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# The effect of immediate neuromuscular training on ankle biomechanics in individuals with functional ankle instability

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**Abstract:** Functional ankle instability arises from recurrent ankle sprains. Neuromuscular training is employed to enhance ankle stability in individuals who experience functional ankle instability. The study involved 24 male university students with functional ankle instability, undergoing ankle neuromuscular training on three surfaces. The OpenSim musculoskeletal model assessed effects on ankle kinematics, kinetics, and muscle activity. Using one-way repeated measures ANOVA and one-dimensional statistical non-parametric mapping to distinguish differences among training surfaces. The study aimed to compare biomechanical characteristics of ankle motion in individuals with functional ankle instability undergoing immediate neuromuscular training on a foam cushion surface versus training on level-ground and artificial turf. Results showed foam cushion training significantly increased tibialis anterior and gastrocnemius medial activation during walking ( $p < 0.05$ ), with no differences observed in peak ankle plantarflexion, peroneus longus, and gastrocnemius lateral. Foam cushion training further increased activation in four muscles and peak ankle plantarflexion moment during jogging and fast running ( $p < 0.05$ ). Furthermore, foam cushion training reduced subtalar mobility ( $p < 0.05$ ) and showed greater dorsiflexion angles during jogging and fast running ( $p < 0.05$ ). Therefore, immediate ankle neuromuscular training on a foam cushion is more advantageous in enhancing ankle stability among individuals with functional ankle instability, positively impacting functional ankle instability improvement.

**Keywords:** functional ankle instability; neuromuscular training; foam cushion surface; muscle; ankle biomechanics

## 1. Introduction

Correct aerobic exercise is beneficial for our health [1]. Running, as a form of aerobic exercise, has become increasingly popular among the general public due to its convenience and affordability, and has become an important form of exercise [2]. While running offers numerous benefits, there is a certain risk of lower limb injuries due to the fact that runners need to load their own body weight and contend with the ground reaction forces (GRF) on surfaces with varying properties [3]. It is worth noting that ankle sprains are a very common lower limb injury in running [4]. In general, when ankle sprains are not properly treated or rehabilitated, they may lead to chronic ankle instability (CAI), with a subset of CAI patients exhibiting mechanical ankle instability or pathologic laxity of the ankle joint. However, there is also a subset of CAI patients who do not have mechanical laxity but instead exhibit functional ankle instability (FAI) [5,6]. The main feature of FAI is that the mechanically stabilized ankle joint sometimes feels “giving way” during normal

activities [7]. Munn et al. found that FAI patients may experience ankle injuries, loss of motor control, and ankle muscle imbalances during exercise [8]. In addition, Daniel et al. found that individuals with FAI exhibit higher ankle kinematic variability during running [9].

It has been shown that the main preventive methods to reduce ankle injuries are external prophylactic supports and preventive exercise program [10]. The first approach typically involves external support, such as elastic bandages, designed to keep the ankle joint within its natural range of motion during exercise [11]. In the second preventive exercise program, neuromuscular training (NMT) is a commonly used method and has been demonstrated to be effective in reducing the risk of ankle sprains [12]. Ankle NMT is a training method that focuses on the nerves and muscles surrounding the ankle joint to improve stability and function [13,14]. Herb et al. found that strengthening ankle stabilizing muscles could help counteract changes in ankle ligament mechanoreceptor activity, thereby enhancing ankle stability [15]. Previous studies had confirmed that training the ankle stabilizing muscles was usually accomplished through NMT, involving a combination of heel lifts, lunges, jumps, and other training techniques [16]. Previous reports had suggested that long-term interventions were required for NMT of FAI [14]. However, whether immediate NMT can enhance ankle joint stability during movement remains inconclusive.

Specifically, NMT is applicable to stable surfaces and various unstable surfaces [17]. Previous studies have suggested that ankle NMT performed on unstable surfaces may be more beneficial in enhancing ankle stability among patients with FAI compared to training on stable surfaces [18]. Unstable surfaces, such as foam cushion (FC) of different densities, BOSU balls, balance boards, etc., are commonly used for the training of individuals with FAI. These devices rely on deformable surfaces, contributing to postural instability in the human body, which allows for increased core stability and muscle control during exercises performed on them [19]. The Ethylene-vinyl acetate FC, a common FC, and is moderately unstable, making it a good choice for FAI individuals who are choosing an unstable surface to exercise for the first time [20]. However, level-ground (LG) and artificial turf (AT), as the more common NMT surfaces in daily life, exhibit relatively strong stability, especially LG [21].

The aim of this work is to investigate potential differences in the biomechanical characteristics of ankle joint movement in FAI individuals with immediate NMT using FC, compared to training using LG and AT. The hypothesis of this study is that FAI individuals would be able to better activate the ankle stabilizing muscles after an immediate of NMT on FC compared to immediate of NMT on LG and AT, thus providing an efficient ankle stabilization training method for FAI individuals.

## **2. Materials and methods**

### **2.1. Participants**

Under the guidance of a specialized clinician, participants were instructed to complete the Identification of functional ankle instability (IdFAI) scale for their dominant leg [22]. The assessment tool consists of 10 items to assess sensations such

as subjective ankle pain, whether the ankle is unstable during daily activities, and recovery time after an ankle sprain. When the IdFAI score is higher than 10, it is considered indicative of FAI, meeting the requirements for participation in the experiment. During the preceding 6 months, participants had not experienced any significant injuries to their lower limbs, with the right leg identified as dominant (identified by the leg habitually used to kick a football). This study was approved by the Ethics Committee of Ningbo University Research Institute (RAGH202305221185), and obtained written consent from all participants and informed them of the requirements, objectives, and procedures of the experiment.

## 2.2. NMT Program

Before commencing the formal test, subjects were instructed to perform NMT barefooted on various surfaces. The combinations of movements for training were derived from previous reports [16,18], and the five most common, simple and highly beneficial movements were selected as shown in **Table 1**. The NMT programs were marked by guidelines and recommendations set forth by the American College of Sports Medicine. All training exercises were conducted under the supervision of a professional fitness coach.

Plantarflexion (PF) refers to the ankle joint movement that directs the foot downward or away from the shin. Dorsiflexion (DF) is the movement that brings the foot closer to the shin or upward. Lunge is a strength training exercise that involves taking a step forward or backward while keeping the torso upright. Vertical jump is a movement where an individual starts from a stationary standing position and rapidly propels their body upward by bending the hips, knees, and ankles. Lateral jump is a movement that involves jumping horizontally from one side to another.

**Table 1.** NMT program.

Program	Number of times (time)	Group (group)	Interval (s)	Time (min)
PF	15	3	20	3
DF	15	3	20	3
lunge	16	2	60	3
vertical jump	15	2	60	3
lateral jump	15	2	60	3

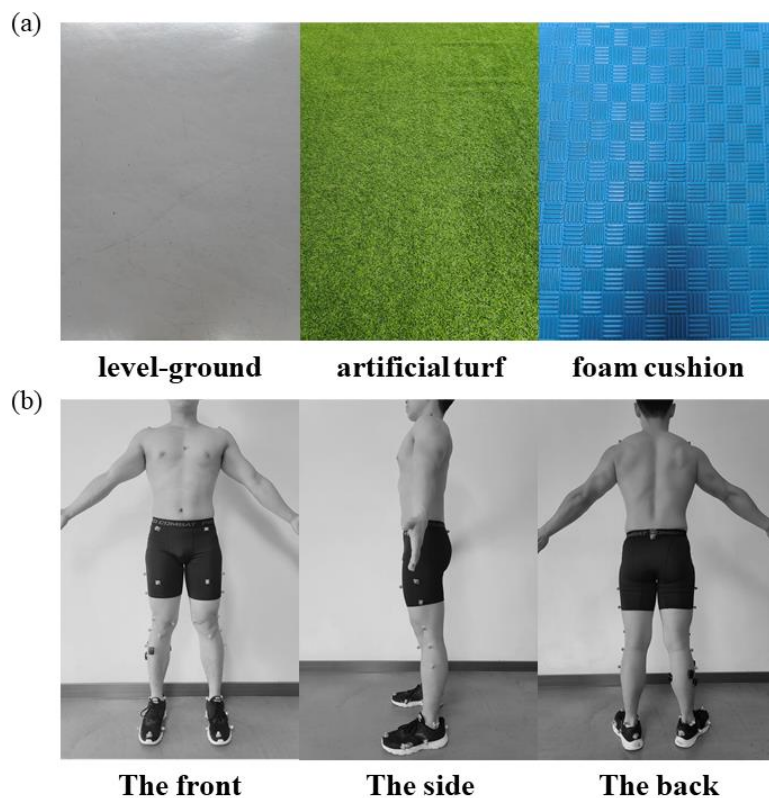
Note: PF indicates plantarflexion, DF indicates dorsiflexion. “Number of times” indicates the number of times each group needs to be completed. “Group” indicates the number of groups to be completed for each program. “Interval” indicates the rest time between two groups. “Time” indicates the total time spent on each program. Lunge, Vertical Jump, and Lateral Jump are sourced from reference [16], the PF and DF are sourced from reference [18].

## 2.3. Experimental design

This experiment consisted of three parts. The initial phase involves obtaining the subject's maximal voluntary contraction (MVC) using the CON-TREX dynamometer (CON-TREX-MJ System, CMV, Dübendorf, Switzerland) [23]. First, the gastrocnemius medial muscle (GMM) and gastrocnemius lateral muscle (GLM) are assessed with the subject while lying on one's back, the limb under study in extension, and immobilized with a knee and mid-foot girdle. The patient is instructed to perform maximal PF for 10 seconds, repeated three times with 30 seconds of rest

between each repetition [24]. The participant actively resists against the force transducer by exerting their maximum force, allowing for the assessment of their muscles' maximal strength performance. Subsequently, the tibialis anterior muscle (TAM) and peroneus longus muscle (PLM) muscles are evaluated in the same position, with the limb immobilized using knee and mid-foot straps. The patient is asked to execute maximal DF for 10 seconds, repeated three times with 30 seconds of rest between each repetition [25,26].

The objective of the second part was to implement the previously described NMT program to participants through various surfaces (**Figure 1a**). Subjects performed ankle NMT in a specified surface as required, barefoot to minimize the possible influence of shoes on the experiment. At the same time, to mitigate the influence of fatigue on the experiment, each participant undergoes only one surface test per day, requiring a total of non-consecutive three days to complete the entire experiment. Additionally, the NMT surfaces for the three days of experimentation are randomly selected from LG, AT, and FC (Among them, LG is a stable surface, AT is a moderately stable surface, and FC is an unstable surface with a density of 85 kg/m<sup>3</sup>) [27].



**Figure 1.** (a) Different degrees of stability with three training surfaces; (b) OpenSim 2392 reflective marker points.

The third part aimed to collect biomechanical data on participants' movements following the NMT intervention in the second part. While conducting the third part of the experiment, all subjects wore uniformly provided leggings and footwear. Thirty-eight reflective markers (12.5 mm in diameter) were set up to be fixed on each participant according to the requirements of the OpenSim (Stanford University,

Stanford, CA, USA) model of gait 2392, and the markers were affixed with tape by the same proficient examiner. (**Figure 1b**). The lab was furnished with an eight-camera Vicon (Vicon Metrics Ltd., Oxford, UK) motion capture system and an embedded AMTI (AMTI, Watertown, MA, United States) force platform fixed in the middle of the path [28]. They were synchronized to record marker trajectories and GRF at 200 Hz and 1000 Hz, respectively. The calibration process for the VICON Motion Capture System adhered to the recommendations provided by the camera manufacturer. Throughout the movement, reflective markers on the subject were concurrently captured by a minimum of two cameras [29]. At the same time, Simultaneous acquisition of EMG signals from TAM, PLM, GMM and GLM at 1000 Hz using a wireless Delsys EMG test system (Delsys, Boston, MA, USA) for data processing and OpenSim model validation [30,31]. Then, the subjects underwent randomized tests of normal walk, jog at a speed of 2.68 m/s, and fast run on a 10-meter track [32–34]. During our tests, we used single beam electronic timing gates (Brower Timing Systems, Draper, Utah, United States, height = 1 m) to control the speed [35]. Meanwhile, during the subject's movement, the experimenter had to make sure that the subject's right leg (heel strike pattern) stepped accurately into the force platform, with an interval of 30 s between each test for fatigue recovery, and the subject was required to complete 3 corresponding data collections at each speed.

#### **2.4. Data processing**

Jang et al. [36] employed OpenSim, an open source software, to calculate and process the GRF and ankle contact forces in a population with ankle instability so as to validate the data collected from the experiment. OpenSim provided a realistic simulation of the range of muscle forces by constructing a personalized skeletal muscle model to predict the muscle forces and joint contact loads generated during exercise [31,37]. This is crucial for a thorough discussion of what kind of training on FC maximizes muscle activation and ankle protection.

In the first place, we employed MATLAB R2018b (The MathWorks, Natick, MA, USA) for processing the experimental data [38]. This resulted in “trc” (labelled trajectories) and “mot” (GRF) files suitable for OpenSim runs. After that, the marker points' weights within the model were manually fine-tuned in OpenSim. Additionally, the model was scaled to align with the anthropometric traits of the subjects, ensuring that the root mean square (RMS) error between the experimental marker points and virtual marker points was below 0.01, with a maximum error of under 0.02. Ultimate, ankle angles and moments were determined through the utilization of inverse kinematics and inverse dynamics algorithms. Meanwhile, Static optimization techniques were employed to gauge the level of muscle activation across major muscle groups, such as the TAM, PLM, GMM and GLM, during exercise.

In Delsys EMG procedures, the initial step involved filtering the raw EMG signals using a fourth-order Butterworth bandpass filter within the frequency range of 100–500 Hz. Amplitude analysis was carried out using RMS calculations, outputting MVC and normalized activity values for each movement. The degree of EMG activation was calculated by the test RMS amplitude/MVC RMS amplitude from 0 (completely inactive) to 1 (fully activated).

## 2.5. Statistical analysis

Statistical efficacy analyses were performed using G\*Power3 (Hamburg, GRE) software with medium effect sizes to reduce the risk of Type II errors and establish the minimum participant requirement for this study. Inputs to adapt the parameters chosen for this experiment: Effect Size was 0.4, Alpha was set at 0.05, Power was set at 0.8, Number of measures was 3, and Nonsphericity was 0.5. The results indicated that a sample size of 12 was adequate to achieve over 80% statistical efficacy in this study.

A Shapiro-Wilk test (SPSS Inc., Chicago, IL, USA) was employed to assess the normality of the mean muscle activation on the ankle surface during exercise in the subjects [39]. One-way repeated measures analysis of variance (ANOVA) was used to assess statistically significant differences in subjects' changes in muscle activation at the ankle surface after different surfaces of intervention. Post-hoc pairwise comparisons were conducted using the Bonferroni method for further analysis. Descriptive statistics are presented as arithmetic mean (Mean) and standard deviations (SD). The significance level was set at  $\alpha < 0.05$  and the above was realized using IBM SPSS Statistics 26 (SPSS, Chicago, IL, USA).

The Shapiro-Wilk test (SPSSs Inc., Chicago, IL, USA) was used to determine the normal distributions of ankle moments, sagittal plane PF and DF, and subtalar joint inversion (INV) and eversion (EVE) at different mating speeds. If the data met the criteria for a normal distribution, a one-way repeated measures ANOVA with one-dimensional statistical parameter mapping (SPM1d) was employed. Otherwise, one-way repeated measures ANOVA with one-dimensional statistical non-parametric mapping (SnPM1d) was conducted [40]. For post hoc pairwise comparisons of significant main effects, Bonferroni adjustment was applied to account for multiple comparisons. This study utilized MATLAB open-source scripts (The MathWorks, Natick, MA, USA) for conducting SPM1d and SnPM1d analyses and the significance threshold for each test was established at 0.05.

## 3. Results

### 3.1. Demographic data

Ultimately, in this experiment, there were a total of 24 participants who met the selection criteria and were willing to sign the informed consent form. Upon comparison, no significant differences in demographic characteristics at baseline were observed among all participants in any parameter (**Table 2**).

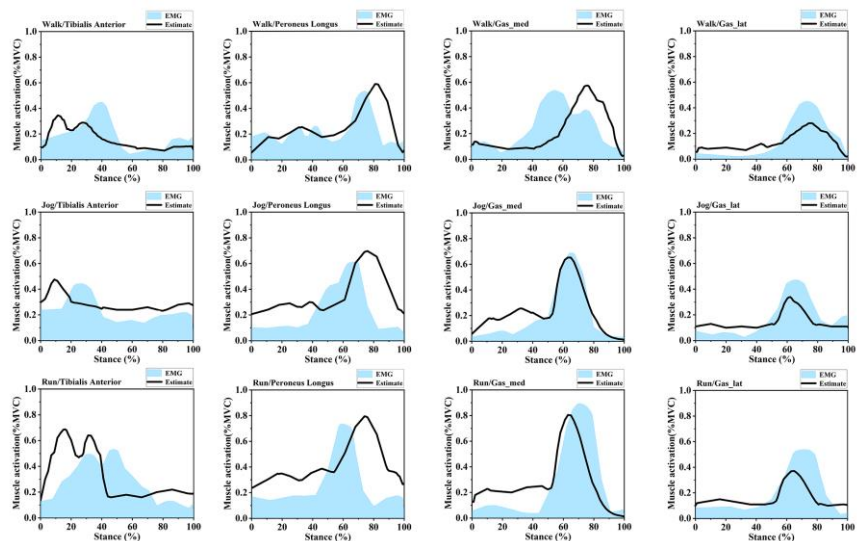
**Table 2.** Overview of demographic characteristics.

Demographic	Results	<i>p</i> -Value
Age(years)	22.00 ± 2.00	<i>p</i> > 0.05
Height(m)	1.76 ± 0.05	<i>p</i> > 0.05
Mass(kg)	73.00 ± 5.33	<i>p</i> > 0.05
Exercise hours per week(h)	10.36 ± 1.15	<i>p</i> > 0.05

### 3.2. Model validation and sensitivity

We evaluated the sensitivity and reliability of the model by comparing the captured surface EMG data with muscle activations calculated by the OpenSim static optimization tool. During the walk, jog and fast run, the RMS calculations for the four muscles in the surface EMG were adjusted to the maximum RMS during the MVC test, which ranged from 0 to 1.

Model-simulated muscle activation is also reported on a scale from 0 to 1, where 0 signifies completely inactive and 1 represents full activation. Comparative results are shown in **Figure 2**, where predicted muscle activation is consistent with surface EMG during the standing phase and compared with previous results, demonstrating good agreement [41].



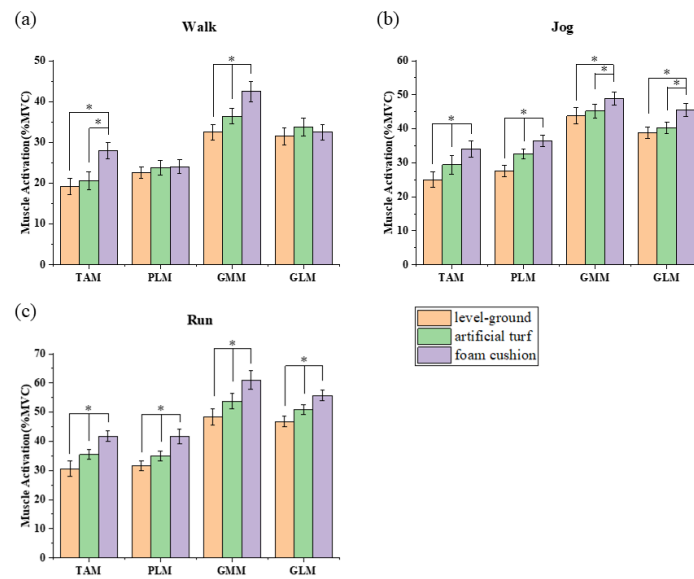
**Figure 2.** Comparison of activation of TAM, PLM, GMM and GLM measured by static optimization estimation (black line) and filtered EMG signals (blue) during the walk, jog and fast run.

Walk denotes normal walk, Jog denotes jog at 2.68 m/s, and Run denotes fast run.

### 3.3. Comparative results of non-parametric tests of muscle activation

At the 0.05 level of significance ( $p < 0.05$ ), the original hypothesis (the sample conformed to the normal distribution) was rejected, indicating that the samples did not conform to a normal distribution. Therefore, we used non-parametric tests. Analysis of the results showed that there was a statistical difference in the mean muscle activation of the subjects after training in different surfaces.

As shown in **Figure 3a**, PLM and GLM were not statistically significant after training on different surfaces during walk. Mean muscle activation was significantly higher in TAM ( $p = 0.001$ ) and GMM ( $p < 0.05$ ) post FC training than post LG and AT training.



**Figure 3.** Mean normalized muscle activation of participants post training in LG, AT, and FC during walk, jog, and fast run. **(a)** Mean normalized muscle activation during the walk; **(b)** Mean normalized muscle activation during the jog; **(c)** Mean normalized muscle activation during the run.

\*Indicates significant differences between the two groups ( $p < 0.05$ ).

Orange indicates LG training, green indicates AT training, purple indicates FC training; TAM, tibialis anterior muscle; PLM, peroneus longus muscle; GMM, gastrocnemius medial muscle; GLM, gastrocnemius lateral muscle.

As shown in **Figure 3b**, the mean muscle activation levels of TAM, PLM, GMM, and GLM during the jog were statistically significant after training at different interfaces. Post hoc pairwise comparisons revealed that mean muscle activation was significantly higher in TAM post FC training ( $p = 0.003$ ) than post LG training ( $p = 0.018$ ) and AT training ( $p = 0.018$ ). Mean muscle activation was significantly higher in the PLM post FC training ( $p = 0.001$ ) than post LG training ( $p = 0.001$ ) and AT training ( $p = 0.002$ ). Mean muscle activation was significantly higher in GMM post FC training ( $p = 0.014$ ) than post LG training ( $p < 0.001$ ) and AT training ( $p < 0.001$ ). Mean muscle activation was significantly higher in GLM post FC training ( $p = 0.003$ ) than post LG training ( $p = 0.003$ ) and AT training ( $p = 0.008$ ).

As shown in **Figure 3c**, the mean muscle activation levels of TAM, PLM, GMM, and GLM during the fast run were significantly different after training at different interfaces. Post hoc pairwise comparisons revealed that mean muscle activation was significantly higher in TAM post FC training ( $p = 0.001$ ) than post LG training ( $p = 0.001$ ) and AT training ( $p = 0.006$ ). Mean muscle activation was significantly higher in the PLM post FC training ( $p < 0.001$ ) than post LG training ( $p < 0.001$ ) and AT training ( $p = 0.008$ ). Mean muscle activation was significantly higher in GMM post FC training ( $p < 0.001$ ) than post LG training ( $p < 0.001$ ) and AT training ( $p = 0.021$ ). Mean muscle activation was significantly higher in the GLM post FC training ( $p < 0.001$ ) than post LG training ( $p < 0.001$ ) and AT training ( $p = 0.001$ ).



### 3.4. Results of the SnPM1d comparison of kinematic and kinetic

#### 3.4.1. Ankle moments

As shown in **Table 3**, there were no significant differences in peak ankle PF moment during walk. However, during jog, the peak ankle PF moment post AT training was significantly greater than LG ( $p = 0.002$ ), and the peak ankle PF moment post FC training was significantly greater than LG ( $p < 0.001$ ) and AT ( $p < 0.001$ ). In fast run, the peak ankle PF moment post AT training was significantly greater than LG ( $p = 0.006$ ), and the peak ankle PF moment post FC training was significantly greater than LG ( $p < 0.001$ ) and AT ( $p = 0.02$ ).

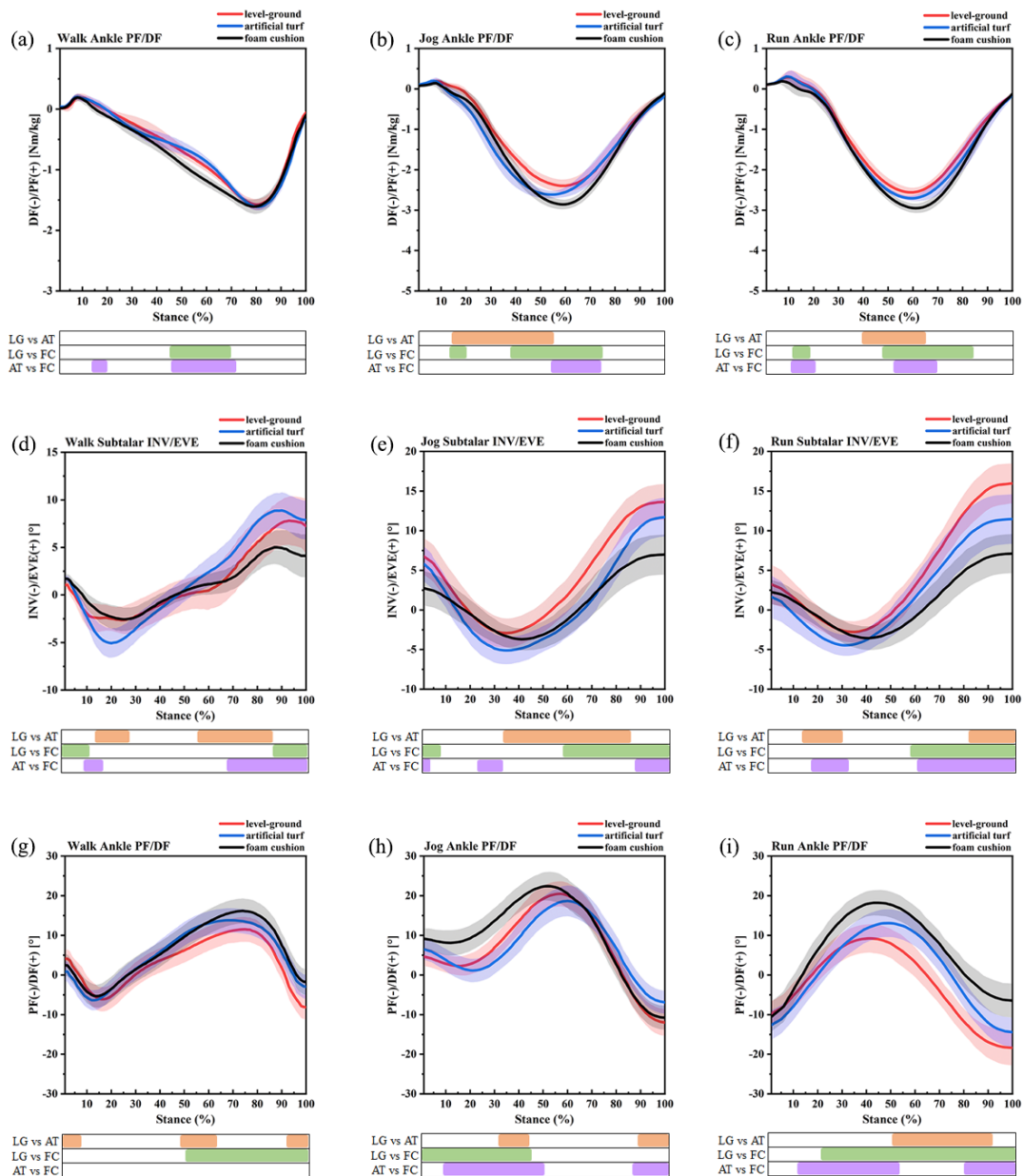
**Table 3.** Peak ankle PF moment under different movement conditions.

Joint Moments (Nm/kg)		LG	AT	FC
Walk	Ankle PF	1.58 ± 0.10	1.62 ± 0.06	1.61 ± 0.12
Jog	Ankle PF	2.41 ± 0.16 <sup>bc</sup>	2.65 ± 0.09 <sup>ac</sup>	2.87 ± 0.12 <sup>ab</sup>
Run	Ankle PF	2.51 ± 0.12 <sup>bc</sup>	2.72 ± 0.10 <sup>ac</sup>	2.96 ± 0.17 <sup>ab</sup>

Note: <sup>a</sup> indicates a significant difference from LG ( $p < 0.05$ ), <sup>b</sup> indicates a significant difference from AT ( $p < 0.05$ ), and <sup>c</sup> indicates a significant difference from FC ( $p < 0.05$ ). Walk denotes normal walk, Jog denotes jog at 2.68 m/s, and Run denotes fast run.

As shown in **Figure 4a**, post hoc paired analyses showed not statistically difference between LG and AT during the walk, and a significantly greater PF moment in FC than in LG during the 46%–70% standing phase ( $p < 0.001$ ). The PF moment was significantly greater for FC than for AT in the 14%–20% ( $p = 0.03$ ) and 47%–73% ( $p < 0.001$ ) standing phases. During the jog (**Figure 4b**), the PF moment of the AT was significantly greater than that of the LG during the 15%–56% standing phase ( $p < 0.001$ ). The FC had a greater PF moment than the LG in the 14%–20% ( $p = 0.035$ ) and 38%–75% ( $p < 0.001$ ) standing phases. During the 54%–74% standing phase, the plantar flexion moment was significantly greater in FC than in AT ( $p < 0.001$ ). During the fast run (**Figure 4c**), the PF moment of the AT was significantly greater than that of the LG during the 40%–65% stance phase ( $p < 0.001$ ). The FC had a greater PF moment than the LG in the 12%–18% ( $p = 0.046$ ) and 47%–83% ( $p < 0.001$ ) standing phases. The PF moment was greater in FC than in AT during the 10%–20% ( $p = 0.038$ ) and 52%–70% ( $p < 0.001$ ) stance phases.

In the figure below, red line denotes LG training, blue line denotes AT training, black line denotes FC training. Orange denotes significant difference in LG compared to AT in SnPM1d analysis, green denotes significant difference in LG compared to FC in SnPM1d analysis, and purple denotes significant difference in AT compared to FC in SnPM1d analysis. Walk denotes normal walk, Jog denotes jog at 2.68 m/s, and Run denotes fast run.



**Figure 4.** Post LG, AT, and FC training, the mean and standard deviation of waveform changes in ankle joint moment, subtalar joint angle, and ankle sagittal plane angle during walk, jog, and fast run. **(a)** Ankle joint DF/PF moment during stance phase of walk; **(b)** Ankle joint DF/PF moment during stance phase of jog; **(c)** Ankle joint DF/PF moment during stance phase of fast run; **(d)** Subtalar INV/EVE during stance phase of walk; **(e)** Subtalar joint INV/EVE during stance phase of jog; **(f)** Subtalar joint INV/EVE during stance phase of fast run; **(g)** Ankle joint DF/PF during stance phase of walk; **(h)** Ankle joint DF/PF during stance phase of jog; **(i)** Ankle joint DF/PF during stance phase of fast run.

### 3.4.2. Subtalar joint INV and EVE

As shown in **Table 4**, post training under different surfaces, comparing the peak INV and EVE angles of the subtalar joint revealed the following results: during the walk, the peak EVE angle of the subtalar joint post FC training was significantly lesser than AT ( $p < 0.001$ ), and the subtalar mobility post FC training was significantly lesser than AT ( $p < 0.001$ ); During the jog, the peak EVE angle of the

subtalar joint post FC training was significantly smaller than LG ( $p < 0.001$ ) and AT ( $p < 0.001$ ). The peak INV angle of the subtalar joint post LG training was significantly lesser than AT ( $p < 0.001$ ). Additionally, the subtalar mobility post FC training was significantly smaller than LG ( $p < 0.001$ ) and AT ( $p < 0.001$ ); During the fast running, the peak EVE angle of the subtalar joint post AT training was significantly smaller than LG ( $p = 0.009$ ), the peak EVE angle post FC training was significantly lesser than LG ( $p < 0.001$ ), and the subtalar mobility post FC training was significantly lesser than LG ( $p < 0.001$ ) and AT ( $p = 0.042$ ).

**Table 4.** Subtalar joint angles peak and mobility.

	Joint kinematics (°)	LG	AT	FC
Walk	Subtalar EVE	8.10 ± 2.67	9.01 ± 1.86 <sup>c</sup>	5.14 ± 1.84 <sup>b</sup>
	Subtalar INV	3.14 ± 1.54	5.12 ± 1.52	2.82 ± 1.01
	Subtalar mobility	11.24 ± 3.08	14.13 ± 2.40 <sup>c</sup>	7.96 ± 2.10 <sup>b</sup>
Jog	Subtalar EVE	13.63 ± 1.34 <sup>c</sup>	11.72 ± 1.86 <sup>c</sup>	7.20 ± 2.47 <sup>ab</sup>
	Subtalar INV	2.97 ± 0.94 <sup>b</sup>	5.15 ± 0.93 <sup>a</sup>	3.88 ± 1.37
	Subtalar mobility	16.60 ± 1.64 <sup>c</sup>	16.87 ± 2.08 <sup>c</sup>	11.09 ± 2.83 <sup>ab</sup>
Run	Subtalar EVE	15.97 ± 1.59 <sup>bc</sup>	11.47 ± 3.12 <sup>a</sup>	7.19 ± 2.49 <sup>a</sup>
	Subtalar INV	2.84 ± 1.55	4.51 ± 1.29	3.65 ± 1.49
	Subtalar mobility	18.81 ± 2.22 <sup>c</sup>	15.98 ± 3.37 <sup>c</sup>	10.84 ± 2.90 <sup>ab</sup>

Note: <sup>a</sup> indicates a significant difference from LG ( $p < 0.05$ ), <sup>b</sup> indicates a significant difference from AT ( $p < 0.05$ ), and <sup>c</sup> indicates a significant difference from FC ( $p < 0.05$ ). Walk denotes normal walk, Jog denotes jog at 2.68 m/s, and Run denotes fast run.

During the walk (**Figure 4d**), the INV angle of the AT was significantly larger than the LG in 13%–27% of the standing phase ( $p < 0.001$ ), and the EVE angle of the AT was significantly larger than the LG in 55%–85% of the standing phase ( $p < 0.001$ ). In the 0%–12% standing phase, FC had a significantly greater EVE angle than LG ( $p < 0.001$ ). The EVE angle of the FC was significantly smaller than that of the LG in the 84%–100% standing phase ( $p < 0.001$ ). During the 8%–17% standing phase, the INV angle was significantly greater in FC than in AT ( $p < 0.001$ ). In the 66%–100% standing phase, FC had a significantly smaller EVE angle than AT ( $p < 0.001$ ).

During the jog (**Figure 4e**), the INV angle of the AT was significantly larger than that of the LG during the 33%–84% standing phase ( $p < 0.001$ ). In the 0%–8% ( $p = 0.002$ ) and 57%–100% ( $p < 0.001$ ) standing phases, the FC had a significantly smaller EVE angle than the LG. In the 0%–4% ( $p = 0.005$ ) and 85%–100% ( $p < 0.001$ ) standing phases, the FC had a significantly smaller EVE angle than the AT. In the 22%–33% ( $p = 0.02$ ) standing phase, the FC had a significantly smaller INV angle than the AT.

During the fast run (**Figure 4f**), the INV angle of the AT was significantly larger than that of the LG during the 14%–30% ( $p < 0.001$ ) standing phase. In the 80%–100% ( $p < 0.001$ ) standing phase, the EVE angle of the AT was significantly smaller than that of the LG. In the 56%–100% ( $p < 0.001$ ) standing phase, the FC had a significantly smaller EVE angle than the LG. During the 17%–32% standing phase, the INV angle of the FC was less than the AT ( $p = 0.004$ ). In the 60%–100%

standing phase, the FC had a significantly smaller EVE angle than the AT.

### 3.4.3. Ankle sagittal plane kinematics

As indicated in **Table 5**, following training under different surfaces, a comparison of ankle joint DF and PF peaks revealed the following outcomes: during the walk, post FC training, the ankle joint DF was significantly greater than LG ( $p = 0.01$ ); During the jog, post FC training, ankle joint DF was significantly greater than LG ( $p = 0.038$ ) and AT ( $p < 0.001$ ). Post AT training, ankle joint PF was significantly smaller than LG ( $p = 0.006$ ) and FC ( $p = 0.34$ ), and ankle joint sagittal plane mobility was significantly smaller than LG ( $p = 0.01$ ) and FC ( $p < 0.001$ ); During the fast run, post AT training, ankle joint DF was significantly greater than LG ( $p < 0.001$ ). Post FC training, ankle joint DF was significantly greater than LG ( $p < 0.001$ ) and AT ( $p = 0.16$ ), and ankle joint PF was significantly smaller than LG ( $p < 0.001$ ) and AT ( $p < 0.001$ ).

**Table 5.** Ankle sagittal plane angle peak and mobility.

Joint kinematics (°)		LG	AT	FC
Walk	Ankle DF	10.10 ± 2.73c	13.18 ± 2.35	15.14 ± 2.58a
	Ankle PF	6.64 ± 2.18	7.37 ± 1.88	5.05 ± 1.79
	Ankle mobility	17.74 ± 3.49	20.55 ± 3.01	20.19 ± 3.14
Jog	Ankle DF	18.58 ± 2.61c	16.71 ± 2.87c	22.52 ± 2.67ab
	Ankle PF	12.78 ± 2.69b	7.90 ± 2.36ac	12.04 ± 2.54b
	Ankle mobility	31.36 ± 3.75b	24.61 ± 3.72ac	34.56 ± 3.69b
Run	Ankle DF	7.14 ± 2.73bc	13.25 ± 3.04ac	18.44 ± 2.62ab
	Ankle PF	18.41 ± 2.70c	14.49 ± 2.74c	8.38 ± 2.98ab
	Ankle mobility	25.55 ± 3.84	27.74 ± 4.09	26.82 ± 3.97

Note: <sup>a</sup> indicates a significant difference from LG ( $p < 0.05$ ), <sup>b</sup> indicates a significant difference from AT ( $p < 0.05$ ), and <sup>c</sup> indicates a significant difference from FC ( $p < 0.05$ ). Walk denotes normal walk, Jog denotes jog at 2.68 m/s, and Run denotes fast run.

Post hoc paired analyses showed that during the walk (**Figure 4g**), the ankle DF angle of the AT was significantly greater than that of the LG in the 0%–7% ( $p = 0.02$ ) and 47%–62% ( $p = 0.003$ ) stance phases, and the ankle PF angle of the AT was significantly less than that of the LG in the 90%–100% ( $p < 0.001$ ) stance phase. The ankle DF angle was significantly greater in FC than in LG during the 50%–92% stance phase ( $p < 0.001$ ), and the ankle PF angle was significantly less in FC than in LG during the 92%–100% stance phase ( $p < 0.001$ ). There was no statistical significance between DF and PF angles of the ankle in AT and FC ( $p > 0.05$ ).

During the jog (**Figure 4h**), the ankle DF angle of the AT was significantly greater than that of the LG during the 31%–44% ( $p = 0.001$ ) stance phase, and the ankle PF angle of the AT was significantly less than that of the LG during the 86%–100% ( $p < 0.001$ ) stance phase. The ankle DF angle was significantly greater in FC than in LG during the 0%–45% standing phase ( $p < 0.001$ ). The ankle DF angle was significantly greater in FC than in AT during the 10%–50% standing phase ( $p < 0.001$ ). The ankle PF angle was significantly greater in FC than in AT during the 83%–100% standing phase ( $p < 0.001$ ).

During the fast run (**Figure 4i**), the ankle DF angle of the AT was significantly

greater than that of the LG during the 50%–74% stance phase ( $p < 0.001$ ), and the ankle PF angle of the AT was significantly less than that of the LG during the 74%–90% stance phase ( $p < 0.001$ ). The ankle DF angle was significantly greater in FC than in LG during the 21%–80% stance phase ( $p < 0.001$ ), and the ankle PF angle was significantly less in FC than in LG during the 80%–100% stance phase ( $p < 0.001$ ). The ankle DF angle of FC was significantly greater than AT in the 12%–52% stance phase ( $p < 0.001$ ), and the ankle PF angle of FC was significantly less than AT in the 78%–100% stance phase ( $p < 0.001$ ).

#### 4. Discussion

Ankle joint NMT is commonly used for FAI training and rehabilitation. However, most of the current studies on ankle NMT for FAI have focused on long-term interventions on the ankle joint [42–46], and there have been few studies on the effects of immediate ankle NMT on the ankle. Simultaneously, surfaces with different levels of stabilization will have different effects on the effectiveness of training [17]. Therefore, in this investigation, we examined the effects of immediate NMT on the ankle joint, conducted on surfaces with different levels of stabilization, on the biomechanical characteristics of the ankle joint in individuals with FAI. The findings of this investigation indicate that ankle NMT in surfaces with different levels of stabilization can differentially affect ankle moments, joint angles, and ankle stabilizer muscle activation in FAI populations. Comparing the three surface types of training, it was found that NMT on the unstable surface FC may have more positive effects on individuals with FAI.

We observed that increased instability of the training surface may increase the average level of activation of the ankle stabilizing muscles in subjects (e.g., **Figures 3**), which is consistent with our previous hypothesis. The PLM around the ankle joint is mainly responsible for controlling the movement of the ankle in the left-right direction, the GMM and GLM are mainly responsible for regulating the PF movement of the ankle joint, and the TAM plays an important role in both INV and DF movements, and ankle joint stability is largely dependent on these four muscles [47]. Therefore, NMT utilizing an unstable surface (e.g., FC), compared to a commonly used stable surface (e.g., LG) in everyday life, will be more conducive to the activation of the four muscles. In addition, different training movements will have different effects on the ankle joint, and an increase in jumping movements may be more conducive to the activation of ankle stabilizing muscles [48]. Increased levels of activation of these muscles during exercise can have a positive effect on ankle stability [49], thereby reducing the risk of ankle injury in FAI individuals during running. It has been reported that TAM, PLM, GMM, and GLM EMG activity is higher after training in the unstable surface compared to the stable surface, which is similar to our results (e.g., **Figure 3**) [50]. This phenomenon may be explained by the fact that during ankle training in unstable environments, the muscles surrounding the joint need to be more actively involved in maintaining balance and stability [51,52]. The mean level of activation of the ankle stabilizing muscles increases to some extent with increasing instability of the training surface, a finding that is supported by a previous study [17], this study found that the level of

activation of the ankle stabilizing muscles in individuals with CAI all increased to some extent with increasing instability of the training surface. However, it has also been shown that training in the unstable surface does not have a significant effect on the level of activation of the muscles surrounding the ankle joint [53]. However, the study was conducted on healthy subjects (no history of ankle sprains), and we hypothesized that the sensitivity of the training effect may be lower in this healthy population, thus producing different results.

When the instability of the training surface is altered, the participant's ankle moment during movement is directly affected. In our current investigation, we observed that participants exhibited greater peak ankle PF moments during exercise as the instability of the training surface gradually increased. This may be due to increased levels of activation of the plantarflexor muscles, including the TAM, around the ankle joint to accommodate unstable surface, resulting in stronger moment generation [47]. However, the increase in PF moment may subject the ankle joint to greater loads, which will increase the risk of ankle injuries [54], which is a point that needs to be worthy of our attention.

Increased levels of TAM activation will produce greater foot DF during gait [55], and it is possible that stretching of the gastrocnemius muscle may also be associated with improved DF of the ankle [56]. In this study, after NMT on a FC, the DF angle of the ankle joint during exercise was significantly increased, which improved the flexibility of the ankle joint in the sagittal plane to some extent. It has been shown that a single session of joint activity can result in a moderate increase in ankle DF mobility, which is similar to the training in this study to increase the ankle DF angle [57]. Of note, increased ankle DF mobility may help reduce the risk of ankle sprains and lower extremity injuries [58]. The geometry of the subtalar joint allows for INV and EVE of the ankle, and it is in this position that most of the foot's EVE and INV is achieved [59]. In our present study, the range of motion of the subtalar joint decreased with increasing instability of the training surface, indicating a reduced ankle INV and EVE angle. This may be associated with an increased activation level of the ankle EVE muscles PLM and INV muscles TAM controlling the ankle joint. Related studies have reported that increased levels of TAM activation may place the lateral ankle at less risk of stress in athletes with CAI [60,61]. Simultaneously, it has been observed that preparatory co-activation of ankle EVE and INV muscles can limit the angular range of ankle INV and EVE, potentially reducing the incidence of ankle injuries [62]. INV and EVE injuries are also common in ankle sports injuries [63], and reducing the incidence of this injury is of great significance for individuals with FAI.

However, this study is subject to certain limitations. The primary findings were as follows: (1) The participants were mainly general FAI individuals, and it is not known whether the results of this study are applicable to athletes with FAI. (2) Our research has primarily concentrated on the dominant leg, overlooking the examination of the non-dominant leg. Given that both legs are frequently engaged in sports activities, comprehending the performance of the non-dominant leg is equally crucial. (3) This experiment mainly considered the sports biomechanics of the ankle joint in walk and run post NMT, but it remains to be considered whether the training method is applicable to other sports. (4) Future related research should focus more

on the movement and timing of training and the effects of fatigue on the experiment. (5) This study focused on male FAI individuals, and future related studies should include females for a more comprehensive understanding of the effects of NMT on the ankle joint.

## **5. Conclusion**

The findings of this study indicate that ankle joint NMT on FC is more effective in activating the ankle stabilizing muscles in individuals with FAI compared to training on LG and AT. And the increased activation level of the ankle stabilizing muscles is beneficial to improve the flexibility of the ankle joint in the sagittal plane and reduce subtalar INV and EVE, which has a positive effect on the motion control and stability of the ankle joint during exercise. However, our study subjects were all male and did not include women, so the findings may only apply to males. It is also worth noting that the duration of the effect of this immediate ankle NMT is unknown. Therefore, we recommend that individuals with FAI incorporate ankle NMT training on an unstable surface into their daily routine. This practice helps to further enhance ankle stability through prolonged training interventions while reducing the risk of sports-related injuries.

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**Data availability statement:** The data are unavailable due to privacy or ethical restrictions.

**Ethical approval:** Written informed consent was obtained from each participant after familiarization with the testing requirements and procedures. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Ningbo University (RAGH202305221185), Approval date 22 May, 2023.

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## Abbreviation

Full name	Abbreviation
chronic ankle instability	CAI
functional ankle instability	FAI
ground reaction forces	GRF
neuromuscular training	NMT
foam cushion	FC
level-ground	LG
artificial turf	AT
Identification of functional ankle instability	IdFAI
plantarflexion	PF
dorsiflexion	DF
maximal voluntary contraction	MVC
gastrocnemius medial muscle	GMM
gastrocnemius lateral muscle	GLM
tibialis anterior muscle	TAM
peroneus longus muscle	PLM
root mean square	RMS
inversion	INV
eversion	EVE
one-dimensional statistical parameter mapping	SPM1d
one-dimensional statistical non-parametric mapping	SnPM1d

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