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IMU and EMG-based Analysis of Kinematic and Neuromuscular Adaptations During Uneven-surface Running for Protocol Design in Trail Footwear Evaluation

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Abstract

The purpose of this study was to investigate the trial order effect on sagittal-plane kinematics and neuromuscular adaptations during repeated-measures footwear tests on an uneven surface to establish reliable evaluation protocols. Eleven habitual trail runners performed four 3-minute running trials on an uneven treadmill, with different footwear presented in a randomized order. Sagittal-plane lower limb kinematics and the activity of six muscles were recorded using IMU motion sensors and surface EMG. Data were analyzed during the final 20 seconds of each trial. No significant differences were observed in most kinematic variables across the four test trials (p>.05), except for the maximum knee flexion angle during the pre-strike phase (p=.041). In contrast, post-strike muscle activity showed a systematic reduction for the vastus medialis (p=.001), gastrocnemius lateralis (p=.003), soleus (p=.040) and biceps femoris (p=.039). Our results indicate that while sagittal-plane kinematic patterns stabilize quickly, the neuromuscular system undergoes a continuous tuning process. This suggests that kinematic stability does not equate to neuromuscular stability, necessitating approximately 10 minutes of familiarization to secure reliable and reproducible data in trail running footwear evaluation protocols.

Key words: trail running, footwear evaluation, neuromuscular adaptation, kinematic optimization, wearable sensor

Introduction

Unlike road running, which typically takes place on even surfaces, trail running is primarily performed on unpredictable terrains characterized by uphill, downhill, and level sections, as well as natural obstacles such as rocks and tree roots (Hamill et al., 2022). Driven by these challenging characteristics, the popularity of trail running has grown rapidly, with global participation increasing by approximately 15% over the past decade (Viljoen et al., 2022). However, the incidence of musculoskeletal injuries in trail running has been reported to be comparable to that of road running, with ankle sprains being the most common injury (Hamill et al., 2022; Vincent et al., 2022). Thus, establishing a reliable scientific protocol to evaluate trail running footwear is essential, not only for injury prevention but also to ensure the validity of biomechanical data in unpredictable environments.

Human biomechanical evaluation of footwear in industry typically employs a repeated-measures design, in which a single participant tests multiple shoe

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conditions. This method is known for its high statistical power, as it controls for inter-individual variability and allows for direct comparison of performance differences across footwear conditions (Flores et al., 2019; Mohr et al., 2022; Sterzing et al., 2015; Willwacher et al., 2013). However, a critical yet often overlooked confounding factor in this design is the 'order effect'—the body's progressive adaptation to novel stimuli throughout repeated trials. While previous studies have attempted to adapt participants through short runs of 120 m or brief warm-ups of a few minutes (Isherwood et al., 2024; Mohr et al., 2022; Paquette et al., 2024), these protocols lack a standardized familiarization period. Consequently, it remains ambiguous whether the observed results stem from the intrinsic effects of the footwear or simply from the body's adaptation process, potentially leading to misinterpretation of footwear performance. To minimize this bias, standardized familiarization periods are essential. However, the current protocols vary widely, ranging from a few minutes to short distance runs, lacking a robust scientific basis for data reliability.

Recent studies indicate that while the kinematic system stabilizes quickly (2-3 minutes) even in novel footwear (Paquette et al., 2024), the neuromuscular system undergoes progressive adaptation over a longer period (approximately 7 minutes) (Mohr et al., 2022). Furthermore, Macdermid et al. (2026) suggested that achieving true structural stability in spatiotemporal variables may require up to 17 minutes, depending on the complexity of the running environment. Therefore, if the adaptation time is set solely based on kinematic stability when evaluating both kinematic variables (e.g., joint angles) and neuromuscular variables (e.g., EMG), it may lead to interpretive errors, as the ongoing neuromuscular adaptation process could be mistaken for the intrinsic effects of the footwear.

This temporal discrepancy requires critical attention, particularly given the specificity of the trail running environment, where unpredictable stimuli occur constantly. For instance, because unstable surfaces induce unpredictable perturbations, runners employ distinct motor control strategies to adapt to the changing environment. Kinematically, runners tend to adopt strategies such as a 'crouched posture' of the lower limbs during the initial stance phase to secure stability (Apps et al., 2017; Hébert-Losier et al., 2015; Menant et al., 2008; Mohr et al., 2023). This serves as a protective mechanism to absorb shock and ensure stability, characterized by increased flexion of the knee and hip upon ground contact and a flatter shoe-ground angle. However, relying exclusively on sagittal-plane kinematic stabilization may be insufficient to conclude that full adaptation has occurred, as it may overlook active defensive strategies in other planes or internal neuromuscular tuning.

From the perspective of neuromuscular adaptation, runners actively modulate muscle activation to secure stability in response to unstable conditions. Honert et al. (2022) demonstrated that during prolonged running, runners exhibit a sophisticated redistribution of mechanical work—specifically, a decrease in positive ankle work and an increase in positive foot work—accompanied by shifts in Tibialis Anterior (TA) EMG frequency. This indicates that the neuromuscular system continuously refines its 'muscle tuning' and control strategies for eccentric contractions and energy absorption even after outer kinematic patterns appear stable. Previous research indicates that runners exert increased muscular control to maintain frontal plane ankle stability, specifically by increasing the activation of the peroneus longus (Apps et al., 2017; Sterzing et al., 2014a). Additionally, runners may exhibit a tendency for decreased activation of the tibialis anterior during the pre-landing phase, which is associated with kinematic changes that maintain a flatter ankle joint at landing (Mohr et al., 2023). As such, neuromuscular adaptation to unstable environments extends beyond simply increasing or decreasing muscle activation, manifesting in diverse forms depending on the characteristics and duration of the task.

Collectively, these findings offer critical insights for the design of trail running footwear evaluation protocols. The temporal discrepancy between kinematic stabilization and neuromuscular adaptation poses a risk of misinterpretation, where measured results may be conflated with the adaptation process rather than reflecting true differences in footwear performance.

Furthermore, while running in unstable environments involves more complex adaptation mechanisms than stable conditions, existing research has largely been confined to stable environments or has lacked a comprehensive, integrated investigation of these two adaptation processes. Recent advancements in wearable inertial measurement unit (IMU) and electromyography (EMG) sensors offer new opportunities to transcend laboratory constraints and simultaneously analyse these complex adaptation mechanisms (Hollis et al., 2019; Khant et al., 2023).

The purpose of this study was to quantify the temporal characteristics of lower limb kinematic changes and neuromuscular adaptations by examining the effects of trial order during a repeated-measures experimental design on an uneven-surfaced treadmill. By focusing on the temporal progression of both sagittal-plane kinematics and neuromuscular activity across successive trials, this study aimed to isolate the systematic adaptation process from intrinsic footwear effects. Thus, this research focuses on designing reliable trail running footwear evaluation protocols by providing a concrete recommendation for the minimum familiarization duration required to ensure data integrity and validity in future biomechanical assessments.

Methods

Participants

Eleven male amateur runners who regularly engaged in trail running participated in this study. Participants had no history of any lower-extremity musculoskeletal injuries within the past six months and presented no physical and medical limitations to exercise. Additional inclusion criteria defined experienced runners as those with a maximum finish time of 45 minutes in an official 10 km race and a history of participating in trail running competitions at least four times per year. The characteristics of the participants are presented in Table 1.

Prior to the start of the test, all participants were fully informed of the study's purpose and experimental procedures, provided written informed consent, and voluntarily participated in the experiment. This experiment was approved by the Public Institutional Review Board (P01-202409-01-035).

Table 1. Participant characteristics

Variable	Mean (std)
Age (yrs)	40.29 (11.52)
Height (m)	1.74 (0.04)
Weight (kg)	73.74 (10.14)
BMI (kg/m^2)	24.30 (3.35)
Trail running experience (yrs)	4.6 (1.2)
Right foot length (mm)	257.10 (6.99)

Data Collection

Data collection was conducted using a treadmill (Woodway PRO XL, USA, Inc.) modified with resin-based artificial stones to simulate an uneven surface, as illustrated in Figure 1.

Following the recommendations of Mohr et al. (2022), participants performed an 8-minute initial familiarization consisting of 3 minutes of walking at 5 km/h followed by 5 minutes of running at 10 km/h. Subsequently, they ran for 3 minutes at 10 km/h in each of four trail running shoe conditions presented in a randomized order. The tested footwear consisted of developmental prototypes sharing a similar morphological framework, with minor variations in specific structural components such as midsole thickness and outsole lug depth. The detailed specifications for each shoe condition are summarized in Table 2.

The 3-minute duration for each trial was specifically selected to capture the temporal discrepancy between kinematic stabilization (Paquette et al., 2024) and neuromuscular adaptation (Mohr et al., 2022). A 5-minute rest period was provided between trials. For analysis, the four repeated measurements were defined as ‘trials’ based on the chronological order of execution,

Figure 1. Modified treadmill surface for uneven terrain simulation

Table 2. Specifications and structural properties of the experimental footwear

Variable	A	B	C	D
Weight (g)	298	304	295	304
Heel Height (mm)	39	39	36	36
Outsole Height (mm)	5.5	5.5	5.5	5.5
Outsole inserted material	Evonik	BTM	Evonik	BTM
Outsole inserted material thickness (mm)	3.5	5.5	3.5	5.5
Peak G (G)	9.99	10.02	10.40	10.17

regardless of the shoe type.

To collect muscle activity and kinematic data during uneven treadmill running, a wireless surface EMG system (Ultium, Noraxon, USA) and wearable IMU sensors (Myomotion, Noraxon, USA) were used. The sampling rates were set to 2000 Hz for EMG data and 200 Hz for kinematic data, with both systems synchronized during measurement. Additionally, an accelerometer (Smartlead, Noraxon, USA) with a measurement range of up to 100 g was incorporated to detect the instant of foot contact. All sensors were attached to the participant's right lower limb, utilizing a total of six EMG sensors, four IMU sensors, and one accelerometer.

The six EMG sensors on the lower limb recorded muscle activity from the tibialis anterior (TA), peroneus longus (PL), soleus (lateral aspect, SOL), gastrocnemius lateralis (GL), vastus medialis (VM), and biceps femoris (BF). The IMU sensors were placed on the dorsal foot, lateral shank, lateral thigh, and posterior pelvis, respectively, in accordance with the Myomotion

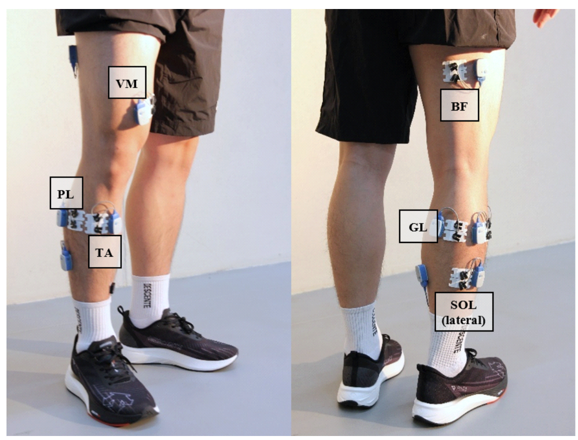

Figure 2. Placement of surface EMG electrodes on the six lower limb muscles

placement guidelines (Noraxon U.S.A., Inc., 2024). The accelerometer was attached to the distal tibia to be positioned as close to the ground as possible. In this study, the signal from the X-axis, corresponding to the vertical axis, was used to determine foot strike events.

Data Processing

All data were collected from the final 20 seconds of each trial. Raw EMG signals were band-pass filtered (20–450 Hz), rectified, and smoothed using a root mean square (RMS) algorithm with an 80 ms moving window. Foot contact events were identified based on the maximum negative peak immediately preceding the maximum impact peak in the vertical axis signal of the accelerometer attached to the distal tibia (Maiwald et al., 2009). Based on this contact point, the phases 200 ms before and 200 ms after impact were defined as the pre-strike and post-strike phases, respectively (Mohr et al., 2022). EMG data were averaged across 20 consecutive gait cycles for each trial and normalized to a reference voluntary contraction (RVC), defined as the individual's maximum RMS value observed across all four measurement conditions (Mohr et al., 2022). Kinematic variables (joint angles) of the lower limb were computed using the sensor fusion algorithm in Noraxon MR3 (ver. 3.18). This system estimates the 3D orientation of the IMU sensors and calculates joint angles based on the relative orientation between sensors on adjacent proximal and distal segments (Oberländer, 2015).

Statistics

All measured variables are presented as means and standard deviations. Based on the results of the Shapiro-Wilk test for normality, either a one-way repeated measures ANOVA or the Friedman test was performed. When sphericity was violated in the repeated measures ANOVA, the Greenhouse-Geisser correction was applied. To ensure a standardized interpretation across both parametric and non-parametric analyses, Hedges' *g* was employed as the universal effect size measure. Hedges' *g* values of 0.2, 0.5, and 0.8 were used to define small, medium, and large effects, respectively (Fritz et al., 2012; Honert et al., 2022). This approach was adopted due to the absence of a robust non-parametric effect size equivalent suitable for the current sample size, as previously noted by Honert et al. (2022). Bonferroni adjustments were used for the post-hoc comparisons. The level of statistical significance was set at α=0.05. All statistical analyses were performed using the Pingouin library (ver. 0.5.3) in Python (ver. 3.11.4).

Results

Kinematic Results

A total of 12 kinematic variables, including the range of motion (ROM) and maximum angles for the ankle, knee, and hip joints, were analyzed for each measurement phase. The statistical results across repeated trials are presented in Table 3. Statistical analysis revealed no significant differences across trials for any kinematic variables (p>.05), with the exception of the maximum knee flexion angle during the pre-strike phase (p=.041). The maximum knee flexion angle during the pre-strike phase exhibited a decreasing trend as the trials progressed, declining from $78.65^{\circ}\pm14.66^{\circ}$ in Trial 1 to $68.01^{\circ}\pm23.22^{\circ}$ in Trial 4. The effect size was found to be medium (Hedges' g=0.506). However, post-hoc tests did not reveal any statistically significant differences between specific trials.

EMG Results

The statistical results for the mean muscle activation of the six lower-limb muscles across measurement phases are presented in Table 4. No significant differences were observed across trials for any of the muscles during the pre-strike phase (p>.05). In the post-strike phase, the vastus medialis (VM) exhibited the most pronounced change, showing a significant main effect with a large effect size (χ^2=16.20, p=.001, Hedges' g=0.742). Post-hoc analysis revealed a systematic reduction in VM activation between Trial 1 and Trial 4 (p=.006).

Table 3. Sagittal-plane joint kinematics of the lower limb across repeated trials (mean±SD; °)

Variable	Phase	Trial 1	Trial 2	Trial 3	Trial 4	Test statistic	*p*-value	Effect size
Ankle dorsiflexion angle ROM	Pre	8.88±2.42	10.56±5.14	8.45±3.79	12.21±5.64	1.718	0.211	-0.708
	Post	27.78±6.94	22.69±5.90	26.99±9.49	30.99±11.18	4.44	0.218	-0.318
Ankle dorsiflexion angle max	Pre	4.55±5.89	4.22±5.81	3.20±8.25	8.84±7.78	1.799	0.171	-0.574
	Post	19.16±4.89	18.86±7.10	17.75±5.41	20.63±4.69	0.936	0.376	-0.283
Knee flexion angle ROM	Pre	65.14±9.21	66.75±15.56	65.39±12.69	56.61±20.77	1.054	0.366	0.490
	Post	22.86±7.10	22.00±6.04	23.42±6.49	22.55±7.03	0.188	0.779	0.040
Knee flexion angle max	Pre	78.65±14.66	80.57±26.88	78.88±13.37	68.01±23.22	8.28	**0.041***	0.506
	Post	39.52±8.78	38.01±10.57	38.67±8.44	35.79±10.49	5.64	0.131	0.356
Hip flexion angle ROM	Pre	15.64±4.79	16.91±5.76	15.94±5.21	15.94±6.07	0.225	0.783	-0.051
	Post	26.76±10.20	21.53±8.26	25.63±9.69	24.48±10.52	1.114	0.361	0.203
Hip flexion angle max	Pre	29.98±12.02	30.40±14.09	30.80±8.80	25.80±15.41	6.84	0.077	0.279
	Post	18.01±10.59	18.62±11.10	18.20±8.39	13.64±12.77	5.04	0.169	0.344

Note. *p<.05. Hedges' g (T1 vs. T4) interpreted as small (0.2), medium (0.5), and large (0.8).

Table 4. Mean activation of lower-limb muscles across repeated trials (mean±SD; %)

Muscle	Phase	Trial 1	Trial 2	Trial 3	Trial 4	Test statistic	*p*-value	Effect size
Tibialis anterior (TA)	Pre	54.28±12.13	50.59±9.75	52.58±12.53	50.89±10.08	2.78	0.439	0.281
	Post	28.76±11.18	28.86±11.84	28.72±12.31	30.14±13.95	1.04	0.796	-0.101
Peroneus longus (PL)	Pre	26.59±9.03	26.47±8.00	27.77±8.78	27.98±11.61	0.37	0.627	-0.123
	Post	60.64±7.82	58.89±6.97	57.52±5.59	54.10±8.52	2.43	0.085	0.738
Soleus (SOL)	Pre	16.29±5.62	17.70±9.19	17.54±8.00	18.36±9.60	0.83	0.400	-0.243
	Post	61.98±8.27	59.29±10.10	57.31±8.60	56.90±8.25	3.92	**0.040***	0.568
Gastrocnemius lateralis (GL)	Pre	19.59±6.44	19.38±6.53	19.57±6.26	19.98±7.57	0.54	0.658	-0.051
	Post	63.63±6.85	61.43±6.35	58.05±6.43	59.40±7.90	5.64	**0.003****	0.528
Vastus medialis (VM)	Pre	34.38±6.19	33.22±6.46	33.89±6.52	32.59±5.64	4.86	0.183	0.279
	Post	39.34±5.00	37.75±4.05	37.35±4.58	35.37±4.87	16.20	**0.001****	0.742
Biceps femoris (BF)	Pre	53.27±9.74	51.73±10.05	50.05±10.01	50.54±10.15	2.60	0.070	0.253
	Post	45.22±18.35	44.64±16.15	41.81±14.64	41.07±15.05	8.35	**0.039***	0.228

Note. *p<.05, **p<.01. Hedges' g (T1 vs. T4) interpreted as small (0.2), medium (0.5), and large (0.8).

Significant main effects were also found during the post-strike phase for the soleus (SOL) (F=3.92, p=.040, g=0.568), gastrocnemius lateralis (GL) (F=5.64, p=.003, g=0.528), and biceps femoris (BF) (χ^2= 8.35, p=.039, g=0.228). For the SOL and GL, medium effect sizes were observed, although their post-hoc comparisons did not reach statistical significance.

Discussion

The primary purpose of this study was to investigate the neuromuscular and kinematic changes across consecutive trials to establish a reliable evaluation protocol for trail running footwear. Given the inherent instability of irregular surfaces, understanding how runners adapt through repeated exposure is crucial for ensuring data reliability in performance testing. Our results revealed that even within four brief trials, runners exhibited sagittal-plane kinematic stabilization and a systematic reduction in muscle activation, particularly in the VM (g=0.742). These findings indicate that a distinct 'trial order effect' exists during the early stages of footwear testing on irregular terrain, demanding a re-evaluation of current familiarization protocols.

The fact that most kinematic variables showed no significant differences between trials suggests that kinematic adaptation stabilized rapidly, aligning with the findings of Paquette et al. (2024). However, our results demonstrated that only the maximum knee flexion angle during the pre-strike phase progressively decreased across trials (p<.001). This sagittal-plane kinematic adjustment, evidenced by a medium effect size (g=0.506), suggests that runners modified their lower-limb posture in anticipation of the irregular surface. Although post-hoc tests did not reveal significant pairwise differences, this likely resulted from the conservative Bonferroni correction applied to our small sample size (N=11). Such a rigorous alpha-level was intentionally maintained to avoid Type I errors, given the high data variability inherent in irregular terrain. These changes represent a stabilization of sagittal-plane kinematics as runners tuned their leg stiffness to better manage the unpredictability of the trial conditions, consistent with a ‘cautious gait’ strategy (Apps et al., 2017; Hébert-Losier et al., 2015; Mohr et al., 2023).

Conversely, the neuromuscular system exhibited distinct temporal characteristics compared to kinematic changes. Mohr et al. (2022) reported a progressive reduction in the activity of six major lower limb muscles as trials progressed, attributing this not to fatigue but to a systematic reduction aimed at achieving a more energetically efficient running pattern. In our study, the neuromuscular data supported a similar adaptation, characterized by a systematic reduction in muscle activation during the post-strike phase. The VM showed the most robust response, with both a significant main effect and a distinct post-hoc reduction (g=0.742, p=.006). Although other muscles such as the SOL (g=0.568) and GL (g=0.528) did not exhibit significant pairwise differences, their medium effect sizes suggest a meaningful trend toward reduced neural drive. This discrepancy between significant main effects and non-significant post-hoc results can be attributed to the limited statistical power of the small sample size and the stringent post-hoc adjustments required by the high variability of irregular surface data.

Unlike previous studies that provided no pre-adaptation period, our study included a 3-minute walking and 5-minute running familiarization. Consequently, while we did not observe a global progressive reduction across all trials as seen in Mohr’s results, the significant decrease in VM between T1 and T4, combined with the medium effect sizes for the SOL and GL, underscores a nuanced adaptive response. We interpret these reductions not as absolute optimization, but as neuromuscular tuning, where the central nervous system progressively diminishes unnecessary muscle co-activation to stabilize the joints more efficiently after repeated exposure to the treadmill's surface. This process likely reflects the specific demands of the ‘uneven surface’ which required higher neuromuscular control than level ground, despite our 8-minute familiarization period based on the recommendation of Mohr et al. (2022). Unpredictable surfaces demand significantly higher neuromuscular control compared to stable ground. In this regard, Sterzing et al. (2014b) confirmed that during irregular surface running, while the activity of sagittal plane muscles was similar to level running, the activity of the PL significantly increased to ensure lateral stability. This suggests that runners mobilize additional resources for compensatory control in response to perturbations. Therefore, while general running adaptation was likely achieved during the warm-up, it is possible that a more conservative adaptation phase to cope with the unstable surface appears to have extended into the initial measurement trials.

The systematic reduction in VM activity is closely related to the aforementioned ‘cautious gait’ strategy for coping with uneven terrain. The VM plays a key role in dynamic knee stabilization (Dixit et al., 2007;

Gawda et al., 2019). Previous studies have reported that runners increase VM activation as an immediate compensatory strategy to enhance knee stability in unpredictable environments (Besier et al., 2003; Blair et al., 2018; Voloshina & Ferris, 2015). Therefore, the high activation observed in the early trials is interpreted as a neuromuscular response serving as an initial protective mechanism to secure joint stability. This interpretation aligns with our kinematic findings regarding the maximum knee flexion angle during the pre-strike phase. Although statistical significance was not reached in post-hoc tests, the observed decrease in Trial 4 (68.0°) compared to Trials 1–2 (approx. 79-80°) (g=0.506) suggests that the high activation in Trial 1 was a preparatory defensive action to pre-flex the knee for shock absorption. As trials progressed, this initial defence was followed by a neuromuscular tuning process that reduced unnecessary muscle recruitment. This adjustment, characterized by a redistribution of roles within the musculoskeletal system rather than a simple reduction in activity, is supported by Honert et al. (2022), who observed a sophisticated internal redistribution of work between the ankle and foot during prolonged running. Similarly, the progressive reduction in activation seen here mirrors the findings of Mohr et al. (2022), where such changes were interpreted as the neuromuscular system learning to improve movement efficiency through repeated exposure, rather than a result of muscle fatigue.

Ultimately, the systematic reduction of muscle activity observed in this study is part of a complex adaptation strategy to cope with unstable environments. Our results, particularly the large effect size for VM, indicate that even after the 8-minute warm-up suggested by Mohr et al. (2022), runners continued to fine-tune their strategies during the initial measurement trials. This suggests that for tasks requiring high neuromuscular demand, such as running on irregular surfaces, a more extensive familiarization period—potentially exceeding the 6–10 minutes typically recommended for stable ground (Macdermid et al., 2026; Mohr et al., 2022; Paquette et al., 2024)—is essential for securing reliable biomechanical data.

This study has several limitations. First, all measurements were conducted on a treadmill at a fixed speed, which may not perfectly replicate the variable speeds and terrain changes characteristic of actual outdoor trail running. Second, despite the participants being experienced runners, the small sample size (N=11) and the application of the conservative Bonferroni correction for post-hoc comparisons may have increased the risk of Type II errors. Although this rigorous alpha-level was necessary to minimize Type I errors given the high data variability of irregular surfaces, it may have obscured statistically significant pairwise differences in variables where Hedges' g indicated meaningful effect sizes. Third, the kinematic analysis was restricted to the sagittal plane, potentially missing adaptation strategies in the frontal and transverse planes that are critical for maintaining lateral stability. Fourth, from a microscopic perspective, the 3-minute trial duration might have been insufficient for complete neuromuscular accommodation to the specific shoe-surface interface. Although the footwear models used in this study shared a fundamentally similar structure with only subtle functional variations, the complex interaction between these slight differences and the irregular terrain may have required a more extended period for the neuromuscular system to reach a fully 'tuned' state. Finally, the study population was limited to males, making it difficult to generalize these results to female runners. Additionally, measurements were performed on the right leg, meaning that potential bilateral asymmetries or compensatory adaptations in the contralateral limb during the familiarization process were not assessed.

In conclusion, this study demonstrates that during running on uneven terrain, distinct temporal characteristics exist between sagittal-plane kinematic stabilization and the systematic reduction of neuromuscular activation. While kinematic patterns stabilized relatively quickly, the neuromuscular system continued to adapt through a systematic reduction in muscle activation. These findings provide important implications for the design of future footwear functional evaluation protocols. To minimize the confounding influence of the 'trial order effect,' our results suggest a more extensive familiarization period (approximately

10 minutes) specifically for unstable terrain testing, based on the observed medium-to-large effect sizes even after an 8-minute warm-up. Future research should explore these neuromuscular tuning mechanisms by incorporating metabolic cost measurements to confirm whether these reductions directly translate to improved running economy or altered co-activation strategies.

Conclusion

This study aimed to establish a reliable experimental protocol for evaluating trail running footwear on uneven terrain by investigating the trial order effect on kinematic and neuromuscular adaptations. The key finding is that during repeated trials on irregular surfaces, runners' sagittal-plane kinematic patterns stabilize relatively quickly, whereas the neuromuscular system undergoes a progressive tuning process. This demonstrates that kinematic stability does not necessarily equate to neuromuscular stability, which has critical implications for footwear studies that concurrently measure kinematics and muscle activity.

Specifically, relying solely on kinematic indicators to determine adaptation periods may lead to interpretive errors, as the ongoing neuromuscular adjustment might be mistaken for the intrinsic effects of the footwear. To ensure data reliability in future research on unstable terrain, we recommend a more extensive global familiarization period (approximately 10 minutes) to allow the neuromuscular system to reach a stable state. Furthermore, considering the shoe-specific neuromuscular adaptation required for specific footwear properties, individual trial durations should potentially be extended beyond the 3-minute period used in this study. Therefore, to secure reliable data, future related research protocols should consider this multi-layered process when evaluating kinematic variables and neuromuscular data concurrently.

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Author Contributions

Conceptualization: S. Park, K. Han
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Formal analysis: S. Park, S. Kim
Investigation: S. Park
Project administration: J. Isherwood
Writing-original draft preparation: S. Park
Writing-review and editing: J. Isherwood, S. Kim, J. Lee, K. Han

Conflict of Interest

The authors declare no conflict of interest.

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