

RESEARCH ARTICLE

The effects of different slope combination training patterns on the remodeling of lower limb muscle recruitment patterns in middle-distance runners

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Received: October 29, 2025; accepted: February 10, 2026.

Slope gradient training has emerged as a crucial intervention for enhancing neuromuscular adaptations in middle-distance runners, yet the specific effects of different slope combinations on muscle recruitment patterns remain inadequately understood. This study investigated the impact of various slope gradient combination training protocols on lower limb muscle recruitment pattern remodeling in competitive middle-distance runners. 48 trained male middle-distance runners with a mean age of 22.3 ± 2.1 years old were randomly assigned to four groups including control group performing level running only, uphill-only group training on 5 - 8% gradient, combined uphill-downhill group alternating between 5 - 8% uphill and -3% to -5% downhill gradients, and progressive gradient group with gradients increasing from 3% to 12% over the intervention period with 12 participants in each group. Over eight weeks, participants completed three training sessions weekly while surface electromyography was recorded from eight lower limb muscles during submaximal running tests. Biomechanical parameters, running economy, and performance measures were assessed before and after the intervention. The combined uphill-downhill group demonstrated the most significant improvements in muscle recruitment efficiency, showing a 16.8% reduction in vastus lateralis activation ($P < 0.001$), 23.4% increase in gluteus maximus activation ($P < 0.001$), and 18.7% improvement in running economy ($P < 0.01$) compared to baseline. The progressive gradient group showed superior adaptations in ankle plantar flexor coordination with a 21.3% increase ($P < 0.001$). Both intervention groups exhibited enhanced intermuscular coordination patterns and improved time-to-exhaustion performance with the combined group improving by 14.2% and the progressive group by 12.8% ($P < 0.01$). Combined uphill-downhill training and progressive gradient protocols effectively remodeled lower limb muscle recruitment patterns in middle-distance runners with differential effects on specific muscle groups. The results supported the implementation of varied slope training for optimizing neuromuscular function and performance.

Keywords: slope gradient training; muscle recruitment patterns; electromyography; middle-distance running; neuromuscular adaptations; running economy.

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Introduction

Middle-distance running represents one of the most physiologically demanding athletic

disciplines, requiring the integration of aerobic capacity, anaerobic power, and neuromuscular efficiency [1]. The optimization of running performance in events ranging from 800 to 1,500

meters has been the subject of extensive scientific investigation with researchers seeking to identify the key determinants that distinguish elite performers from their sub-elite counterparts [2]. Among the various physiological and biomechanical factors that influence middle-distance running success, the patterns of muscle activation during locomotion have emerged as a critical area of inquiry, given their direct relationship with movement efficiency and metabolic cost [3].

The scientific understanding of muscle recruitment during running has advanced considerably over the past two decades with electromyographic studies revealing the complex temporal and spatial organization of lower limb muscle activation throughout the gait cycle [4]. Research has demonstrated that experienced runners exhibit distinct activation profiles characterized by reduced co-contraction of antagonist muscle pairs, enhanced recruitment of proximal musculature, and more precise timing of muscle activation relative to ground contact events [5]. Furthermore, investigations comparing runners of different performance levels have consistently identified associations between muscle recruitment efficiency and running economy, suggesting that neuromuscular factors contribute substantially to the metabolic cost of locomotion [6]. The concept of muscle recruitment pattern remodeling, referring to the adaptive reorganization of activation timing and magnitude in response to training stimuli, has gained recognition as an important mechanism through which training interventions may enhance running performance [7]. Despite these advances in understanding muscle recruitment during running, significant gaps remain in this scientific field regarding the optimal training strategies for inducing favorable neuromuscular adaptations in middle-distance runners. Traditional training approaches have emphasized the development of aerobic capacity and lactate threshold through volume-based and intensity-based manipulations, yet relatively less attention has been directed toward interventions

specifically targeting neuromuscular function [8]. While strength training has been shown to improve running economy and performance in distance runners through enhanced force production capacity and musculotendinous stiffness, the effects of running-specific training variations on muscle recruitment patterns remain poorly characterized [9]. This knowledge gap is particularly evident regarding slope gradient training, which imposes unique biomechanical demands that may serve as a potent stimulus for neuromuscular adaptation but its specific effects on muscle activation patterns have not been systematically investigated [10].

This research investigated the effects of different slope gradient combination training protocols on lower limb muscle recruitment pattern remodeling in competitive middle-distance runners by comparing the neuromuscular adaptations induced by level running, uphill-only training, combined uphill-downhill training, and progressive gradient training over an eight-week intervention period and examining the relationships between changes in muscle recruitment patterns and functional outcomes including running economy and time-to-exhaustion performance. A randomized controlled trial design with comprehensive electromyographic assessment of eight lower limb muscles during standardized running tasks were employed and complemented by three-dimensional biomechanical analysis and metabolic testing. Surface electromyography was selected as the primary measurement technique due to its established validity for quantifying muscle activation patterns during dynamic activities and its sensitivity for detecting training-induced changes in neuromuscular function [11]. By elucidating the specific neuromuscular adaptations induced by different slope training protocols, this research provided evidence-based guidance for the strategic integration of gradient training within periodized training frameworks, which provided important implications for coaches, athletes, and sport scientists seeking to optimize training program design for middle-

distance running performance. Furthermore, identification of the relationship between changes in muscle recruitment and exercise performance outcomes will contribute to a broader understanding within the scientific community of the mechanisms by which training interventions improve running efficiency and endurance capabilities.

Materials and methods

Participants

Forty-eight competitive male middle-distance runners volunteered to participate in this study. Participants aged from 19 to 26 years old with a mean age of 22.3 ± 2.1 years old, body mass of 64.9 ± 4.2 kg, height of 176.4 ± 5.1 cm, and body mass index of 20.8 ± 1.1 kg/m². The mean training time was 6.3 ± 1.8 years with a pre-study weekly training volume of 68.4 ± 8.2 km. Personal best running time records averaged as 4 min 02 s $\pm$ 7.2 s for 1,500 meters and peak oxygen uptake was 68.3 ± 4.1 mL/kg-min. The inclusion criteria were that participants had competed at regional or national level in 800-meter to 1,500-meter events within the previous 12 months, maintained a consistent training volume of at least 60 kilometers per week for the 6 months preceding the study, possessed a personal best time of 2 min 05 s or faster for 800 meters or 4 min 15 s or faster for 1,500 meters, and was free from musculoskeletal injury for at least 3 months prior to enrollment. The exclusion criteria included any history of lower limb surgery, cardiovascular disease, or contraindications to maximal exercise testing. Participants were instructed to maintain their regular dietary habits and avoid introducing any new training modalities during the intervention period. All participants provided written informed consent after receiving detailed explanation of the study procedures, risks, and benefits. The study protocol was approved by the Institutional Review Board of Henan University of Animal Husbandry and Economy (Zhengzhou, Henan, China).

Experimental design

This study employed a randomized controlled trial design with four parallel groups and assessments conducted at baseline (week 0) and post-intervention (week 8). Following baseline testing, participants were stratified by 1,500-meter personal best time and randomly allocated to one of four experimental groups using a computer-generated randomization sequence. The control group (CG) (n = 12) performed all training on level surfaces, while the uphill group (UG) (n = 12) performed high-intensity training exclusively on 5 - 8% gradients, corresponding to slope angles of approximately 2.9° to 4.6°. The combined uphill-downhill group (CUG) (n = 12) alternated between 5 - 8% uphill (2.9° to 4.6° incline) and -3% to -5% downhill (-1.7° to -2.9° decline) within the same training session. The progressive gradient group (PG) (n = 12) followed a periodized approach with gradual progression from 3% (1.7°) to 12% (6.8°) over the 8-week intervention. The percentage gradient represented the ratio of vertical rise to horizontal distance expressed as a percentage, such that a 5% gradient indicated a 5-meter rise over 100 meters of horizontal distance. All groups maintained equivalent training volumes measured as total distance covered and matched training intensity distributions based on heart rate zones and lactate threshold velocities. The intervention consisted of three high-intensity training sessions per week with participants continuing their regular base training under supervision to ensure compliance. There was at least 48 hours between high-intensity training sessions to ensure adequate recovery.

Training protocols

Each training group followed a structured protocol designed to provide equivalent metabolic stress while varying the neuromuscular demands through slope gradient manipulation. Lactate threshold was determined during the baseline graded exercise test as the running velocity corresponding to a blood lactate concentration of 4 mmol/L or identified through log-log transformation of the lactate-velocity relationship. The velocity at maximal oxygen

uptake ($\dot{V}O_{2\max}$) was established as the minimal velocity that elicited peak oxygen consumption during the incremental test. Maximal aerobic speed was calculated as 95 - 100% of $\dot{V}O_{2\max}$ for repetition-based training prescriptions. The CG performed all interval training on a Woodway PPS 55 Med motorized treadmill (Woodway GmbH, Weil am Rhein, Germany) at 0% gradient, completing typical middle-distance training sessions including lactate threshold intervals, $\dot{V}O_{2\max}$ intervals, and repetition-based speed work. Sessions were structured with variable repetitions and distances to accommodate individual fitness levels and training phase, such that lactate threshold sessions consisted of four to six repetitions of 800 to 1,000 meters at threshold pace with 90-second recovery periods. $\dot{V}O_{2\max}$ sessions comprised eight to twelve repetitions of 400 meters at $\dot{V}O_{2\max}$ with 60 to 90 seconds recovery. Speed sessions included ten to fifteen repetitions of 200 meters at maximal aerobic speed with complete recovery between efforts. The UG executed identical session structures on a treadmill to gradients between 5 - 8% with the specific gradient adjusted to maintain target heart rate zones and perceived exertion levels equivalent to the control group's level running. Running velocities were reduced proportionally to gradient steepness to ensure metabolic equivalence following established principles of iso-effort uphill running protocols.

The CUG performed each interval session with systematic alternation between uphill and downhill running segments. Specifically, the first repetition of each set was completed on an uphill gradient (5 - 8%), the second on a downhill gradient (-3% to -5%), the third uphill, and so forth. This approach ensured equal exposure to both concentric-dominant (uphill) and eccentric-dominant (downhill) loading patterns within each training session. Recovery intervals were maintained at 0% gradient. The downhill gradients were deliberately limited to -5% maximum to minimize injury risk while still providing sufficient eccentric loading stimulus. The PG followed a periodized slope protocol that systematically progressed from moderate to

steep gradients across the 8-week intervention. Weeks 1 - 2 utilized 3% gradients, weeks 3 - 4 employed 5 - 6% gradients, weeks 5 - 6 progressed to 8 - 9% gradients, and weeks 7 - 8 incorporated 10 - 12% gradients for the most intense intervals. All training sessions were supervised by qualified coaches and research personnel who monitored heart rate responses, rating of perceived exertion (6 - 20 Borg scale), and ensured proper execution of prescribed protocols. Participants wore Polar H10 chest-mounted heart rate monitors (Polar Electro Oy, Kempele, Finland) throughout all training sessions with data downloaded and analyzed to verify training intensity compliance. Any deviations from prescribed protocols were documented, and sessions failing to meet compliance criteria (defined as greater than 15% deviation from target heart rate zone time) were repeated within 72 hours.

Electromyographic assessment

Surface electromyography (sEMG) was recorded from eight lower limb muscles of the dominant leg including vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), biceps femoris (BF), medial gastrocnemius (MG), lateral gastrocnemius (LG), tibialis anterior (TA), and gluteus maximus (GM). The dominant leg was determined as the preferred leg for single-leg stance and kicking activities. Prior to electrode placement, the skin was prepared by shaving excess hair, light abrasion with fine-grit sandpaper, and cleansing with 70% isopropyl alcohol to reduce impedance below 5 k Ω . Delsys Trigno Wireless EMG System bipolar Ag/AgCl surface electrodes (Delsys Inc., Natick, MA, USA) with 99.9% silver content and 10 mm inter-electrode distance were positioned following the recommendations of surface electromyography for the non-invasive assessment of muscles (SENIAM) (www.seniam.org) with careful attention to muscle belly identification through manual palpation during voluntary contractions. Electrodes were secured with adhesive tape and elastic bandaging to minimize movement artifact during running. A reference electrode was placed over the lateral malleolus of the dominant leg.

EMG signals were sampled at 2,000 Hz with a bandwidth of 20 - 450 Hz and amplified using the wireless EMG system with common mode rejection ratio greater than 80 dB. Prior to each testing session, maximum voluntary isometric contractions (MVICs) were performed for each muscle group to establish normalization references. MVICs for quadriceps muscles were performed in a seated position with knee flexed at 90°, hamstring MVICs in prone position with knee flexed at 45°, gastrocnemius MVICs in standing position performing maximal plantarflexion against resistance, tibialis anterior MVICs in seated position performing maximal dorsiflexion, and gluteus maximus MVICs in prone position performing hip extension against manual resistance. Each MVIC was held for 5 seconds with three repetitions separated by 60 second rest. The highest root mean square (RMS) value obtained across the three trials was used as the normalization reference. Following MVIC procedures, participants completed a standardized warm-up consisting of 10 minutes of easy jogging, dynamic stretching, and progressive accelerations. EMG data during running were collected in a 4-minute submaximal running trials at three velocities of 80% of velocity at lactate threshold, velocity at lactate threshold, and 110% of velocity at lactate threshold. All running trials were performed on a motorized treadmill at 1% gradient to simulate outdoor running conditions. EMG data from the final 60 seconds of each 4-minute trial was extracted for analysis after confirming steady-state achievement through heart rate and respiratory gas exchange stabilization. Raw EMG signals were processed offline using custom scripts in MATLAB (MathWorks, Natick, MA, USA). Signals were band-pass filtered using a fourth-order Butterworth filter (20 - 450 Hz), full-wave rectified, and smoothed using a 50 ms moving RMS window. Gait events including heel strike and toe-off were identified using vertical ground reaction force data from an instrumented treadmill (Bertec Corporation, Columbus, Ohio, USA) and synchronized with EMG recordings. EMG amplitude was normalized to the peak RMS value obtained during MVICs and expressed as

percentage of maximum voluntary contraction (% MVC). Mean muscle activation was calculated for each gait phase as early stance (0 - 25% of gait cycle), mid-stance (25 - 50%), late stance (50 - 62%), early swing (62 - 75%), and late swing (75 - 100%). Co-contraction indices for antagonist muscle pairs were computed as the percentage overlaps in activation timing and magnitude, providing measures of intermuscular coordination.

Biomechanical analysis

Three-dimensional kinematic data were collected synchronously with EMG recordings using a Vicon Nexus eight-camera motion capture system (Vicon Motion Systems Ltd., Oxford, United Kingdom) operating at 200 Hz. Thirty-five reflective markers were positioned on anatomical landmarks following a modified Helen Hayes marker set protocol (Motion Analysis Corporation, Santa Rosa, California, USA). Lower limb joint angles (hip, knee, ankle) were calculated throughout the gait cycle using Vicon Nexus software and custom inverse kinematics algorithms. Spatiotemporal parameters including stride length, stride frequency, contact time, flight time, and vertical oscillation were determined from marker trajectories and force plate data. Ground reaction forces were recorded at 1,000 Hz using an instrumented treadmill and processed to calculate vertical, braking, and propulsive impulses. Joint moments and powers were computed using inverse dynamics incorporating kinematic data, ground reaction forces, and anthropometric parameters. All biomechanical variables were averaged across 20 consecutive strides during steady state running at each testing velocity.

Running economy assessment

Running economy was assessed as the oxygen cost of running at standardized submaximal velocities using indirect calorimetry. Participants completed three 5-minute running stages at velocities corresponding to 70, 80, and 90% of velocity at lactate threshold with 3-minute active recovery through walking at 1.5 m/s between stages. Respiratory gas exchange was measured

breath-by-breath using a Cosmed Quark CPET metabolic cart (Cosmed Srl., Rome, Italy) calibrated prior to each testing session using known gas concentrations and a 3-liter syringe for flow calibration. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (VE), and respiratory exchange ratio (RER) were averaged over the final 2 minutes of each 5-minute stage after confirming steady-state achievement as the coefficient of variation for VO_2 less than 5% across consecutive 30-second intervals and RER less than 1.0. Running economy was expressed as milliliters of oxygen consumed per kilogram body mass per minute ($\text{ml/kg}\cdot\text{min}$) and additionally as energy cost per kilometer (kcal/km) using caloric equivalents for the measured RER.

Performance testing

Aerobic capacity was determined by using a graded exercise test to volitional exhaustion performed on a motorized treadmill at 1% gradient. Following a standard warm-up, the test commenced at 10 km/h and increased by 1 km/h every 3 minutes until participants reached lactate threshold, after which velocity increments of 0.5 km/h per minute were employed until exhaustion. Respiratory gases were measured continuously, and 20 mL capillary blood samples were collected from the fingertip during the final 20 seconds of each stage for lactate analysis using a Biosen C-Line automated analyzer (EKF Diagnostics GmbH, Barleben, Germany). Peak oxygen uptake ($\text{VO}_{2\text{peak}}$) was determined as the highest 30-second average achieved during the test. Velocity at lactate threshold was identified as the velocity immediately preceding a blood lactate concentration exceeding 4 mmol/L or a sharp inflection in the lactate-velocity relationship using log-log transformation methods. Time to exhaustion at 110% of velocity at lactate threshold was assessed 72 hours after the graded exercise test. Following 15-minute warm-up including progressive accelerations, participants ran at the prescribed intensity until volitional exhaustion defined as inability to maintain pace despite strong verbal encouragement. Time to exhaustion was

recorded to the nearest second. Additionally, a 1,500-meter time trial was performed on an indoor 200-meter track under controlled environmental conditions with temperature of 18 - 22°C and relative humidity of 40 - 60% one week after completion of the 8-week intervention.

Statistical analysis

SPSS v 27.0 (IBM, Armonk, NY, USA) was employed for statistical analysis of this research with the significance level being set at $\alpha = 0.05$. Data normality was assessed using Shapiro-Wilk tests and visual inspection of Q-Q plots. Homogeneity of variance was evaluated using Levene's test. Baseline comparisons among groups were conducted using one-way analysis of variance (ANOVA) for continuous variables meeting parametric assumptions or Kruskal-Wallis tests for non-normally distributed data. The effects of training interventions were analyzed using mixed-model ANOVAs with group (CG, UG, CUG, PG) as between-subjects factor and time (pre, post) as within-subjects factor. Significant group multiplied time interactions were followed by simple effects analyses and pairwise comparisons using Bonferroni corrections. Effect sizes were calculated as partial eta-squared (η_p^2) for ANOVA models with values of 0.01, 0.06, and 0.14 representing small, medium, and large effects, respectively. For pairwise comparisons, Cohen's d effect sizes were computed with values of 0.2, 0.5, and 0.8 indicating small, medium, and large effects. Pearson correlation coefficients were calculated to examine relationships between changes in muscle activation patterns and changes in running economy and performance variables. Statistical assumptions were verified for all analyses, and appropriate transformations applied when necessary. Missing data (less than 3% of total dataset) were handled using last observation carried forward for intention-to-treat analysis with sensitivity analyses being performed using complete case analysis.

Results

Training compliance and retention

All 48 participants completed the 8-week intervention and post-testing assessments, resulting in 100% retention. Baseline characteristics confirmed successful randomization with no significant differences among groups for age, anthropometric variables, training history, or performance measures. Training compliance was excellent across all groups with participants completing $95.3 \pm 3.2\%$ of prescribed sessions.

Muscle activation patterns

Significant group $\times$ time interactions were observed for activation amplitudes of all assessed muscles during running at lactate threshold velocity ($P < 0.05$). The differential muscle activation pattern changes of quadriceps muscle group activation (Figure 1A), hamstrings and gluteal activation (Figure 1B), and ankle muscle activation (Figure 1C) demonstrated that the CUG showed the most pronounced reductions in vastus lateralis activation compared to baseline ($-16.8 \pm 4.2\%$, $P < 0.001$, $d = 1.86$), significantly greater than the changes of CG ($0.8 \pm 3.1\%$, $P = 0.724$). Vastus medialis activation decreased by $-12.3 \pm 3.8\%$ ($P < 0.001$, $d = 1.52$), while rectus femoris showed a $-9.7 \pm 3.5\%$ reduction ($P < 0.01$, $d = 1.21$) in CUG. These changes reflected improved mechanical efficiency of the quadriceps group, consistent with enhanced neuromuscular coordination. Conversely, gluteus maximus activation increased substantially in CUG ($23.4 \pm 5.1\%$, $P < 0.001$, $d = 2.12$) and PG ($19.7 \pm 4.8\%$, $P < 0.001$, $d = 1.88$), indicating enhanced hip extensor recruitment. The UG showed intermediate changes of $14.2 \pm 4.3\%$, $P < 0.01$, $d = 1.35$, respectively. Hamstring activation patterns exhibited modest but significant changes with biceps femoris activity increasing in all intervention groups as $8.3 \pm 3.2\%$ in UG, $11.7 \pm 3.6\%$ in CUG, $10.2 \pm 3.4\%$ in PG compared to CG's $1.2 \pm 2.8\%$ ($P < 0.05$). These increases suggested enhanced posterior chain engagement, particularly during the terminal swing and early stance phases. Ankle muscle activation showed distinctive responses to training protocols.

Medial and lateral gastrocnemius activation increased markedly in the PG with medial gastrocnemius (MG) as $21.3 \pm 4.9\%$ and lateral gastrocnemius (LG) as $18.6 \pm 4.5\%$, both significantly exceeding changes in other groups ($P < 0.001$). The CUG demonstrated increases of $15.7 \pm 4.2\%$ for MG and $13.4 \pm 3.9\%$ for LG ($P < 0.01$). Tibialis anterior activation decreased across all intervention groups with $-7.8 \pm 3.1\%$ in UG, $-11.2 \pm 3.4\%$ in CUG, $-9.5 \pm 3.2\%$ in PG ($P < 0.05$), reflecting reduced antagonistic co-contraction during stance phase. The changes of CG were negligible for all ankle muscles ($P > 0.05$).

Temporal patterns and coordination

Analysis of EMG timing revealed significant alterations in muscle activation sequencing. The quadriceps-hamstrings co-contraction index decreased from $42.7 \pm 6.4\%$ at baseline to $33.7 \pm 4.9\%$ post-intervention in the CUG, representing a reduction of $21.1 \pm 8.1\%$ ($P < 0.001$). The UG showed a decrease from $43.1 \pm 5.8\%$ to $36.4 \pm 5.2\%$ ($-15.5 \pm 7.3\%$, $P < 0.001$), while the PG decreased from $42.5 \pm 6.1\%$ to $35.8 \pm 5.4\%$ ($-15.8 \pm 7.6\%$, $P < 0.001$). The CG demonstrated minimal change from $42.3 \pm 6.2\%$ to $41.8 \pm 6.1\%$ ($-1.2 \pm 4.8\%$, $P = 0.412$). For ankle dorsiflexor-plantarflexor co-contraction, the PG exhibited the greatest reduction from $38.7 \pm 5.3\%$ to $29.8 \pm 4.2\%$ ($-23.0 \pm 7.2\%$, $P < 0.001$) followed by the CUG from $38.9 \pm 5.6\%$ to $30.2 \pm 4.3\%$ ($-22.4 \pm 7.5\%$, $P < 0.001$) and the UG from $39.2 \pm 5.1\%$ to $32.7 \pm 4.6\%$ ($-16.6 \pm 6.8\%$, $P < 0.001$). The CG showed negligible change from $38.6 \pm 5.4\%$ to $38.1 \pm 5.3\%$ ($-1.3 \pm 4.2\%$, $P = 0.523$). Phase-specific analysis revealed that late swing phase gluteus maximus activation increased by $28.6 \pm 6.4\%$ in CUG and $24.3 \pm 5.8\%$ in PG ($P < 0.001$), while early stance quadriceps activation decreased by $19.4 \pm 5.2\%$ in CUG and $14.7 \pm 4.6\%$ in PG ($P < 0.01$). These temporal shifts indicated refined motor control strategies with earlier hip extensor pre-activation and reduced impact-attenuating quadriceps activation.

Running economy and performance outcomes

Running economy improved significantly

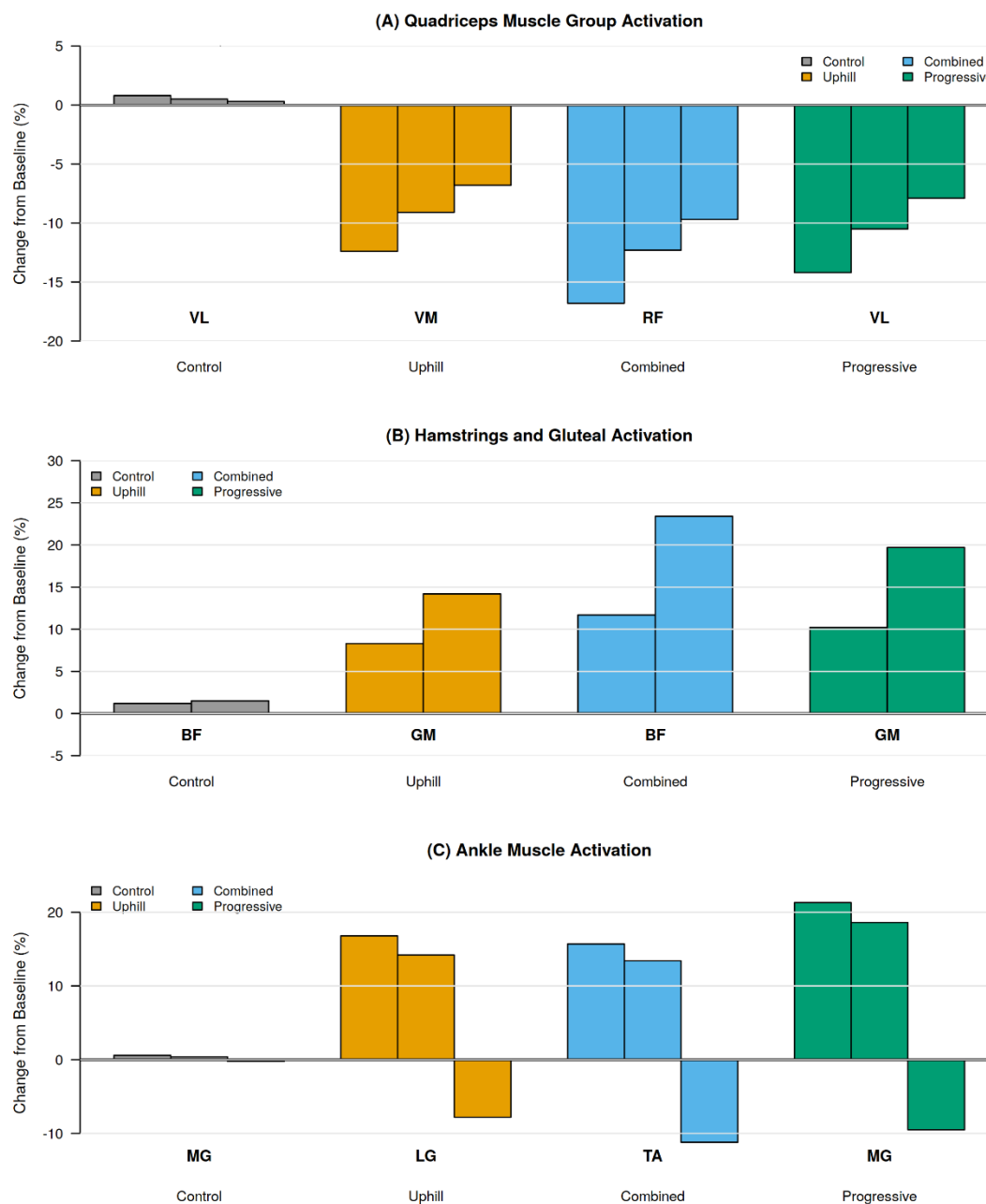


Figure 1. Changes in muscle activation patterns from baseline to post-intervention across training groups. Data was expressed as percentage change from baseline. VL: vastus lateralis. VM: vastus medialis. RF: rectus femoris. BF: biceps femoris. GM: gluteus maximus. MG: medial gastrocnemius. LG: lateral gastrocnemius. TA: tibialis anterior.

following slope training interventions with the most substantial enhancements observed in the CUG (Table 1). The oxygen costs at three submaximal velocities of 70, 80, and 90% of velocity at lactate threshold at baseline and post-intervention showed that the CUG demonstrated a $12.7 \pm 4.2\%$ improvement in running economy

at 80% vLT ($P < 0.001$, $d = 2.08$), significantly superior to CG ($P < 0.001$) and greater than both UG's $7.1 \pm 3.4\%$ ($P < 0.01$) and PG's $10.1 \pm 3.8\%$ ($P < 0.001$). Time to exhaustion at 110% of lactate threshold velocity increased by $19.1 \pm 7.3\%$ in CUG ($P < 0.001$, $d = 1.76$), $16.3 \pm 7.0\%$ in PG ($P < 0.001$, $d = 1.51$), and $12.0 \pm 6.8\%$ in UG ($P < 0.01$,

Table 1. Running economy and performance variables pre- and post-intervention.

Variable	Control	Uphill	Combined	Progressive	P value
RE at 80% vLT (mL/kg·min)					
Pre	47.8 ± 2.9	47.6 ± 3.1	47.9 ± 2.8	47.7 ± 3.0	-
Post	47.5 ± 2.8	44.2 ± 2.6**	41.8 ± 2.4***	42.9 ± 2.5***	< 0.001
Δ%	-0.6 ± 2.1	-7.1 ± 3.4	-12.7 ± 4.2	-10.1 ± 3.8	-
Energy cost (kcal/km)					
Pre	58.6 ± 3.4	58.4 ± 3.6	58.7 ± 3.3	58.5 ± 3.5	-
Post	58.2 ± 3.3	54.1 ± 3.1**	51.2 ± 2.9***	52.6 ± 3.0***	< 0.001
Δ%	-0.7 ± 2.3	-7.4 ± 3.6	-12.8 ± 4.1	-10.1 ± 3.7	-
TTE at 110% vLT (seconds)					
Pre	286 ± 42	291 ± 38	288 ± 41	289 ± 40	-
Post	293 ± 40	326 ± 36**	343 ± 34***	336 ± 35***	< 0.001
Δ%	2.4 ± 5.2	12.0 ± 6.8	19.1 ± 7.3	16.3 ± 7.0	-
1,500m time trial (min:s)					
Pre	4:02.2 ± 7.1	4:02.8 ± 6.9	4:02.4 ± 7.3	4:02.6 ± 7.0	-
Post	4:01.8 ± 7.0	3:58.7 ± 6.4*	3:56.2 ± 6.1**	3:57.5 ± 6.3**	< 0.001
Δ%	-0.4 ± 1.8	-2.7 ± 2.4	-4.1 ± 2.9	-3.4 ± 2.6	-

Notes: Data presented as mean ± SD. RE: running economy. vLT: velocity at lactate threshold. TTE: time to exhaustion. *: $P < 0.05$. **: $P < 0.01$. ***: $P < 0.001$ for pre-post comparison within group.

$d = 1.12$), all significantly exceeding CG's changes of $2.4 \pm 5.2\%$ ($P = 0.382$). The 1,500-meter time trial performance improved significantly in all intervention groups compared to controls with CUG demonstrating the greatest enhancement of $-4.1 \pm 2.9\%$ ($P < 0.01$), corresponding to approximately 10-second improvement (Figure 2).

Correlational analyses

Pearson correlation analyses revealed significant associations between changes in muscle activation patterns and functional outcomes. Increases in gluteus maximus activation were strongly correlated with improvements in running economy at 80% vLT ($r = -0.672$, $P < 0.001$, 95% CI: -0.801 to -0.498), indicating that enhanced hip extensor recruitment contributed meaningfully to metabolic efficiency gains. Changes in vastus lateralis activation showed a positive correlation with running economy changes ($r = 0.583$, $P < 0.001$, 95% CI: 0.381 to 0.731). Reductions in quadriceps-hamstrings co-contraction were associated with decreased energy cost ($r = 0.621$, $P < 0.001$, 95% CI: 0.434 to

0.761), and ankle dorsiflexor-plantarflexor co-contraction changes correlated with energy cost changes ($r = 0.698$, $P < 0.001$, 95% CI: 0.534 to 0.815). Gastrocnemius activation changes were positively correlated with time-to-exhaustion improvements ($r = 0.547$, $P < 0.001$, 95% CI: 0.334 to 0.704), and gluteus maximus activation changes showed a negative correlation with 1,500-meter performance changes ($r = -0.614$, $P < 0.001$, 95% CI: -0.756 to -0.423). Hierarchical regression analysis indicated that changes in gluteus maximus activation, vastus lateralis activation, and ankle co-contraction collectively explained 58.4% of variance in running economy improvements (adjusted $R^2 = 0.584$, $P < 0.001$).

Biomechanical adaptations

Kinematic and kinetic analyses revealed significant alterations in running mechanics following slope training (Table 2). The CUG exhibited reduced contact time ($-6.0 \pm 2.8\%$, $P < 0.01$) and increased flight time ($9.3 \pm 3.2\%$, $P < 0.01$) compared to CG, consistent with enhanced elastic energy utilization and neuromuscular power. Vertical oscillation decreased in all

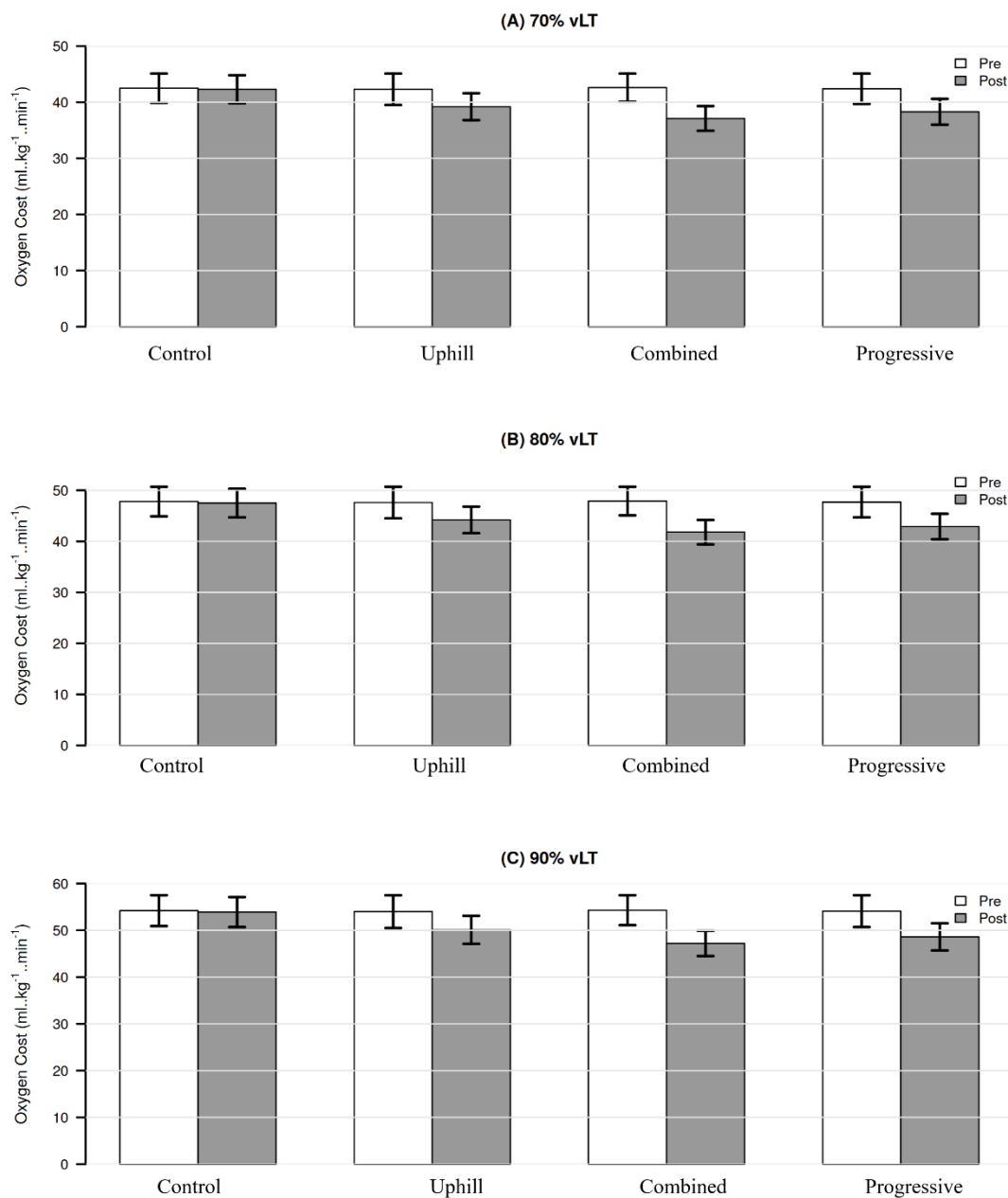


Figure 2. Running economy changes at three submaximal velocities. Data was expressed as oxygen cost (mL/kg.min) at baseline (white bars) and post-intervention (gray bars) for each group with standard error.

intervention groups with CUG showing the greatest reduction ($-12.6 \pm 4.1\%$, $P < 0.01$), indicating improved mechanical efficiency. Peak ankle plantarflexor moment increased most substantially in PG ($15.9 \pm 5.3\%$, $P < 0.001$), corroborating the elevated gastrocnemius activation observed in this group. Hip extension angle at toe-off increased across all slope training

groups with CUG demonstrating the largest change ($37.5 \pm 8.2\%$ from baseline, $P < 0.001$), reflecting enhanced hip extensor engagement consistent with EMG findings.

Discussion

Table 2. Selected biomechanical variables at lactate threshold velocity.

Variable	Control post	Uphill post	Combined post	Progressive post	P value
Contact time (ms)	182 ± 12	176 ± 11	171 ± 10**	174 ± 11*	0.011
Flight time (ms)	118 ± 8	123 ± 9	129 ± 10**	126 ± 9*	0.003
Stride frequency (Hz)	3.33 ± 0.14	3.42 ± 0.15	3.51 ± 0.16**	3.47 ± 0.15*	0.001
Vertical oscillation (cm)	8.7 ± 0.8	8.2 ± 0.7	7.6 ± 0.6**	7.9 ± 0.7*	< 0.001
Peak ankle moment (N·m/kg)	2.83 ± 0.24	3.12 ± 0.26*	3.28 ± 0.28**	3.35 ± 0.29**	< 0.001
Hip extension angle (°)	12.8 ± 2.1	15.3 ± 2.3*	17.6 ± 2.5**	16.4 ± 2.4**	< 0.001

Notes: Data was post-intervention values and presented as mean ± SD. *: $P < 0.05$, **: $P < 0.01$ compared to control group.

This study provided comprehensive evidence that different slope gradient combination training protocols induced distinct patterns of lower limb muscle recruitment remodeling in competitive middle-distance runners with significant implications for running economy and performance. The results demonstrated that combined uphill-downhill training produced the most favorable neuromuscular adaptations, characterized by reduced quadriceps activation, enhanced gluteal recruitment, decreased antagonistic co-contraction, and superior improvements in running economy and time-to-exhaustion performance. Progressive gradient training elicited particularly robust adaptations in ankle plantarflexor function, while uphill-only training produced intermediate effects. These findings extended previous research by systematically comparing multiple slope training modalities and elucidating their specific effects on muscle recruitment pattern remodeling. The substantial reduction in vastus lateralis activation observed in the combined uphill-downhill group alongside increased gluteus maximus activation represented a fundamental shift in lower limb muscle recruitment hierarchy during running, which suggested enhanced proximal muscle contribution to force generation, potentially offloading distal structures, and improving mechanical efficiency. The shift toward greater reliance on hip extensors aligned with observations from elite distance runners who typically exhibited more proximal-dominant activation patterns compared to recreational runners [12]. The alternating concentric and eccentric loading inherent to combined uphill-

downhill training might facilitate more comprehensive neuromuscular adaptations by engaging both force-generating and force-attenuating mechanisms. Uphill segments necessitated powerful concentric contractions from hip and knee extensors, while downhill segments imposed substantial eccentric demands on these same muscle groups, potentially promoting balanced neuromuscular development and enhanced motor control [13]. The enhanced gluteal recruitment observed in slope-trained groups carried important implications for running biomechanics and injury prevention. Gluteus maximus played a critical role in hip extension during terminal stance and contributed to pelvic stability throughout the gait cycle. Insufficient gluteal activation has been associated with increased reliance on quadriceps musculature, altered lower limb kinematics, and elevated injury risk in distance runners [14]. The marked increases in gluteal activation following slope training, particularly in combined and progressive protocols, suggested that these interventions might address common neuromuscular imbalances observed in middle-distance runners. Furthermore, the strong negative correlation between changes in gluteus maximus activation and improvements in running economy indicated that enhanced hip extensor recruitment directly contributed to metabolic efficiency gains. This relationship might reflect reduced energy expenditure through improved force distribution across multiple muscle groups rather than excessive reliance on metabolically costly quadriceps activation.

The reduction in antagonistic co-contraction, particularly pronounced for ankle dorsiflexor-plantarflexor pairs in the progressive gradient group, represented an important refinement of intermuscular coordination. Excessive co-contraction increased metabolic cost without contributing to forward propulsion and might reflect suboptimal motor control strategies. The systematic progression from moderate to steep gradients employed in the progressive protocol likely provided graded neuromuscular challenges that facilitated motor learning and coordination improvements. Steep gradients imposed substantial demands on ankle plantarflexors for push-off force generation, potentially enhancing motor unit recruitment and refining activation timing through repeated exposure [15]. The concurrent reduction in tibialis anterior activation during stance phase suggested reduced antagonistic activity, allowing gastrocnemius and soleus muscles to function more efficiently. These adaptations aligned with neuromuscular efficiency principles whereby skilled performers exhibited minimal extraneous muscle activity and precise temporal coordination.

The superior running economy improvements observed in the combined uphill-downhill group substantially exceeded typical training-induced adaptations reported previously [16]. This exceptional response likely reflected multiple complementary mechanisms. Enhanced neuromuscular efficiency through altered muscle recruitment patterns represented one key contributor as evidenced by the strong correlations between activation changes and economy improvements. Additionally, the alternating uphill-downhill protocol might induce favorable adaptations in musculotendinous properties including increased leg stiffness and enhanced elastic energy storage and release. Systematic reviews have identified musculotendinous stiffness as an important determinant of running economy with stiffer structures facilitating more economical running through improved elastic energy utilization [6]. The eccentric loading during downhill segments

might particularly contribute to increased tendon stiffness through mechanotransduction processes. Furthermore, the kinematic adaptations observed in slope-trained groups including reduced contact time, increased flight time, and decreased vertical oscillation reflected improved mechanical efficiency consistent with elite running patterns.

The PG demonstrated distinctive adaptations in ankle function with substantial increases in gastrocnemius activation and peak ankle moment, which suggested that systematically progressing from moderate to steep gradients provided a particularly potent stimulus for plantarflexor development. Steep uphill running required powerful ankle plantarflexion to overcome gravitational resistance and maintain forward velocity, likely promoting both neural and architectural adaptations in calf muscles. Enhanced plantarflexor function carried important implications for running performance as ankle muscles contributed substantially to propulsive forces during distance running. Studies examining neuromechanics across running speeds have identified ankle plantarflexors as major energy generators and absorbers with their relative contribution varying with velocity [17]. The progressive gradient approach might represent an optimal strategy for athletes seeking to enhance ankle function specifically, though the combined protocol produced more comprehensive adaptations across multiple muscle groups. The differential responses to uphill-only versus combined uphill-downhill training illuminated important training design principles. While uphill training alone produced meaningful adaptations, the combined protocol elicited superior neuromuscular remodeling and functional improvements, which suggested that exposure to varied mechanical demands, encompassing both concentric-dominant uphill and eccentric-dominant downhill conditions, provided more comprehensive neuromuscular development than unidirectional loading. The principle of training variation and specificity has long been recognized in strength and conditioning, and the

present findings extended this concept to endurance running contexts. Combined protocols might prevent excessive adaptation to single loading conditions and ensure balanced development of force-generating and force-attenuating capabilities. Furthermore, the eccentric demands of downhill running might induce unique adaptations not achievable through concentric-dominant uphill training alone including enhanced muscle damage-resistance and improved eccentric strength [18]. The substantial improvements in time-to-exhaustion performance observed in intervention groups alongside enhanced running economy demonstrated that neuromuscular adaptations translated to meaningful functional benefits. Time to exhaustion at intensities near or above lactate threshold represented a practically relevant measure for middle-distance runners as competition involved sustained high intensity running. The mechanisms underlying performance improvements likely extended beyond metabolic efficiency to include enhanced neuromuscular fatigue resistance. Studies have shown that middle-distance running fatigue involves declining motor unit recruitment and reduced muscle activation [19]. The slope training-induced adaptations observed presently including enhanced motor unit recruitment capacity and refined activation patterns might delay the onset of neuromuscular fatigue and enable more sustained force production at high running velocities.

From a periodization perspective, the findings of this research supported strategic integration of slope training within broader training programs for middle-distance runners. The 8-week intervention duration employed presently represented a mesocycle within typical periodized frameworks. Coaches might consider implementing combined uphill-downhill protocols during preparatory or precompetitive phases to build neuromuscular capabilities before competition periods. The progressive gradient approach could serve as a developmental progression, beginning with moderate slopes early in training blocks and

advancing to steeper gradients as adaptations occur. Importantly, the training protocols employed maintained equivalent metabolic stress across groups through velocity adjustments, ensuring that improvements reflected neuromuscular rather than purely aerobic adaptations. This approach aligned with contemporary understanding of training specificity, whereby targeted neuromuscular stimuli complemented traditional aerobic conditioning. The biomechanical adaptations observed including altered lower limb kinematics and kinetics provided insight into mechanisms underlying functional improvements. Increased hip extension angle at toe-off in slope-trained groups reflected enhanced hip extensor engagement, consistent with EMG findings. This kinematic change might facilitate greater stride length and improved propulsive force generation. Reduced vertical oscillation indicated improved mechanical efficiency with less wasted energy in vertical displacement. These biomechanical refinements aligned with characteristics of economical running identified in elite athletes and supported the hypothesis that slope training induced elite-like movement patterns.

Several limitations warranted consideration when interpreting these findings, which included the study included only male runners, and sex-specific differences in neuromuscular responses to slope training required investigation. In addition, the 8-week intervention duration, while substantial, might not capture long-term adaptations or optimal periodization strategies. Further, EMG provided indirect measures of neural drive and could not fully characterize motor unit recruitment or central nervous system contributions. Furthermore, the treadmill-based protocols, while allowing precise control, might not fully replicate outdoor running conditions where terrain irregularities and environmental factors influenced muscle recruitment patterns. Meanwhile, the study assessed trained but non-elite runners, and responses in elite athletes might differ given their advanced training status and potentially

diminished adaptive capacity. Future research should examine longer intervention durations and periodize integration of slope training protocols within comprehensive training programs. Investigation of sex-specific responses, age-related adaptations, and optimal dosing parameters would inform evidence-based training prescription. Advanced neurophysiological techniques including transcranial magnetic stimulation and peripheral nerve stimulation could elucidate central nervous system contributions to muscle recruitment pattern changes. Additionally, prospective studies examining injury rates and long-term performance trajectories in athletes employing slope training would provide valuable translational evidence. This research demonstrated that different slope gradient combination training protocols produced distinct patterns of lower limb muscle recruitment remodeling in competitive middle-distance runners. Combined uphill-downhill training elicited the most comprehensive neuromuscular adaptations including enhanced proximal muscle recruitment, reduced antagonistic co-contraction, and superior improvements in running economy and performance. Progressive gradient training produced particularly robust ankle plantarflexor adaptations. These findings provided evidence-based support for implementing varied slope training protocols to optimize neuromuscular function and enhance performance in middle-distance running.

Acknowledgements

This research was funded by the Soft Science Research Project of Science and Technology Department of Henan in 2024 (Grant No. 242400410293), the Research and Practice Project of Higher Education Teaching Reform in Henan Province in 2024 (Grant No. 2024SJGLX0545).

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