristics of the upper and lower fiber bundles of the ATFL and the anatomical relationship between the ATFL and the CFL, functional differences may exist between the two fiber bundles.

This study aimed to clarify the impact of the upper and lower fiber bundles of the ATFL on the stabilizing function of the talocrural joint when anterior drawer stress and inversion stress were applied using Thiel-fixed cadavers and a Telos stress device (Telos SE; Aimedica MMT, Japan). Based on morphological characteristics, we hypothesized that during anterior drawer stress, the upper fiber bundle of the ATFL affects stability in the plantarflexed position, whereas the lower fiber bundle affects stability in the dorsiflexed position. During inversion stress, we hypothesized that only the lower fiber bundle would affect stability.

Results

For all angles, the ICC was above 0.98 for anterior drawer stress and above 0.97 for inversion stress. According to the criteria described by Landis et al.²⁵, these results were classified as “almost perfect,” confirming adequate reproducibility (Table 1).

For the anterior drawer stress, both the upper- and lower-cut groups showed a significant increase in the separation rate in the cut condition compared to that in the intact condition at 30-degree plantar flexion (Table 2). No significant differences for the other angles were observed. For inversion stress, no significant differences were observed between conditions in the upper-cut group; however, in the lower-cut group, only at dorsiflexion 0 degree did the separation rate significantly increase in the cut condition compared to that in the intact condition (Table 3). No significant differences in either anterior drawer stress or inversion stress at all angles were noted between the upper- and lower-cut groups (Tables 2 and 3, respectively).

Discussion

This study aimed to clarify the roles of the upper and lower fiber bundles of the ATFL in ankle joint stability under anterior drawer and inversion stresses using Thiel-embalmed cadavers and a Telos stress device. The main findings of this study are twofold. First, under anterior drawer stress, both the upper- and lower-cut

Stress method	Ankle joint angle	Stress strength (N)	First (mm)	Second (mm)	ICC (1,3)	Reliability	MDD95%
Anterior drawer stress	0-degree dorsiflexion	0	19.14 ± 0.14	19.02 ± 0.16	0.99	Almost perfect	0.11
		120	19.96 ± 0.13	19.72 ± 0.15	0.98	Almost perfect	0.10
	15-degree plantar flexion	0	20.15 ± 0.06	20.13 ± 0.23	0.99	Almost perfect	0.11
		120	20.92 ± 0.21	20.62 ± 0.22	0.99	Almost perfect	0.17
	30-degree plantar flexion	0	20.45 ± 0.19	20.76 ± 0.21	0.99	Almost perfect	0.16
		120	20.94 ± 0.23	20.96 ± 0.16	0.99	Almost perfect	0.15
Inversion stress	0-degree dorsiflexion	0	19.58 ± 0.18	19.41 ± 0.18	0.99	Almost perfect	0.14
		120	20.26 ± 0.21	20.19 ± 0.16	0.99	Almost perfect	0.14
	15-degree plantar flexion	0	18.93 ± 0.09	19.02 ± 0.22	0.98	Almost perfect	0.12
		120	19.71 ± 0.13	19.75 ± 0.11	0.98	Almost perfect	0.09
	30-degree plantar flexion	0	19.63 ± 0.20	19.69 ± 0.17	0.99	Almost perfect	0.15
		120	20.07 ± 0.12	20.18 ± 0.18	0.97	Almost perfect	0.12

Table 1. Inter-session reliability and MDD95% of the anterior drawer stress and Inversion stress. $n = 10$. Values are presented as means ± standard deviations. ICC intraclass correlation coefficient, MDD95% minimal detectable difference at the 95% confidence interval.

Ankle joint angle	Superior cut group		Inferior cut group	
	Intact condition	Cut condition	Intact condition	Cut condition
0-degree dorsiflexion	3.04 ± 2.04	4.62 ± 4.94	3.43 ± 3.65	2.32 ± 0.72
15-degree plantar flexion	3.64 ± 2.51	5.31 ± 4.13	2.04 ± 1.95	2.00 ± 1.16
30-degree plantar flexion	2.17 ± 1.48	6.61 ± 3.41 ^a	2.32 ± 1.41	3.44 ± 0.87 ^b

Table 2. Comparison of the lateral malleolus–talus distance between the intact and cut conditions in Anterior drawer stress. Values are presented as means ± standard deviations. ^a $P=0.013$ vs. intact condition in the superior cut group. ^b $P=0.04$ vs. intact condition in inferior cut group.

Ankle joint angle	Superior cut group		Inferior cut group	
	Intact condition	Cut condition	Intact condition	Cut condition
0-degree dorsiflexion	3.38 ± 1.44	4.39 ± 2.28	1.36 ± 0.91	3.01 ± 0.80 ^a
15-degree plantar flexion	2.26 ± 1.15	3.82 ± 2.92	2.09 ± 1.26	3.39 ± 2.12
30-degree plantar flexion	2.33 ± 2.07	3.89 ± 3.22	2.69 ± 0.73	3.46 ± 2.35

Table 3. Comparison of the lateral malleolus–talus distance between the intact and cut conditions in Inversion stress. Values are presented as means ± standard deviations. ^a $P=0.049$ vs. intact condition in the inferior cut group.

groups demonstrated significantly increased separation rate in the cut condition compared to that in the intact condition only at 30-degree plantar flexion. Furthermore, no significant differences at any angle were observed between the upper- and lower-cut groups. Second, under inversion stress, only the lower-cut group at 0-degree dorsiflexion demonstrated a significantly increased separation rate in the cut condition compared to that in the intact condition.

Thus, under anterior drawer stress, both the upper- and lower-cut groups exhibited significantly increased separation rates in the cut condition compared to that in the intact condition only at 30-degree plantar flexion. Furthermore, no significant differences at any angles were observed between the two groups. These results contradicted our initial hypotheses. Based on anatomical findings⁹, the upper fiber bundle of the ATFL is longer and wider than the lower fiber bundle, and the angle with the sagittal plane differs. We hypothesized that the two-fiber bundles would behave differently. However, both the upper and lower fiber bundles of the ATFL insert at adjacent sites on the anterior border of the lateral malleolus and the neck of the talus^{8,12}. Despite observing statistically significant differences, the difference was only approximately 3 mm in length, approximately 2 mm in width, with an angle of the sagittal plane of approximately 5–10 degrees (approximately 20–30 degrees)⁹. However, these differences are relatively small. Additionally, mechanical studies have shown that when considering the ATFL as a single ligament, its tension increases as the ankle dorsiflexion angle increases²⁶, and the tension becomes stronger as the plantar flexion angle increases¹⁶. Therefore, the results of this study suggest that the increase in the separation rate only at 30-degree plantar flexion and the lack of significant differences between the two groups may be attributed to these anatomical and mechanical factors.

By contrast, anatomical studies have suggested that the upper fiber bundle relaxes during ankle dorsiflexion, whereas the lower fiber bundle and arcuate fibers connecting it to the CFL maintain isometric tension throughout both the dorsiflexion and plantar flexion ranges²⁰. Additionally, previous studies using simulations have reported that in all types of ATFL (single-, two-, and three-fiber bundles), the upper fiber bundle stretches during both dorsiflexion and plantar flexion, whereas the lower fiber bundle shortens during plantar flexion and stretches during dorsiflexion¹⁸. Hence, the functions of the upper and lower fiber bundles may differ, although simulation-based research has limitations. Furthermore, a study using fresh cadavers has reported that the upper fiber bundle of the ATFL is particularly important for ankle stability, serving to inhibit both internal rotation and anterior displacement of the talus, using a robotic system with six degrees of freedom²¹. However, the role of lower fiber bundles has not been thoroughly examined. Based on these findings, both the upper and lower fiber bundles of the ATFL may contribute to resisting anterior drawer stress in the plantarflexed position.

Regarding inversion stress, an increase in the separation rate was observed only at 0-degree dorsiflexion after cutting the lower fiber bundle of the ATFL. Anatomical studies have reported that in all cases, the ATFL and CFL are continuous at the fibular portion (100%)^{9,10} and that arciform fibers connecting the ATFL and CFL are present in the superficial layer of all ankles (100%)^{11,12}. In biomechanical studies, one previous study observed that the CFL becomes taut at dorsiflexion angles more than 18 degrees and is nearly relaxed at other angles²⁷. Furthermore, this study argued that the changes in ligament length during ankle flexion and extension were slight and that the CFL likely plays a major role in stabilizing ankle flexion and extension²⁸. However, although Sarrafian and Kelikian²⁹ have reported that the CFL is taut in dorsiflexion and relaxed in plantar flexion, they have also noted that some specimens showed a reversal of motion, whereas in others, the tension in this ligament remained constant in all positions. Thus, no consensus on the CFL function has been established. In our previous study³⁰, three-dimensional CFL reconstructions were used to simulate and examine the differences in the angles of the CFL considering the long axis of the fibula and how they affect CFL function. The CFL function changed according to the difference in the angles of the CFL with respect to the long axis of the fibula. Specimens in the

CFL20-degree and CFL30-degree groups contracted with plantar flexion and were stretched with dorsiflexion. By contrast, the specimens in the CFL40-degree, CFL50-degree, and CFL2-degree groups were stretched with plantar flexion and contracted with dorsiflexion. In the present study, all the specimens belonged to the CFL20-degree and CFL30-degree groups. Therefore, cutting the lower fiber bundle of the ATFL, which is continuous with the CFL, resulted in a decrease in CFL tension, leading to an increase in the separation rate during inversion stress at 0-degree dorsiflexion.

This study had some limitations. First, Thiel-fixed cadavers were used. Although they have maintained flexibility and biomechanical properties similar to those of living organisms^{31,32}, whether similar results can be obtained in patients with ATFL injuries in future studies needs to be determined. Second, as all cadavers were from elderly individuals around 90 years old, whether the present findings apply to individuals without soft tissue degeneration and reflect the actual situation of the human body is uncertain. Third, the sample size was small. Therefore, anatomical variations may have influenced the results. Fourth, to identify the individual fiber bundles of the ATFL, skin, subcutaneous tissue, and part of the joint capsule were excised. The removal of these soft tissues may have influenced joint stability. However, because the intact condition was set after excising these soft tissues in this study, the impact of soft tissue removal on the results was likely small. Fifth, this study did not measure rotational instability. A previous study showed that the upper and lower fiber bundles of the ATFL contribute to restraining internal rotation and anterior translation of the talus during plantarflexion, and that the entire ATFL is involved in restraining anterior translation, internal rotation, and inversion of the talus²¹. In addition, tests involving internal rotation, such as the anterolateral drawer test^{33,34} and the reverse anterolateral drawer test^{35,36}, have been proposed. Future investigations are needed to examine rotational instability related to the upper and lower fiber bundles of the ATFL. Lastly, as this study only sought to clarify the role of ligaments as static stabilizing factors, dynamic stabilizing factors such as muscle groups were not evaluated.

Methods

Cadavers

Ten ankles from five Japanese Thiel-embalmed cadavers (mean age at death, 90 ± 3 years; eight males and two females; all two-fiber bundle types) donated to a university anatomy program were evaluated. Formalin-fixed cadavers used for gross anatomical studies can be dissected; however, their mobility is limited, making it difficult to evaluate the dynamics of the suprapatellar bursa and other parts of the body. In this study, we focused on Thiel-fixed cadavers, which can be dissected, as well as formalin-fixed cadavers^{37,38} which have mobility and mechanical properties similar to those of living human bodies^{31,32}. The exclusion criteria were as follows: presence of surgical or traumatic history in the foot and ankle, ankle plantar flexion range of motion of less than 30 degrees, ankle dorsiflexion range of motion of less than 0 degree, and difficulty in setting the measurement position.

Anatomical procedure

First, an incision of approximately 2 cm in diameter was created in the skin over the anterolateral aspect of the ankle, and the upper and lower fiber bundles of the ATFL were identified after removing some of the subcutaneous tissue and part of the lower extensor retinaculum. Next, the joint capsule and soft tissues were carefully dissected and the upper and lower fiber bundles of the ATFL were exposed (Fig. 1).

Experimental protocol

The specimens were divided into two groups as follows: one in which only the upper fiber bundle of the ATFL was severed (upper-cut group, five specimens), and one in which only the lower fiber bundle of the ATFL was severed (lower-cut group, 5 specimens). Measurements were obtained under two conditions: the intact condition, in which the upper and lower fiber bundles of the ATFL were exposed, and the cut condition, in which either the



Fig. 1. Example of anatomic dissection of lateral ankle ligaments through the skin window. **A** Foot and ankle joint. **B** Enlarged figure of the area enclosed in the square in **A**. 1: Anterior talofibular ligament of superior fascicles. 2: Anterior talofibular ligament inferior fascicles. 3: Calcaneofibular ligaments.

upper or lower fiber bundle of the ATFL was severed. The severance points for the upper and lower fiber bundles of the ATFL were set at the middle of the fiber bundle length of each bundle.

Experimental procedure

An ultrasound diagnostic device (Aplio500, Toshiba Medical Systems, Tochigi, Japan) with a 10-MHz linear probe was used to evaluate the instability of the talocrural joint. The stress load and limb positioning were fixed using a Telos stress device (Telos SE; Aimedica MMT, Japan). Stress loading was performed using two methods: anterior drawer and inversion stress. For anterior drawer stress, the specimen was placed in a side-lying position with the knee joint flexed at 30 degrees, and forward pulling stress was applied approximately 3 cm proximal to the lateral malleolus^{39,40}. For inversion stress, the specimen was placed in the supine position with the knee joint flexed at 30 degrees, and eversion stress was applied approximately 5 cm proximal to the medial malleolus^{40,41}. Both anterior drawer and inversion stresses were applied under three conditions: dorsiflexion at 0 degree, plantar flexion at 15 degrees, and plantar flexion at 30 degrees, with the stress intensity set to 120 N^{39–42}. Two examiners performed the measurements: one performed the ultrasound evaluation, and the other operated the Telos stress device (Fig. 2).

Ultrasound imaging was performed by placing the probe on the anterolateral aspect of the ankle joint, with the joint surface cartilage of the talocrural joint and talar neck as bone landmarks to capture images (Fig. 3)²⁸. Three ultrasound images were captured within 10 s with the Telos stress device set to a 0 N load. Subsequently, stress was applied at 10 N/s, and three ultrasound images were captured when the load reached 120 N. To account for potential changes in the mechanical properties of the ATFL due to repeated stress, a 10-min interval was allowed when changing the position and a 5-min interval when adjusting the ankle joint angle (Fig. 4).

The images were analyzed using the ImageJ software (NIH), where the distance between the apex of the joint surface cartilage of the talocrural joint and the apex of the neck of the talus was measured as the external malleolus–talus distance (mm). The average of the three measurements of the external malleolus–talus distance



Fig. 2. Position of the foot during measurement. For anterior drawer stress (A), the specimen was fixed in the lateral position with the knee joint flexed at 30 degrees, and anterior drawer stress was applied approximately 3 cm proximal to the lateral malleolus. For inversion stress (B), the specimen was positioned in supine with the knee joint flexed at 30 degrees, and inversion stress was applied approximately 5 cm proximal to the medial malleolus. The angle conditions for both the anterior drawer and inversion stresses included 0-degree dorsiflexion, 15-degree plantar flexion, and 30-degree plantar flexion, with a stress intensity of 120 N.

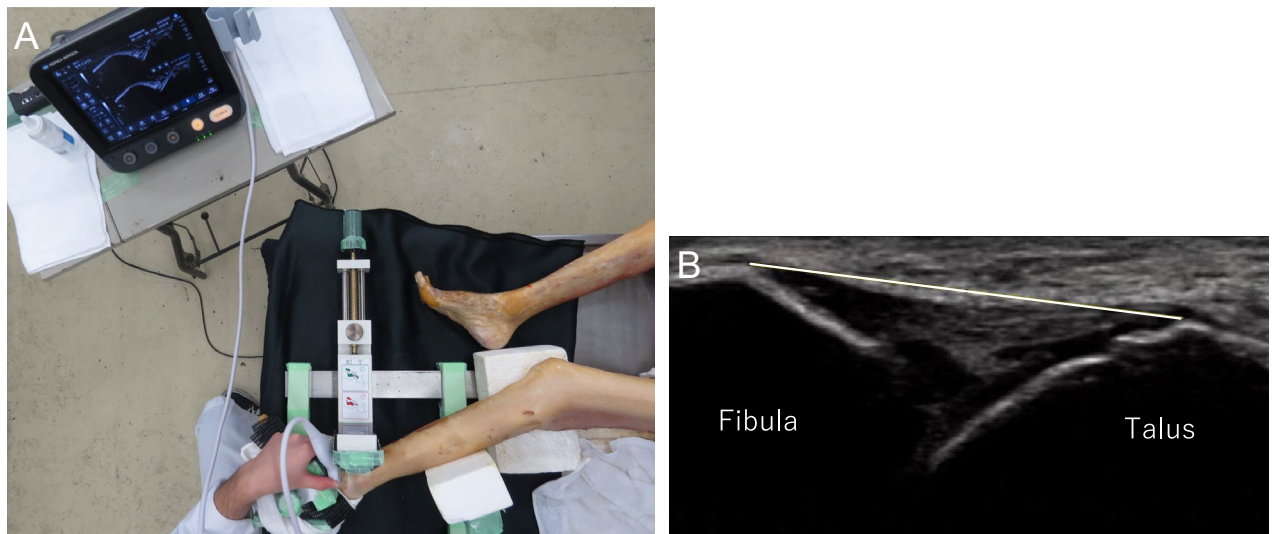


Fig. 3. Ultrasound image for measurement of the anterior talofibular ligament length. The ultrasound image captured directly over the origin and insertion of the anterior talofibular ligament allowed the examiner to use a straight-line measurement tool to draw a line from the anterolateral aspect of the lateral malleolus to the talus, which corresponds to the anatomic attachment sites of the ligament.

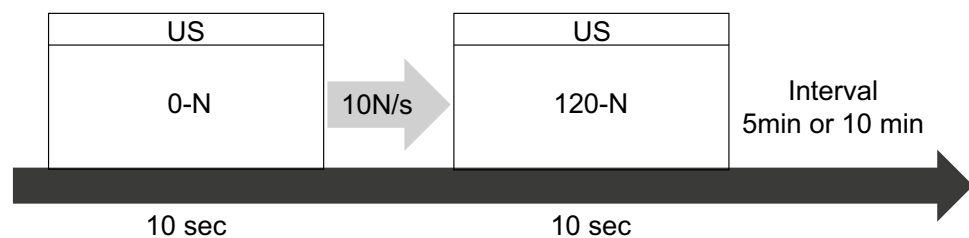


Fig. 4. Protocol for measuring the anterior talofibular ligament length. US: Three ultrasonographic images were obtained within 10 s, and the anterior talofibular ligament length was calculated as the mean value from the three images. 0-N starting limb position, 120-N, 120-N of valgus stress applied using a Telos stress device.

from the captured images was used as the representative value. Based on previous studies^{39,40}, the separation rate (%) of the external malleolus–talus distance at 0 N and 120 N was calculated using the following formula.

$$\text{Separation rate (\%)} = \frac{\{\text{lateral malleolus} - \text{talus distance at 120 N} - \text{lateral malleolus} - \text{talus distance at 0N}\} \times 100}{\text{lateral malleolus} - \text{talus distance at 0 N}}$$

To verify the reproducibility of the measurements, we performed two measurements using the same ultrasonographic evaluation procedure on three bodies (6 ft and one female) without dissection. The second measurement was performed more than 2 h after the first measurement.

Statistical analysis

The reliability of the ultrasonographic evaluation was assessed using the intraclass correlation coefficient (ICC)^{1,3}. Following the criteria of Landis et al.²⁵, an ICC of 0.81 or higher was considered “almost perfect.” The minimum detectable change (MDC95%) was calculated as follows:

$$MDC = \text{standard error} \times \sqrt{2} \times 1.96$$

For all ankle joint angles, the separation rates under the intact and cut conditions were compared using a paired *t* test. For all ankle joint angles, the separation rates between the upper- and lower-cut groups were compared using unpaired *t* tests. The significance level was set at 5%.

Conclusion

Both the upper and lower fiber bundles of the ATFL may be involved in restraining the anterior drawer stress at 30-degree plantar flexion. Additionally, the lower fiber bundle may play a role in restraining the inversion stress at 0-degree dorsiflexion.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available because of limitations of ethical approval involving patient data and anonymity but are available from the corresponding author upon reasonable request.

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Author contributions

M.E. and Y.S. designed the study, collected data, and drafted the manuscript. K.S., T.I. H.Y., and R.H. analyzed the data and made critically revised the manuscript. M.K., C.S., and T.T. made essential revisions to the manuscript. N.S. supervised the study, contributed to data analysis and interpretation, and critically revised the manuscript. All the authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

All experiments were performed in accordance with the 1964 Declaration of Helsinki, and all cadavers were donated for study purposes to the Niigata University Graduate School of Medical and Dental Sciences in Niigata, Japan. This study was approved by the Ethics Committee of the Niigata University Graduate School of Medical and Dental Sciences. Informed consent was obtained from the families of all participants.

Additional information

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