UNIVERSITA’ DEGLI STUDI DI VERONA

DEPARTMENT OF

NEUROSCIENCES, BIOMEDICINE AND MOVEMENT SCIENCES

DOCTORAL PROGRAM IN

NEUROSCIENCE, PSYCHOLOGICAL AND PSYCHIATRIC SCIENCES, AND MOVEMENT SCIENCES

WITH THE FINANCIAL CONTRIBUTION OF

TRERÈ INNOVATION S.R.L.

XXXVIII° Cycle /2022

TITLE OF THE DOCTORAL THESIS

Trail running performance and biomechanics: insights to support innovative off-road footwear development

S.S.D. MEDF-01/B

Coordinator: Prof. Alberto Gajofatto
Signature ____________________

Tutor: Prof.ssa Chiara Zoppirolli
Signature Chiara Zoppirolli

Tutor: Prof. Alessandro de Nunzio
Signature Alessandro Marco De Nunzio

Doctoral Student: Dott. Simone Bettega
Signature ____________________

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Sommario

Negli ultimi anni la performance nel trail running ha suscitato un crescente interesse scientifico, poiché questa disciplina presenta richieste energetiche, biomeccaniche e neuromuscolari specifiche, diverse da quelle della corsa su strada tradizionale. La presenza di pendenze importanti in salita e discesa, terreni irregolari e instabili e durate di gara prolungate e molto variabili richiedono agli atleti continui aggiustamenti del pattern di corsa, la capacità di tollerare elevati carichi eccentrici e un marcato accumulo di fatica. Tuttavia, la maggior parte delle conoscenze disponibili deriva da studi sulla corsa in pianura, spesso su superfici regolari, e i test di laboratorio tradizionali colgono solo in parte la complessità del trail running. Gli studi più ecologici, condotti all'aperto o in gara, non consentono invece un controllo accurato di velocità, meccanica del passo e caratteristiche del terreno, rendendo difficile isolare i meccanismi alla base della performance. Alla luce di queste considerazioni, la tesi utilizza il trail running come modello sperimentale e applica un approccio integrato, che combina esperimenti controllati e dati di gara per analizzarne le principali richieste fisiologiche, biomeccaniche e neuromuscolari.

Il **Capitolo 1** fornisce il background generale sul trail running, descrive i principali vincoli imposti dalla salita, dalla discesa e dal terreno irregolare e sintetizza le evidenze disponibili sui determinanti della performance nella corsa off-road, presentando inoltre gli obiettivi generali e le ipotesi della tesi. Il **Capitolo 2** esamina gli effetti del terreno irregolare in condizioni altamente standardizzate, vincolando velocità, frequenza del passo e punto di appoggio per valutare in che modo l'irregolarità della superficie influenzi il costo della corsa e l'attivazione muscolare degli arti inferiori, con particolare attenzione ai muscoli stabilizzatori della caviglia. Il **Capitolo 3** analizza cinematica e performance nei tratti in salita e in discesa di una gara di Skyrunning in atleti maschi ben allenati ed élite, quantificando il contributo relativo di salita e discesa al risultato finale e descrivendo come i parametri spaziotemporali differiscano tra livelli di performance e si modifichino con la fatica. Il **Capitolo 4** affronta le differenze tra i sessi nel trail running competitivo, confrontando atlete donne con atleti maschi con la stessa performance relativa o assoluta, al fine di identificare differenze sesso-specifiche nella performance in salita e in discesa e nella cinematica di corsa. Il **Capitolo 5** indaga gli effetti della manipolazione della

frequenza del passo in discesa sulla fatica neuromuscolare, sulla successiva performance in salita e sulla durability in trail runner ben allenati ed élite. Utilizzando un protocollo salita–discesa–salita per confrontare una la naturale frequenza del passo degli atleti con una frequenza aumentata durante la corsa in discesa; ed analizza le possibili variabili alla base della durability in nei soggetti coinvolti. Il **Capitolo 6** presenta il lavoro svolto in collaborazione con Treré Innovation. Diversi prototipi di scarpe da trail running sono stati testati per esplorare in che modo le scelte di design possano influenzare la biomeccanica di corsa, la percezione di comfort e la performance in condizioni off-road. Il capitolo 7 presenta un progetto esplorativo svolto durante un periodo di ricerca all'estero, in cui vengono analizzate la variabilità e la prevedibilità della prestazione di atleti élite nelle gare di trail running. Infine, il **Capitolo 8** propone una discussione generale dei principali risultati, affronta i limiti principali del lavoro e suggerisce possibili direzioni future per la ricerca sulla performance e sulla durability nel trail running.

Abstract

The analysis of trail running performance has gained increasing attention in recent years, as trail running has specific energetic, biomechanical and neuromuscular demands that differ from those of traditional road running. Steep uphill and downhill sections, irregular and unstable ground, and prolonged race durations require athletes to continuously adjust their movement patterns and to tolerate high eccentric loads and cumulative fatigue. However, most of the available knowledge on running comes from level, even-surface conditions, and classical laboratory tests only partially capture the complexity of trail running. Field studies, in turn, often lack detailed control over speed, stride mechanics and terrain properties, making it difficult to isolate the mechanisms underlying performance.

For these reasons, there is a need for an integrated approach combining controlled experimentation and ecologically valid race data, with a particular focus on uneven terrain, uphill and downhill segments, sex differences and fatigue-related performance decline (durability). The present thesis was designed within this framework, keeping trail running as the common denominator and addressing its demands from complementary physiological, biomechanical and neuromuscular perspectives. **Chapter 1** provides the general background on trail running, outlines the specific constraints imposed by uphill, downhill and uneven surfaces, and summarises current evidence on performance determinants in off-road running, as well as the overall aims and hypotheses of the thesis. **Chapter 2** examines the effects of uneven terrain under highly standardised conditions, constraining speed, step frequency and foot placement to investigate how surface irregularity influences the cost of running and lower-limb muscle activation, with a focus on ankle stabiliser muscles. **Chapter 3** analyses kinematics and performance in uphill and downhill sections during a Skyrunning race in well-trained and elite male athletes, quantifying the relative importance of ascent and descent to overall race outcome and describing how spatiotemporal parameters differ across performance levels and evolve with fatigue. **Chapter 4** addresses sex differences in competitive trail running, comparing female athletes with male counterparts matched for either relative or absolute performance to identify sex-related differences in uphill and downhill performance and running kinematics, and to assess how these differences change after controlling for

speed. **Chapter 5** investigates the effects of manipulating downhill stride frequency on neuromuscular fatigue, subsequent uphill performance and durability in well-trained and elite trail runners, using an uphill–downhill–uphill protocol to compare preferred versus increased stride frequency during a prolonged steep descent and to explore cardiometabolic decoupling as a marker of durability. **Chapter 6** reports the applied work carried out with Treré Innovation, where different trail shoe prototypes were tested to explore how shoe design may influence running biomechanics, comfort and performance in off-road conditions. **Chapter 7** presents an exploratory project conducted during an international research stay, where race-to-race performance variability and predictability are explored using a database of elite trail running results from multiple competitions. Finally, **Chapter 8** presents a general discussion of the main findings, addresses the principal limitations of the work and proposes future directions for research on trail running performance and durability.

Acknowledgements

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List of Abbreviations

AFT	Advanced Footwear Technologies
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
Cr	Energy Cost of Running
CT	Contact Time
CV	Coefficient of Variation
DH	Downhill
E-T	Even Terrain
EMG	Electromyography
F	Females
GRF	Ground Reaction Forces
HR	Heart Rate
ISF	Increased Step Frequency
ITRA	International Trail Running Association
LMM	Linear Mixed Model
PSF	Preferred Step Frequency
RER	Respiratory Exchange Ratio
Rf	Respiratory frequency
SD	Standard Deviation

SF	Stride Frequency
SL	Stride Length
ST	Stride Time
UE-T	Uneven Terrain
UH	Uphill
VCO_2	Carbon dioxide production
VE	Minute ventilation
VO_2	Oxygen consumption
VO_2max	Maximal oxygen uptake
vVO_2max	Running velocity at VO_{2max}
WA	World Athletics
WMRA	World Mountain Running Association

Publications related to the present thesis

The results of this dissertation produced the following papers and communication

Papers

Bettega S., Callovini A., Bernard V., Fornasiero A., Viscioni G., Bortolan L., Pellegrini B., Schena F., Zoppirolli C. Sex differences in performance and kinematics during competitive trail running. *Submitted*

Bettega, S., Pellegrini, B., Viscioni, G., Fornasiero, A., Bortolan, L., Schena, F., & Zoppirolli, C. (2025) Kinematics and performance on uphill and downhill trail running in elite and well-trained athletes. *International Journal of Sports Science & Coaching*, 0(0). doi 10.1177/17479541251352176

Bettega, S., Bortolan, L., Stella, F., Tarperi, C., Schena, F., Pellegrini, B., & Zoppirolli, C. (2025). Energetic and neuromuscular impact of running on even or uneven surfaces in standardized laboratory conditions. *Journal of electromyography and kinesiology*, 81, 102987. doi: 10.1016/j.jelekin.2025.102987

Oral Communications

Bettega S., Viscioni G., Bortolan L., Pellegrini B., Schena F., Zoppirolli C. Influence of Performance Level and Fatigue on Kinematic Parameters during Uphill and Downhill Trail-Running. *SISMES XV National Congress*. Chieti, 19 – 21 Sep 2024 – Short talk

Poster

Bettega S., Viscioni G., Bortolan L., Pellegrini B., Schena F., Zoppirolli C. Kinematic Analysis of Trail-Running Performance: Gender Differences and Sectional Variation in Uphill and Downhill Terrains. *European College of Sport Science Congress*. Glasgow, 2 – 5 Jul 2024.

Bettega S., Bortolan L., Stella F., Pellegrini B., Schena F., Zoppirolli C. Energetic and Neuromuscular Impact of Running on Even and Uneven Surfaces in Standardized Laboratory Condition. *SISMES XIV National Congress*. Napoli, 2 – 4 Nov 2024.

Other scientific papers and communications

Papers and oral communications not related to this dissertation

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Cuniberti G. C., Callovini A., Mattivi M., Palumbo M., Forrer T., **Bettega S.**, Pedrinolla A., Danese E., Ugel S., Fornasiero A., Zoppirolli C., Schena F., Pellegrini B., Bortolan L. Effectiveness of Compressive Garments on Recovery and Performance in Trail Runners: A Controlled Study. *Submitted*

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Nardello, F., Bernabè, R., **Bettega, S.**, Zandomeneghi, M., & Zamparo, P. (2022). Kinematics of Backward Standing Starts in Elite Cyclists: The Effect of Initial Crank Angle. *Research Quarterly for Exercise and Sport*, 94(2), 476–484. doi: 10.1080/02701367.2021.2009109

Oral Communications

Bettega S., Fornasiero A., Callovini A., Pellegrini B., Schena F., Zoppirolli C. Understanding Performance Variability in Elite Trail-Runners across Different Competitions. *European College of Sport Science Congress*. Rimini, 1 – 4 Jul 2025

Bettega S., Palumbo M., Modena R., Viscioni G., Bortolan L., Pellegrini B., Schena F., Zoppirolli C. Roller Ski Interval Training in Biathletes: Assessing Whether Athletes Meet High-Intensity Targets on the Track. *10th International Congress on Science and Skiing*. Cavalese, 28 Jan – 1 Feb 2025

Poster

Bettega S., Skroce K., D'Emanuele S., Boccia G., Schena F., Tarperi C. The acute effects of unpredictable slope surface variations in well-trained runners. *SISMES XIII National Congress*. Milano, 4 – 6 Nov 2022.

Chapter 1 – Background, aims and hypotheses

1.1 Trail running: Growth and scientific relevance

Trail running has emerged as one of the fastest-growing endurance disciplines worldwide, combining the challenges of prolonged effort with the variability of natural environments. Unlike road or track running, trail running takes place on uneven, often unpredictable terrain, involving continuous changes in slope, surface, and technical demands. These characteristics not only increase the physiological and mechanical requirements placed on athletes but also make trail running a unique context in which performance is determined by a complex interaction of energetic, neuromuscular, and biomechanical factors (Ehrström et al., 2018; Lemire, Hureau, et al., 2021; Pastor et al., 2022). The rapid expansion of competitive events and the professionalization of the sport have further highlighted the need for scientific research to better understand the determinants of trail running performance and to guide training, injury prevention, and footwear innovation.

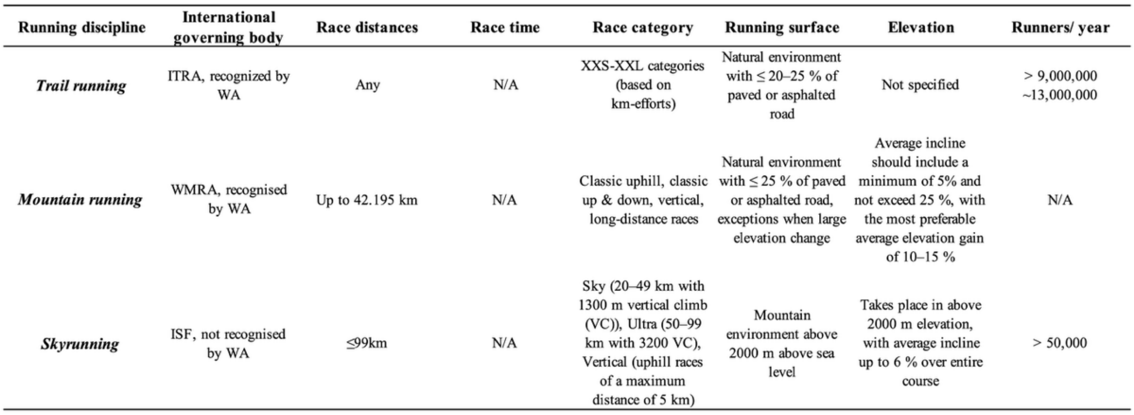
More broadly, off-road running events have become increasingly popular over the last decades, with participation growing substantially and many new competitions appearing worldwide (Cejka et al., 2014; Scheer, 2019; Scheer et al., 2020).

Within this broad context, off-road running includes a wide range of disciplines beyond trail running, such as mountain running, skyrunning, and ultramarathon running. Although they all share the natural and often unpredictable environment, these disciplines differ considerably in terms of terrain, elevation profiles, race formats, and physical demands (Scheer et al., 2020).

Trail running represents the most widespread form of off-road running and is generally defined as running competitions held in natural environments such as mountains, forests, deserts, or coastal areas, typically on surfaces like dirt roads, forest trails, or single tracks. Only a limited portion of the course is allowed on paved sections, usually not exceeding 20–25% of the total distance. Unlike other disciplines, trail running places no restrictions on distance or elevation gain, resulting in a wide diversity of race formats. To provide structure to this variability, the International Trail Running Association (ITRA) classifies events using the concept of ‘km-effort,’ which combines distance and vertical gain. Based on this system, races are grouped into categories ranging from XXS to XXL, offering athletes and organizers a standardized way to describe the demands of a given course (ITRA; Scheer et al., 2020; WA).

Mountain running shares several characteristics with trail running, as both disciplines take place predominantly on natural terrain and are regulated under World Athletics. While the majority of events are held off-road, mountain running may occasionally include paved segments when courses involve substantial elevation changes. Unlike trail running, however, distances are more strictly defined, typically ranging from 1 km up to the marathon distance. Courses are characterized by sustained elevation gain, with an average gradient between 5% and 25%, most often around 10–15%. Events are classified into different formats, including classic uphill races, up-and-down races, vertical kilometre events, and longer-distance competitions, with altitude changes ranging from 50 to 250 m per kilometre. The discipline is governed by the World Mountain Running Association (WMRA), which oversees international competitions and sets the official regulations for race formats (Scheer et al., 2020; WA; WMRA).

Skyrunning represents the most technical expression of off-road running, taking place in high-altitude mountain environments, typically above 2000 m. Races often include steep ascents and descents on highly technical terrain, which may involve glaciers, moraines, or scrambling sections requiring the use of ropes or fixed equipment. The discipline is governed by the International Skyrunning Federation (ISF), which categorizes races into three main formats: Sky (20–49 km with at least 1300 m of vertical climb), Ultra (50–99 km with at least 3200 m of vertical climb), and Vertical (uphill-only events up to 5 km in distance). Compared to trail running and mountain running, skyrunning places greater emphasis on technical difficulty and extreme alpine settings, making it a particularly demanding sport from both a physical and a technical standpoint (ISF; Scheer et al., 2020).



Running discipline	International governing body	Race distances	Race time	Race category	Running surface	Elevation	Runners/ year
Trail running	ITRA, recognized by WA	Any	N/A	XXS-XXL categories (based on km-efforts)	Natural environment with ≤ 20–25 % of paved or asphalted road	Not specified	> 9,000,000 ~13,000,000
Mountain running	WMRA, recognised by WA	Up to 42.195 km	N/A	Classic uphill, classic up & down, vertical, long-distance races	Natural environment with ≤ 25 % of paved or asphalted road, exceptions when large elevation change	Average incline should include a minimum of 5% and not exceed 25 %, with the most preferable average elevation gain of 10–15 %	N/A
Skyrunning	ISF, not recognised by WA	≤99km	N/A	Sky (20–49 km with 1300 m vertical climb (VC)), Ultra (50–99 km with 3200 VC), Vertical (uphill races of a maximum distance of 5 km)	Mountain environment above 2000 m above sea level	Takes place in above 2000 m elevation, with average incline up to 6 % over entire course	> 50,000

Table 1 Trail running, mountain running and skyrunning race characteristics. Adapted from Scheer et al., 2020.

In summary, trail running, mountain running, and skyrunning represent the three main disciplines of off-road running, each characterized by specific rules, environments, and performance demands. Despite their differences, what unites these sports is the intrinsic variability of terrain and elevation, which clearly distinguishes them from traditional road or track running. Unlike flat and predictable courses, off-road disciplines expose athletes to continuous changes in slope, surface, and technical requirements, making performance highly dependent on a multifactorial interaction of physical and mechanical capacities. This complexity not only highlights the scientific relevance of studying these sports, providing insights into the energetic, biomechanical, and neuromuscular strategies required, but also offers practical implications for coaches and practitioners aiming to optimize training, injury prevention, and race performance. Building on this perspective, the following section will explore in greater detail the energetic and biomechanical demands of uneven terrain, which form the foundation for understanding how athletes adapt and succeed in off-road running.

1.2 Energetic and biomechanical demands of uneven terrain

Running over natural and uneven terrain imposes substantially different energetic and mechanical constraints compared to level or paved running. Variations in surface regularity, slope, and ground compliance require continuous adjustments in stride mechanics and muscle activation patterns, influencing both the metabolic cost and biomechanical efficiency of locomotion. Unlike road running, where energy expenditure and movement patterns remain relatively stable, off-road running involves frequent changes in step length, contact time, and joint kinematics as athletes adapt to terrain irregularities and gradient transitions. These adaptations increase the energetic demands of locomotion (Bettega, Bortolan, et al., 2025; Gantz & Derrick, 2018; Voloshina & Ferris, 2015), as stabilizing muscles, particularly around the ankle and knee, must counteract unpredictable perturbations and maintain balance and propulsion (Bettega, Bortolan, et al., 2025; Gantz & Derrick, 2018; Nicot et al., 2022).

From a performance perspective, the interplay between metabolic efficiency and mechanical control becomes critical, since small inefficiencies in force production or postural stability can accumulate over time, contributing to physiological strain, local neuromuscular fatigue, and influencing pacing strategies. Understanding how terrain variability affects both the energetic and biomechanical aspects of running is therefore essential to interpret athlete performance in off-road environments and to guide the design of specific training strategies, injury prevention approaches, and footwear solutions.

1.2.1 Energetic adaptations to running on uneven terrain

Uneven surfaces pose greater challenges to human locomotion compared to flat and even terrains, influencing both the energetic cost and the biomechanical mechanisms of movement. Walking and running on uneven terrain have been widely investigated within the scientific community, as these conditions provide valuable insights into how humans adapt their gait, posture, and energy expenditure in response to environmental variability (Dhawale & Venkadesan, 2023; Gantz & Derrick, 2018; Skroce et al., 2023; Svenningsen et al., 2020; Voloshina et al., 2013; Voloshina & Ferris, 2015; Zamparo et al., 1992).

Locomotion on uneven or deformable surfaces consistently imposes greater metabolic demands than movement on firm, level ground. Early evidence from Zamparo et al. (1992) demonstrated that both oxygen consumption and heart rate increase markedly when walking or running on sand, with energy cost rising by 60–200% for walking and 10–40% for running (Zamparo et al., 1992). These findings were attributed to a reduced recovery of mechanical and elastic energy at each stride, leading to inefficient energy transfer and higher muscular work. Similar effects have been observed in outdoor running conditions, where surface irregularity, gradient, and vegetation contribute to fluctuating physiological load. In female athletes, Creagh and Reilly (1998) reported highly variable heart rate responses during off-road events such as fell running and orienteering, reflecting the uneven energetic requirements imposed by changing terrain and technical complexity (Creagh et al., 1998).

Comparisons between athletes specialized in different environments have further highlighted the role of specific training. Jensen et al. (1999) found that running economy decreased by approximately 40–50% when moving from level paths to rough terrain, although this impairment was smaller in orienteers compared to track runners, suggesting an adaptive improvement in metabolic efficiency resulting from regular exposure to uneven ground (Jensen et al., 1999).

Controlled laboratory studies have confirmed these trends under standardized conditions. Using modified treadmills simulating uneven terrain, Voloshina et al. (2013, 2015) and Gantz et al. (2018) reported increases in metabolic rate of about 5–10% during running and up to 28% during walking (Gantz & Derrick, 2018; Voloshina et al., 2013; Voloshina & Ferris, 2015). These rises were mainly attributed to greater joint mechanical work and muscle coactivation needed to stabilize the limbs, rather than to increased impact forces. However, more recent studies on experienced trail runners have shown attenuated or even negligible differences. Dahwale et al. (2023) observed only a small (~5%) and statistically non-significant rise in metabolic power during running on undulating natural terrain (Dhawale & Venkadesan, 2023), while Skroce et al. (2023) found no significant differences in oxygen uptake, lactate concentration, or heart rate between flat treadmill running and simulated mountain-trail conditions through unpredictable roll variations (Skroce et al., 2023).

Taken together, the literature indicates that uneven surfaces generally increase the energetic cost of locomotion, though the magnitude of this effect depends on surface compliance, irregularity, and the athlete’s technical proficiency. Trained runners appear able to partially compensate through neuromuscular and mechanical adaptations that help preserve running economy even in challenging terrain. These findings highlight that energy expenditure in off-road running is influenced not only by speed but also by the stabilizing demands imposed by surface variability.

1.2.2 Biomechanical adaptations to running on uneven terrain

The increased metabolic demands associated with running on uneven terrain are closely linked to changes in the mechanical and neuromuscular behaviour of the lower limbs. Variations in surface regularity and compliance require runners to continuously adjust stride mechanics, joint kinematics, and muscle activation to maintain stability and propulsion. These biomechanical adjustments not only contribute to the higher energetic cost of locomotion but also play a key role in determining performance, fatigue development, and injury risk in off-road running.

The irregularities and compliance of natural terrain alter stride kinematics, muscle activation, and joint loading patterns compared to level and uniform surfaces. These responses represent both a response to maintain dynamic stability and a potential source of increased energetic cost.

From a kinematic perspective, uneven terrain induces greater variability in step parameters and joint motion. Studies using uneven treadmills have shown that both walking and running lead to increased variability in step length and width, accompanied by shorter steps and modified leg stiffness compared with smooth running (Voloshina et al., 2013; Voloshina & Ferris, 2015). The increased irregularity in foot placement contributes to higher mediolateral accelerations during the stance phase (Nicot et al., 2022), reflecting the continuous need for corrective movements to maintain balance. Field-based analyses of road, path and forest running confirmed these findings, showing that athletes adopt longer stance times, greater vertical pelvis oscillation, and altered joint angles at contact when transitioning from road to off-road surfaces (Hébert-Losier et al., 2015). These adjustments are not random but reflect surface-specific movement strategies that differ between elite and amateur athletes, with more experienced runners exhibiting shorter stance durations and reduced vertical displacement, indicating a more efficient stabilization strategy.

At the neuromuscular level, uneven surfaces elicit substantial changes in muscle activation patterns. Electromyographic analyses reveal that running on irregular terrain increases both the mean activity and variability of muscle activation, particularly in the thigh and shank muscles (Müller et al., 2010, 2014; Skroce et al., 2023; Voloshina & Ferris, 2015) demonstrated that these adaptations are largely governed by anticipatory (feed-forward) control, where pre-activation of key stabilizing muscles, especially the gastrocnemius medialis, is modulated according to the expected surface height or irregularity. Visual feedback plays a secondary role, fine-tuning muscle activation before foot strike to reduce destabilizing impacts. This anticipatory control mechanism allows runners to adjust limb stiffness and landing configuration even when terrain irregularities are only partially visible. Similarly, Dhawale et al. (2023) showed that during natural trail running, athletes do not actively aim for flatter ground but rely on automatic regulation of leg compliance and retraction rate to maintain stability without precise foot placement, suggesting that stability is achieved through intrinsic mechanical responses rather than conscious trajectory control (Dhawale & Venkadesan, 2023).

From a mechanical standpoint, the interaction between surface irregularity and leg stiffness is crucial for stability and energy transfer. During walking, uneven surfaces increase the mechanical work performed at the knee and hip and reduce leg stiffness, reflecting the greater muscular effort required to maintain balance on unstable ground (Voloshina et al., 2013). In contrast, during running, these mechanical adjustments follow an opposite trend. Voloshina and Ferris (2015) reported that runners tend to increase overall leg stiffness when moving over irregular terrain, a strategy that may enhance control during unpredictable impacts while maintaining propulsion efficiency (Voloshina & Ferris, 2015). More recent findings by Gantz et al. (2018) confirmed that runners modify foot and knee angles at ground contact, increasing eversion and knee flexion to maintain shock attenuation despite the irregular surface (Gantz & Derrick, 2018). Changes in trunk posture may further redistribute joint loads, as shown by AminiAghdam et al. (2022), where anterior trunk lean reduced knee loading and shifted energy absorption toward the hip, potentially representing a strategy to cope with terrain-induced perturbations (AminiAghdam et al., 2022).

Overall, the literature indicates that running on uneven terrain involves a complex interplay of anticipatory control, altered kinematics, and neuromuscular co-activation to maintain stability. These mechanisms allow skilled runners to negotiate irregular surfaces efficiently, yet they also impose additional mechanical and energetic costs. Understanding these biomechanical adaptations is essential for interpreting performance and injury mechanisms in off-road running, as well as for informing training strategies and footwear design aimed at improving stability and efficiency in natural environments.

1.3 Uphill and downhill running: specific physiological and biomechanical demands

Building upon the influence of terrain irregularity discussed above, changes in slope represent an additional and defining feature of off-road running, deeply altering the energetic and mechanical requirements of locomotion. Moving uphill or downhill markedly alters the physiological and biomechanical requirements of locomotion, challenging the runner's ability to efficiently produce, absorb, and control mechanical energy. Uphill running primarily demands greater muscular and metabolic effort to overcome gravity, whereas downhill running shifts the mechanical load toward eccentric control and impact attenuation. These contrasting demands not only affect the energetic cost of running but also require specific neuromuscular strategies and coordination patterns to maintain efficiency and stability.

In trail and mountain running, where long ascents and descents often alternate within the same course, these adaptations become central to performance. Understanding the physiological and mechanical responses to different gradients is therefore essential for interpreting off-road running performance and for developing targeted approaches in training, pacing, and equipment design.

The physiological study of running on inclines dates back to 1938 and first demonstrated the steep rise in energetic cost when moving uphill (Margaria, 1938). Decades later, researchers expanded this work by systematically examining how gradient and gait interact to influence metabolic demand (Minetti et al., 1994, 2002). The growing popularity of uphill-only competitions has further driven scientific interest in understanding how runners cope with the mechanical and energetic challenges imposed by gravity.

From a metabolic standpoint, running uphill is markedly more demanding than running on level terrain, with oxygen consumption and energy cost rising almost linearly with gradient (Margaria, 1938; Minetti et al., 1994, 2002). At an incline of about +45%, the energetic requirement may reach nearly five times that observed on flat ground. Interestingly, this higher metabolic cost occurs despite lower vertical ground reaction forces, reflecting the shift from elastic to concentric muscle work to overcome gravity (Gottschall & Kram, 2005). According to Minetti et al. (2002), the mechanical efficiency of locomotion above +15% resembles that of purely concentric muscle contractions, indicating a predominance of positive muscle work during steep ascents (Minetti et al., 2002).

Conversely, downhill running displays the opposite pattern: the energy cost progressively decreases with negative slopes, reaching its minimum around −20%. At these gradients, the mechanical efficiency approaches that of eccentric muscle contractions, meaning that running downhill is largely sustained by negative (eccentric) work (Minetti et al., 2002).

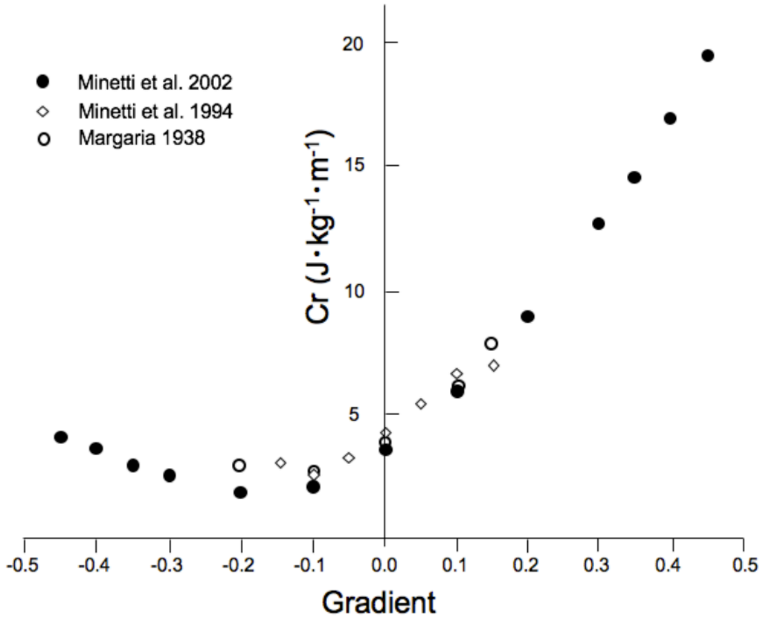


Figure 1 Metabolic energy cost of running as a function of the gradient from the works of Margaria (1938), Minetti et al. (1994a) and Minetti et al. (2002). Adapted from Minetti et al. (2002).

Ground reaction forces (GRF) also vary substantially with gradient. Gottschall and Kram (2005) reported that downhill running markedly increases both vertical and horizontal impact peaks, while uphill running reduces them, reflecting greater braking demands in descent and enhanced shock absorption in ascent (Gottschall & Kram, 2005). These mechanical differences arise from a redistribution of work with slope. In level running, gravitational and kinetic energy oscillations are balanced and efficiently recycled through elastic storage (Cavagna & Kaneko, 1977), whereas inclines require net energy generation and declines demand energy dissipation (Minetti et al., 1994; Snyder et al., 2012).

From a physiological perspective, uphill running is strongly dependent on aerobic capacity, with VO_2max emerging as the primary determinant of performance. The high metabolic cost of sustained positive work against gravity means that athletes with greater aerobic power can maintain faster speeds at steep inclines. In fact, elite-level performances in vertical races often require exceptionally high VO_2max values, approaching 86–89 $mL \cdot kg^{-1} \cdot min^{-1}$ in top male athletes (Fornasiero et al., 2022). Beyond aerobic capacity, additional predictors such as maximal strength and body mass index (BMI) have also been linked to uphill performance, underlining the interplay between oxygen delivery (Bascuas et al., 2024), muscular force production, and body composition (Lemire, Hureau, et al., 2021). Collectively, these findings suggest that while VO_2max remains the central physiological determinant, uphill running success depends on a broader set of factors that enable athletes to translate aerobic capacity into efficient propulsion on steep gradients.

In contrast to uphill locomotion, downhill running imposes unique physiological and mechanical constraints that go beyond aerobic capacity. Although VO_2max and maximal strength contribute to performance in both conditions, Lemire et al. (2021) identified leg musculotendinous stiffness as a specific predictor for downhill running (Lemire, Hureau, et al., 2021), underlining the role of elastic properties in absorbing and reusing energy under eccentric loading. The eccentric nature of muscle contractions during descent, particularly in the quadriceps, exposes athletes to high mechanical stress and impact forces (Giandolini, Horvais, et al., 2016), which can accumulate over prolonged efforts. These loads contribute to muscle damage, neuromuscular fatigue, and impairments in both maximal voluntary force and the rate of force development (RFD), reflecting a decline in the ability to rapidly produce force (Bontemps et al., 2020; Coratella et al., 2024; Varesco et al., 2022). Stride mechanics further modulate these demands: while longer strides may help sustain velocity on steep descents (Bettega, Pellegrini, et al., 2025), they also amplify impact forces and eccentric strain compared to shorter steps at equal speed (Baggaley et al., 2020; Eston et al., 2000). Collectively, these findings suggest that downhill performance is influenced more by the ability to manage eccentric stress, maintain neuromuscular function, and optimize stride strategies to balance speed with mechanical load, rather than by metabolic limitations.

1.4 Determinants of performance in trail running: an integrated approach

Building on the evidence presented in the previous sections, it becomes clear that trail running performance cannot be explained solely by the traditional determinants of endurance running such as aerobic capacity, running economy, and lactate threshold (Coyle, 1999). Unlike road or track running, trail competitions combine the energetic, biomechanical and neuromuscular demands of uneven terrain (section 1.2) with the contrasting challenges of prolonged uphill and downhill sections (section 1.3). This integration of highly variable conditions results in a performance model that is both more complex and more context-dependent than that observed in level endurance running.

In traditional endurance running, performance can be predicted with remarkable accuracy based on a few physiological markers. Classic models suggest that maximal oxygen uptake (VO_2max), fractional utilization of VO_2max, and running economy together explain 90–95% of performance variance across distances ranging from 800 m to the marathon, and across different athlete populations from amateur to international level runners (Di Prampero et al., 1986; Ingham et al., 2008; McLaughlin et al., 2010). In contrast, trail running presents a far more complex scenario where prediction models account for a much smaller proportion of performance variability. For example, only 48% of performance variance could be explained by the classical endurance performance (VO_2max, lactate threshold and running economy at 0% grade) model in a short trail race (27 km, 1400 m D+) (Ehrström et al., 2018), and this predictive power increased only to 62% in an ultra-distance event (166 km, 9500 m D+) (Pastor et al., 2022). Consequently, the performance model for this discipline must integrate multiple physiological and mechanical domains, reflecting the multifactorial and context-specific nature of competing in highly variable outdoor environments. Moreover, performance determinants may differ across competitive levels, requiring specific consideration of how elite, sub-elite, and well-trained athletes adapt and excel under the unique demands of trail running.

Ehrström et al. (2018), studying highly trained runners in a short-distance trail race, confirmed that aerobic capacity (VO_2max) plays a role (20.4%), but highlighted that other variables, including biomechanical and neuromuscular markers, improved model prediction (Ehrström et al., 2018). Similarly, Lemire et al. (2021) demonstrated that the determinants of performance diverge when uphill and downhill sections are analysed separately: while uphill performance is primarily associated with vVO_2max, body mass index, and maximal strength, downhill performance is better explained by vVO_2max, strength, and leg musculotendinous stiffness (Lemire, Hureau, et al., 2021). These findings reinforce the view that mechanical and neuromuscular factors acquire specific relevance depending on the slope.

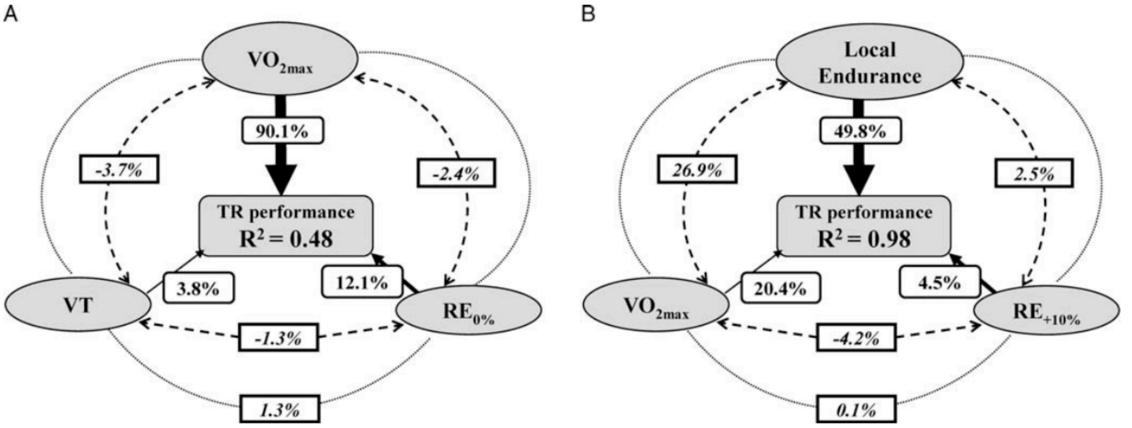


Figure 2 Graphic representation of commonality regression models for predicting TR performance. A, Commonality analysis for the classic endurance running model. B, Commonality analysis for the adapted and specific model to TR (Ehrström et al., 2018).

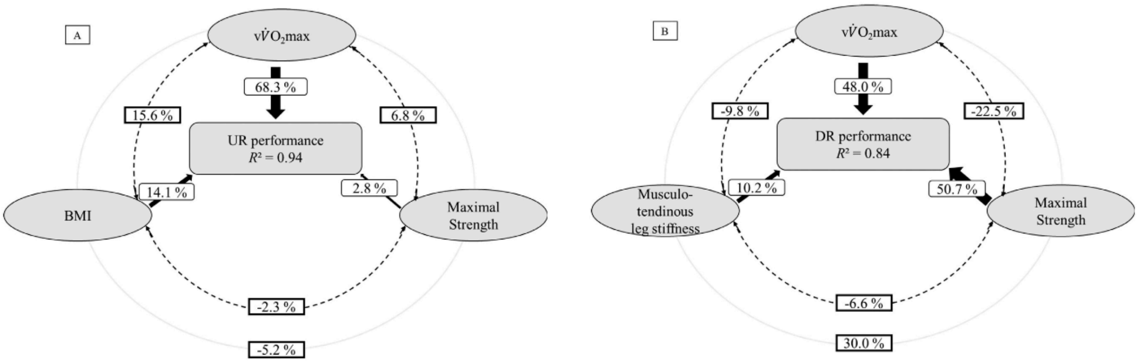


Figure 3 Prediction models of isolated UR and DR time trial performances. Panel A: commonality analysis for the UR performance. Panel B: commonality analysis for the DR performance (Lemire et al., 2021).

In recreational populations, aerobic parameters appear to retain a stronger predictive role. Sabater-Pastor et al. (2022) showed that VO_2max explained a large portion of variance in both short (<55 km) and medium (~100 km) races, while additional contributions came from lipid utilization, muscle strength, and body composition. However, for races longer than 140 km, no linear model could adequately explain performance, indicating that fatigue-related performance maintenance, pacing, and resilience neuromuscular damage may dominate over classical physiological predictors (Pastor et al., 2022).

Finally, focusing on uphill-only competitions, Fornasiero et al. (2022) reported that the relationship between performance and VO_2max in vertical kilometer races was better described by an exponential rather than linear model, with top-level male and female athletes requiring ~86–89 and ~75–80 $ml \cdot kg^{-1} \cdot min^{-1}$ respectively to be competitive at the international level (Fornasiero et al., 2022).

Collectively, these studies suggest that while aerobic capacity remains an important determinant, especially among less trained runners and at shorter distances, the predictive model of trail running performance becomes increasingly multifactorial with higher athletic level and longer, more technical races. Neuromuscular characteristics, mechanical efficiency, body composition, and the tolerance to eccentric loading emerge as critical factors, highlighting the need for discipline-specific models that go beyond the classical framework established for flat endurance running.

1.5 Durability in endurance exercise: conceptual framework and relevance for trail running

Within this multifactorial framework, an additional and increasingly relevant dimension is the athlete's ability to preserve physiological and mechanical performance as fatigue develops, a concept now commonly referred to as durability. In endurance exercise, durability can be understood as the ability to preserve physiological function during prolonged exercise, or, more specifically, to limit the time-dependent deterioration of key physiological-profiling characteristics as exercise continues (B. Hunter et al., 2025; Maunder et al., 2021) . This perspective extends the traditional interpretation of endurance performance, which has largely relied on variables such as maximal oxygen uptake, exercise economy, and metabolic thresholds assessed in a rested condition, by recognising that these same variables are not fixed over time and may progressively decline as fatigue accumulates (B. Hunter et al., 2025; Maunder et al., 2021). Accordingly, durability has been proposed as an additional dimension of endurance performance, reflecting the extent to which an athlete is able to maintain, as far as possible, fresh-state physiological characteristics during prolonged exercise (B. Hunter et al., 2025; Maunder et al., 2021).

The relevance of durability in endurance sports lies in the fact that performance depends not only on the absolute value of physiological capacities in a fresh state, but also on how stable these capacities remain over the course of exercise (Jones, 2024). Experimental evidence shows that prolonged exercise can reduce critical power, lactate threshold, and gas exchange threshold, thereby altering the exercise intensity that can be sustained over time and increasing the likelihood of performance decline if the required external workload remains unchanged (Brownstein et al., 2022; Clark et al., 2018, 2019; Jones, 2024; Stevenson et al., 2022). In this context, the key implication of durability is that two athletes with similar physiological profiles in a rested condition may differ substantially in performance if one is better able to preserve physiological state, mechanical effectiveness, and sustainable output as fatigue develops (B. Hunter et al., 2025; Jones, 2024). For this reason, durability is increasingly viewed as a meaningful component of endurance performance, with important implications for exercise regulation, performance prediction, and the interpretation of fatigue-related changes during prolonged exercise (B. Hunter et al., 2025; Maunder et al., 2021).

Despite its growing relevance, the terminology surrounding durability remains debated, and clear conceptual boundaries are still being refined. Recent attempts to distinguish durability from related constructs have highlighted both the usefulness of this emerging framework and the risk of conceptual overlap when terms are not anchored to sufficiently precise physiological definitions (Colosio et al., 2025; Meixner et al., 2025; Zanini et al., 2025). In this context, durability is most consistently used to describe a time-dependent deterioration in performance or in performance-related physiological characteristics during prolonged exercise, thereby introducing an explicitly temporal dimension into the interpretation of endurance performance (Colosio et al., 2025; Maunder et al., 2021; Meixner et al., 2025). This temporal component is particularly important because it distinguishes durability from the broader concept of fatigue, which refers to the acute impairment of performance associated with an increased perceived effort and, eventually, a reduced ability to produce the required force or power (Meixner et al., 2025). In other words, fatigue describes the state or process of impairment, whereas durability refers to the extent to which performance and its underpinning physiological characteristics can be preserved as exercise duration increases. Although exercise intensity influences how durability and fatigability are assessed, it should not be considered the primary criterion distinguishing the two. In particular, durability may also be examined through highly demanding performance tasks, provided that the focus remains on the time- or work-dependent deterioration of performance or physiological function.

A further distinction should be made with fatigability. Although the two concepts are closely related and are sometimes discussed as part of the same continuum, recent commentaries suggest that fatigability has traditionally been more closely associated with the development of fatigue within a task, and, in its classic physiological interpretation, with the reduction in maximal force or power output, without necessarily requiring the explicit time-dependent framework that characterizes durability (Colosio et al., 2025). Accordingly, durability is better understood as the progressive loss of performance capacity across prolonged exercise, whereas fatigability more generally reflects the susceptibility to fatigue and the rate at which performance deteriorates under load (Colosio et al., 2025; Meixner et al., 2025). In a similar way, fatigue resistance may be considered a broader and less specifically operationalized term describing the ability to tolerate or delay fatigue development, but it does not by itself indicate whether the phenomenon is being assessed through prolonged time-dependent deterioration, repeated efforts, or maximal-output loss. Likewise, pacing should not be considered synonymous with durability, since pacing primarily refers to the conscious or subconscious regulation of effort across an exercise bout, whereas durability concerns the physiological and performance consequences of accumulated work. Finally, performance decline is best viewed as the observable outcome that may emerge during exercise, but not as a mechanistic construct in itself: such decline may reflect limited durability, altered pacing, fatigue development, or the interaction among these factors. Taken together, these distinctions support the use of durability as a specific concept referring to the preservation of performance over time during prolonged exercise, while also acknowledging that its exact boundaries relative to adjacent terminology remain an active subject of discussion in the current literature (Colosio et al., 2025; Meixner et al., 2025; Zanini et al., 2025).

In the context of the present thesis, durability is considered primarily as a short-term manifestation of fatigue-related performance maintenance under controlled conditions. Although this approach does not directly reproduce the physiological, mechanical, and tactical complexity of prolonged trail or ultra-endurance competitions, it provides a useful framework for examining how downhill-induced fatigue and muscle damage may influence subsequent performance capacity.

1.6 Sex differences in trail running performance and kinematics

Sex differences in endurance performance have long been recognized, with elite male athletes typically outperforming females by 8–12% across running distances ranging from middle to long events (Cheuvront et al., 2005; Coast et al., 2004; Joyner & Coyle, 2008). These differences are primarily attributed to physiological factors, including higher maximal oxygen uptake (VO_2max), greater haemoglobin concentration, and lower body fat percentage in males (D. R. Bassett, 2000; Sparling & Cureton, 1983). However, participation trends in trail running have shown a steady rise in female involvement, increasing from ~18% in 2013 to ~26% in 2019 (ITRA), highlighting the growing importance of understanding sex-related performance characteristics in this discipline.

Trail running differs from road running not only in terrain variability but also in the alternation of prolonged uphill and downhill sections, which may magnify or attenuate sex-related disparities. Uphill sections, where performance is strongly linked to aerobic capacity, often highlight the physiological advantages of male athletes (G. P. Millet et al., 2025). In contrast, downhill running imposes high eccentric loading and braking forces on the lower limbs, raising questions about whether men and women respond differently to the associated neuromuscular stress and fatigue.

In addition to aerobic capacity, several neuromuscular factors contribute to sex-specific responses during off-road running. Men typically exhibit greater maximal strength and higher rates of force development (RFD), along with higher tendon stiffness, particularly in the patellar and Achilles tendons (Kubo et al., 2003; Onambélé et al., 2007). These characteristics may enhance energy storage and recoil, as well as the ability to tolerate eccentric braking during downhill running. Conversely, lower tendon stiffness in women may influence shock absorption and joint stabilization strategies on irregular terrain. Moreover, well-established sex differences exist in neuromuscular fatigue: women generally show lower peripheral fatigue and greater resistance to task failure in sustained contractions (Sandra. K. Hunter, 2014).

Beyond physiology, kinematic and spatiotemporal factors such as stride length, contact time, and duty factor are influenced by body size and limb proportions more than by sex per se (Roche-Seruendo et al., 2019). Several kinematic differences between sexes have been consistently reported. Women generally run with higher step frequency and shorter stride length than men at matched speeds (Daniels & Daniels, 1992; Schache et al., 2014) these patterns may influence stability, braking behaviour, and energy cost on uneven surfaces. However, trail running adds further complexity: runners must adapt these parameters dynamically in response to irregular surfaces, technical obstacles, and cumulative fatigue (Scheer et al., 2020; Vermand et al., 2022). Some evidence suggests that women may compensate for lower absolute aerobic power with more economical pacing strategies and potentially superior fatigue resistance, especially in longer events (Besson et al., 2022; Le Mat et al., 2023). This aligns with observations in ultradistance competitions, where sex gaps narrow with increasing race duration, suggesting that women may preserve their performance more effectively under prolonged physiological and mechanical stress (Nikolaidis et al., 2017).

Despite growing interest, most studies on sex differences in running mechanics have been performed under controlled laboratory conditions, often on treadmills, which only partially reflect the real-world demands of trail running. Understanding how male and female athletes adjust their mechanics in natural environments, particularly during uphill and downhill sections, is therefore essential to explain performance differences, inform training strategies, and optimize injury prevention approaches in off-road disciplines.

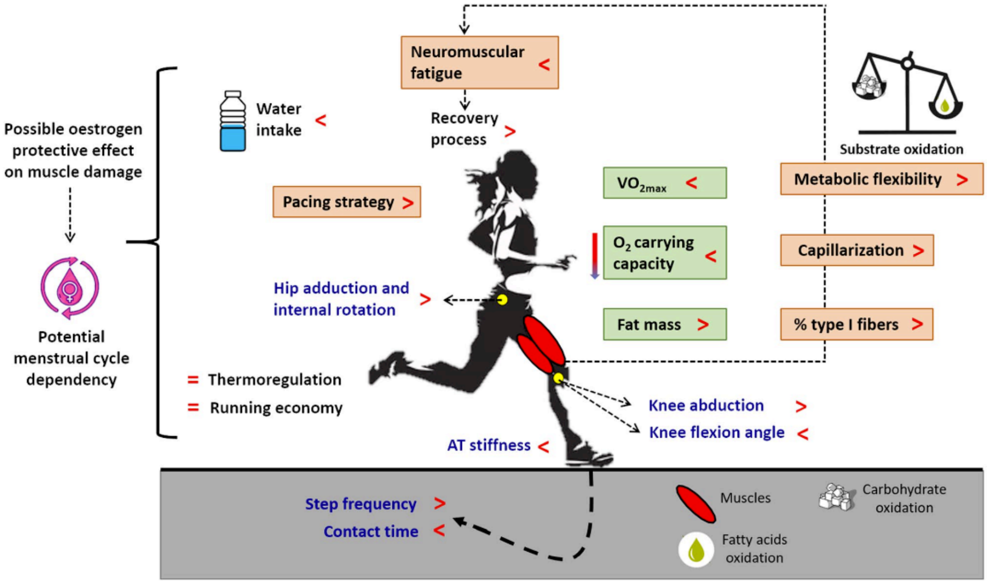


Figure 4 Schematic overview of sex-related differences in endurance running. Factors conferring potential advantages to males or females are highlighted in green and orange, respectively. Symbols indicate: > superior in females, < inferior in females, = no difference. Abbreviations: AT, Achilles tendon; O_2, oxygen; VO_2max, maximal oxygen uptake (Besson et al., 2022).

1.7 Footwear in trail running

In trail running, footwear plays a critical role, where athletes face highly variable conditions including uneven terrain, steep gradients, and prolonged effort. Unlike road running shoes, trail running footwear must balance cushioning, traction, protection, and stability, while also influencing running mechanics and energy expenditure. Research in this area has increasingly focused on how shoe design can support performance, reduce injury risk, and facilitate adaptation to the specific demands of off-road environments.

Over the last decade, innovation in road running footwear has accelerated, driven by the search for marginal performance improvements. The development of so-called advanced footwear technologies (AFT) has been central to this progress, typically combining lightweight and resilient foams with embedded stiff plates (Burns & Joubert, 2024; Frederick, 2022). This design approach increases longitudinal bending stiffness and improves energy return, ultimately enhancing running economy and efficiency. Indeed, AFT have been shown to improve running economy by up to ~4% (Hoogkamer et al., 2018; I. Hunter et al., 2019; Langley & Langley, 2024), which translates into performance gains exceeding 2% in competitive settings (Hébert-Losier & Pamment, 2023).

While the advantages of AFT for economy and performance are well documented in road running, it is uncertain whether these effects extend to off-road contexts. Trail running imposes additional challenges compared to road events, as athletes must cope with prolonged efforts across natural terrains that alternate between flat, uphill, and downhill sections. These environmental and mechanical challenges have driven the development of footwear specifically designed for trail conditions. As a result, the performance benefits observed with AFT on the road cannot be assumed to apply directly to trail running, where grip, protection, and stability may play an equally, if not more, critical role than energy return.

Recent investigations have highlighted that footwear innovations beneficial in road running may not directly translate to trail environments. For instance, stiff carbon plates embedded in midsoles, long recognized for improving efficiency on level, predictable terrain (Chollet et al., 2023; Day & Hahn, 2020), show limited benefits when conditions change. Jaboulay and Giandolini (2025) tested amateur trail runners using footwear with and without carbon plates during both treadmill running and laboratory simulations of unstable ground (Jaboulay & Giandolini, 2025). Results revealed that carbon plates did not affect energy cost on level uneven terrain but increased metabolic power by ~2% during uphill running. Moreover, no differences were found in joint mechanics or subjective plantar feedback between conditions. These findings indicate that the benefits of increased longitudinal bending stiffness observed in road running may not extend to trail-specific scenarios, where uneven surfaces and steep gradients alter the metabolic and mechanical demands of locomotion. While increased longitudinal bending stiffness from carbon plates appears detrimental in uphill trail running and offers no clear advantage on unstable terrain (Jaboulay & Giandolini, 2025), recent findings suggest that advances in midsole foams may provide more consistent benefits. Muzeau et al. (2025) reported that shoes equipped with compliant AFT foams reduced oxygen consumption by ~1–2% compared with traditional foams, particularly on flat and uphill conditions, while effects were negligible in downhill running (Muzeau et al., 2025). These results suggest that, unlike plates, innovative foams may provide incremental improvements in trail running economy, although their effectiveness remains dependent on gradient and terrain complexity.

In summary, while road-derived AFT has reshaped the performance landscape of marathon running, its transferability to trail remains limited. The available evidence indicates that stiff plates may not be advantageous in off-road contexts, whereas novel foams could offer modest benefits in selected conditions. Future footwear development for trail running should therefore prioritize terrain-specific demands, such as traction, stability, and protection, while cautiously integrating technologies originally conceived for road performance.

1.8 Aims and structure of the thesis

As highlighted in the preceding sections, trail running represents a complex and multifactorial endurance discipline, where performance emerges from the interaction of energetic, biomechanical, and neuromuscular demands across highly variable terrains and slopes. Unlike road running, no single determinant can fully explain success in this sport, making it necessary to adopt an integrated approach to better understand the specific requirements of trail performance.

The present doctoral project was developed with the overarching aim of investigating the physiology and biomechanics of trail running under both laboratory and field conditions, with a specific focus on: *i)* the challenge of running on uneven terrain, *ii)* performance and kinematics of uphill and downhill running in relation to sex and performance level, *iii)* effect of downhill running kinematics on general trail running performance. The ultimate goal was to provide novel insights into how athletes adapt to uneven terrain, prolonged ascents and descents, and cumulative fatigue, thereby contributing to the scientific understanding of performance determinants in this discipline. In parallel, these findings may inform applied fields such as training strategies, injury prevention, and footwear development.

The thesis is structured around four main studies, each addressing a specific perspective on trail running performance:

Chapter 2 - Running on uneven ground: Energetic cost and neuromuscular challenges

Aim: To investigate how surface irregularity (even vs. uneven terrain) influences the energetic cost and neuromuscular demands of running under standardized conditions.

Hypotheses: i) Running on uneven terrain requires greater activation of lower leg muscles compared to even terrain, given the increased need for foot stabilization. ii) The higher stabilization demands result in an elevated metabolic cost during uneven running. iii) Uneven terrain induces greater variability in muscle activation patterns, reflecting the continuous adjustments required for stability.

Novelty: the novelty of this chapter lies in the strict standardization of running mechanics across conditions. Unlike previous work conducted on natural enviroments or laboratory setups, this study controls all key spatiotemporal variables (running speed, step frequency, step length, duty factor, and stride timing), allowing us to isolate the pure effect of surface irregularity on energetic and neuromuscular responses.

Impact: This approach provides new causal insight into how uneven ground affects running physiology and neuromuscular control. By ensuring identical running biomechanics across surfaces, the chapter reveals the true, baseline physiological cost of instability and irregular foot–ground interactions.

Chapter 3 – Kinematics and performance on uphill and downhill trail running in elite and well-trained athletes

Aim: To examine how uphill (UH) and downhill (DH) sections contribute to overall trail running performance and to assess kinematic adaptations across performance levels during a race setting.

Hypotheses: i) Downhill running would represent the most decisive determinant of final race performance. ii) Higher-level athletes would maintain higher speeds across the race, supported by longer stride lengths and a reduced duty cycle, despite the effects of fatigue.

Novelty: This chapter provides a unique in-race analysis of running kinematics and performance determinants in a competitive trail running event. Furthermore, the athlete sample includes well-trained to elite competitors, allowing for a detailed examination of how high-level athletes modulate stride mechanics and maintain speed under race-specific demands.

Impact: By focusing on athletes ranging from well-trained to elite, this chapter offers insights into performance-relevant kinematic strategies that distinguish higher-level competitors. The findings may inform: 1) pacing strategies, especially the management of speed and stride mechanics in UH and DH segments; 2) training priorities, such as developing downhill technical efficiency, maintaining stride length under fatigue, and optimising duty factor modulation. Overall, this work contributes practical and scientifically grounded knowledge for enhancing performance in competitive trail running.

Chapter 4 – How sex differences shape performance and running kinematics in competitive trail running

Aim: To analyse sex differences in trail running by comparing female athletes with male counterparts of similar absolute and relative performance, focusing on running kinematics across uphill and downhill sections and their evolution with fatigue.

Hypotheses: i) Male athletes will exhibit higher uphill running speeds than females, reflecting known physiological differences in aerobic capacity; ii) Downhill sections will highlight sex-specific mechanical patterns, with males showing longer strides and higher speeds, and females relying more on increased step frequency and braking control; iii) Fatigue will affect running kinematics differently between sexes, with women maintaining a more stable pacing and stride profile across race segments.

Novelty: This chapter provides a high-resolution, in-race analysis of sex differences in trail running, using male and female athletes matched for both absolute and relative performance to isolate sex-specific characteristics from performance-level effects. This work examines real uphill and downhill running during competition, capturing kinematic adaptations under true race fatigue and detailing sex-related differences in stride mechanics, duty factor, and braking strategies.

Impact: The resulting dataset, collected in a competitive setting with well-trained and elite athletes, offers a rare and valuable contribution to the literature on sex-specific biomechanics and performance determinants in off-road running.

Chapter 5 – Effects of downhill stride frequency on neuromuscular fatigue, uphill performance, and durability in trail runners

Aim: To examine whether manipulating stride frequency during downhill running can mitigate muscle damage and improve subsequent uphill performance in well-trained and elite trail runners, while identifying the neuromuscular and physiological factors associated with greater durability.

Hypotheses: i) Increasing stride frequency during downhill running will reduce mechanical impacts and muscle damage, leading to better preservation of neuromuscular function. ii) Athletes adopting higher stride frequencies will show smaller declines in maximal force and faster recovery, resulting in reduced performance loss during the subsequent uphill time trial. iii) Short-term durability (i.e., Δ time performance decline) will be associated with neuromuscular markers of damage and fatigue, reflecting the interaction between biomechanical strategy, muscle function, and performance resilience.

Novelty: This chapter offers a highly ecologically valid investigation of downhill-induced fatigue by integrating a controlled manipulation of stride frequency within a protocol that closely mimics real trail-running demands. In contrast to previous studies that examined downhill running in isolation or under simplified laboratory conditions, this work incorporates a preceding uphill segment performed at high intensity, reproducing the typical race scenario in which downhill sections are entered in a pre-fatigued state. The combination of an uphill effort, a standardized downhill fatigue task, and a subsequent uphill performance test enables a mechanistic examination of how downhill strategy influences neuromuscular function and uphill performance capacity. The inclusion of well-trained and elite trail runners further strengthens the relevance of the findings, providing insight into biomechanical adaptations in athletes who most closely represent the demands of competitive off-road racing.

> Impact: The results of this chapter contribute to a deeper understanding of how fatigue develops during downhill running in trained athletes, and whether stride-frequency manipulation can attenuate eccentric load, muscle damage, and subsequent performance loss. By identifying neuromuscular and physiological factors associated with greater durability, this work offers practical implications for optimizing pacing and downhill technique, informing training strategies aimed at reducing fatigue carry-over into uphill segments, and supporting performance-oriented decisions in competitive trail running.

Together, these studies aim to provide an integrated overview of trail running performance, highlighting the interplay between energetic cost, biomechanical strategies, and neuromuscular constraints. While each investigation addressed a distinct research question, collectively they contribute to a broader understanding of how athletes manage the unique challenges of this sport.

Finally, the thesis concludes by discussing the findings in relation to the existing literature, emphasizing their implications for performance modelling in trail running, and outlining directions for future research.

Chapter 2 – Running on uneven ground: energetic cost and muscular involvement

2.1 Abstract

Running on natural surfaces typically increases metabolic energy expenditure and alters biomechanics, as athletes adapt their running style to maintain efficiency. To further investigate these demands under controlled conditions, we examined the energetic and neuromuscular effects of running on even (E-T) and uneven terrain (UE-T) terrain by creating smooth and rough conditions on a standardized circuit. Ten adults (32.1 ± 7.6 years; body mass 62.2 ± 7.0 kg; height 167.5 ± 4.2 cm) ran on an iterative-8-shaped path under both conditions in a counterbalanced order. UE-T terrain was simulated by attaching solid hemispheres to a perforated mat, whereas in E-T condition visible marks guided foot placement. By standardizing gait patterns with foot placement targets and a metronome, we were able to minimize the influence of step variability and isolate the effect of terrain. Participants performed two 6-min trials at self-selected low intensity while maintaining the same step frequency, through the use of a metronome to ensure consistency in running speed, patterns and foot placement across conditions. Cardio-metabolic parameters were measured continuously, and surface electromyography was recorded from six leg muscles. Compared with E-T, running on UE-T elicited significantly higher cardio-metabolic responses, with elevated oxygen cost (+18%), energy cost (+23%), respiratory frequency (+7%), ventilation (+19%), heart rate (+10%), and rating of perceived exertion (+50%) (all $p < 0.05$). Electromyographic activity also increased for the tibialis anterior (+22%) and peroneus longus (+10%) in the UE-T condition.

Our findings indicate that running on uneven terrain elicits both higher metabolic responses and greater activation of ankle stabilizer muscles compared to even surfaces. These results emphasize the additional energetic and neuromuscular demands of uneven ground, reflecting the challenges faced in off-road and trail running due to challenging foot support. Such insights contribute to a better understanding of how surface variability influences metabolic demand and thus performance and may support the development of targeted strategies for injury prevention and training optimization.

2.2 Introduction

It is in general known that greater metabolic energy expenditure is required when running on natural surfaces compared to running on smooth and hard terrains (Jensen et al., 1999; Pinnington & Dawson, 2001; Zamparo et al., 1992). Specifically, running outdoors on natural surfaces can affect running biomechanics, as runner adapt their running style to minimize the energy cost of running (Lieberman et al., 2015; Snyder & Farley, 2011).

Trail running is a perfect example of a sport where athletes compete on uneven terrain, with participation growing considerably in recent years, nearly doubling between 2009 and 2017 in the US alone (Scheer et al., 2020). It is defined as a foot race conducted in natural environ- ments, such as mountains, deserts, forests, or coastal areas, traversing diverse terrains like dirt roads, forest trails, single tracks, and beach sand. Paved or asphalt roads account for no more than 20–25 % of the total course (Scheer et al., 2020). Trail running has been shown to significantly increase mediolateral foot acceleration compared to treadmill running, particularly during the foot–ground contact phase, as quantified by the range of acceleration in the mediolateral axis across each step (Nicot et al., 2022). These variations in surface and track slope during off-road running require athletes to make continuous adjustments to their running speed, which in turn increases the variability of heart rate responses (Creagh et al., 1998).

The CV of biomechanical parameters like muscle activation and stride kinematics further highlights differences between even and off- road conditions. Biomechanical alterations, as evidenced by EMG muscle activation analysis, have been explored in previous studies investigating muscular changes resulting from surface perturbations during walking or running (Müller et al., 2010; Skroce et al., 2023; Voloshina & Ferris, 2015). An increased mean value and variability of muscle activation have been noted in thigh muscles when running on uneven terrain (Voloshina & Ferris, 2015). Additionally, augmented variability in EMG activity has been observed in lower leg muscles such as the peroneus longus, tibialis anterior, soleus, and lateral gastrocnemius in comparison to running on even surfaces (Skroce et al., 2023; Voloshina & Ferris, 2015).

Given the difficulties in reproducing the outdoor conditions and constraints when studying off-road running, researchers have sought to mimic real outdoor settings in different ways under controlled labora- tory setups (Dhawale & Venkadesan, 2023; Gantz & Derrick, 2018; Schrøder Jakobsen et al., 2022; Voloshina & Ferris, 2015); by utilizing modified treadmills with wooden blocks of different dimensions (from 1.27 to of 3.81 cm of height) attached to the treadmill belt (Voloshina & Ferris, 2015) or by using indoor pathways made with fiberglass and epoxy that texturally resembled weathered rock (Dhawale & Venkadesan, 2023). These setups presented intrinsic limits when the research aim is to compare running in outdoor conditions with running on an even surface. As an example, the athletes could choose their preferred running pattern in terms of step positioning (ground contact), thus introducing possible biases in the data analysis: a great inter-individual EMG variability could emerge when running on the previously mentioned setups. In such situations, it is difficult to understand whether EMG differences between even and uneven conditions are due to different foot positioning, different running surfaces or both. Furthermore, in treadmill setups, the reduced visibility of the uneven elements could limit athletes from effectively controlling their foot trajectory approaching ground contact. Finally, indoor pathways were often presented as linear and short, forcing athletes to 180°◦ changes of direction, thus impeding continuous running at a constant speed.

Thus, this study aimed to analyse the effect of surface when running on even or uneven terrain, by comparing the energetic and neuromuscular demands of running with the same pattern on standardized smooth and rough terrain. The experimental design was conceived to maintain the same movement speed and the same foot positioning in the two conditions. We hypothesized that: i) an increased activation of lower leg muscles rather than thigh muscles is required when running on uneven than on even terrains when the same step sequence is guaranteed; ii) a higher metabolic effort should be observed in uneven running conditions, due to the increased muscle involvement during foot stabilization; iii) elevated requirements of foot stabilization could lead to augmented muscle activation variability in uneven terrain.

2.3 Material and Methods

2.3.1 Experimental setup

To explore the energetic and neuromuscular effects of running on even (E-T) versus uneven (UE-T) terrains, we conducted a controlled study using a standardized circuit featuring both smooth and rough conditions. Ten adult participants were asked to run on an 'iterative-8-shaped' path. For the UE-T condition, we created a rough surface by attaching solid hemispheres of different sizes to a perforated foam mat. In contrast, the E-T condition involved a smooth surface with visible markers to guide foot placement. Each participant ran two 6-minute trials on both terrains, with a 10-minute rest between trials, in a counterbalanced sequence. They ran at a self-selected, low-intensity speed while maintaining a consistent running pattern, with a metronome used to keep their step frequency and foot placement timing consistent across both terrain types, reducing the variability that would have been introduced if foot positioning during the stance phase had been different and inconsistent between the two conditions.

2.3.2 Subjects

Ten physically active adults, 5 males and 5 women, volunteered to participate in this study (mean ± standard deviation, age: 32.1 ± 7.6 years; height: 167.5 ± 4.2 cm; body mass: 62.2 ± 7 kg). Participants were moderately active in running activity, with familiarity in walking and running on off-road terrain, and none of them had any history of injuries affecting their ability to walk or run. Before taking part in the study, participants provided informed written consent and the study protocol was approved by the ethical committee of the University of Verona and it was conducted according to the ethical standards of the Helsinki Declaration.

2.3.3 Procedures

Subjects performed two 6-min run trials on an "iterative-8-shaped" path (length = 30.5 m) (Fig. 5) in a laboratory environment, with a 10- min rest in between. Two different running conditions were created on a perforated semi-rigid foam mat with a thickness of 5 cm. To mimic the uneven running terrain (UE-T), solid hemispheres with non-slip surfaces and of different dimensions (small Ø: 240 mm, medium Ø: 340 mm, and large Ø: 400 mm) created for this purpose (Powerstone, Key Stone, Folgaria, Italy) were fixed to the foam mat using proper fixing devices. The average distance between the hemispheres was 0.86 ± 0.10 m, which was fixed for all subjects and corresponded to 52 % of the subjects' average height. The hemispheres of different sizes were randomly alternated on the path, ensuring that within a complete lap, participants performed an equal number of steps on stones of different sizes with each leg.

To replicate a comparable even terrain (E-T), visible tape was used to mark areas on the foam mat, matching the circumference of the hemispheres in the UE-T condition. Participants were instructed to place their feet consistently on the upper part of the hemispheres in the UE-T condition, while in the E-T condition, foot placement was required within the tape-marked areas. Subjects always started their runs from the same point (the yellow stone in Fig. 5) and were instructed to begin with the same leg for all trials, ensuring consistency in step pattern and minimizing unnatural movements, even during turns.

Before data collection, a 15-minute warm-up session was conducted to allow participants to familiarize themselves with the running conditions. During this warm-up, performed on the UE-T condition, participants ran at a self-selected low-intensity pace. A metronome was synchronized to their self-selected step frequency during the final minutes of the warm-up, which was then used in both experimental conditions to ensure the same step frequency and running speed.

For all trials, electromyographic analysis was conducted on the dominant leg, which was determined prior to testing. Subjects ran in their own pair of running shoes, primarily road running shoes, as the surfaces in the experiments were mostly smooth.

Bipolar electrodes (24x24mm; CDE-C, OTBioelettronica, Torino, Italy) were placed on the dominant leg, on the muscle belly of *vastus lateralis* (VLa), *biceps femoris* (BFe), *tibialis anterior* (TAn), *peroneus longus* (PLo), *soleus* (Sol) and *gastrocnemius medialis* (GMe), with an inter- electrode distance of 2 cm, accordingly to the SENIAM guidelines (Hermens et al., 2000) to minimize cross talks and geometrical artifacts also during dynamic contractions (Rainoldi et al., 2000). Before this procedure, the interested skin zones were hair-shaved, slightly abraded, and cleaned with alcohol to reduce impedance.

Moreover, the athletes were prepared for the cardio-metabolic measurements with a facial mask, a heart rate belt (Garmin, KS, USA) and a portable metabolimeter (K5, Cosmed, Roma, Italy), that was previously calibrated following the manufacturer's guidelines and fixed to the athletes' back through a specific support.

The EMG (DUE Pro, OT Bioelettronica, Torino, Italy) signals were sampled at 2048 Hz, hardware amplified (gain 1000 V/V ± 1 %), converted and transmitted wirelessly via Bluetooth (Wireless G USB adapter) to a computer for the storage process (OT Biolab 2.0.6484.0EM acquisition software, OT Bioelettronica, Torino, Italy). The cardio-metabolic data were acquired in a breath-by-breath mode to measure oxygen consumption (VO_2), carbon dioxide production (VCO2), minute ventilation (VE), respiratory frequency (Rf), respiratory exchange ratio (RER), and heart rate (HR).

EMG and cardio-metabolic measurements were taken continuously during the trials. At the end of each trial, the subjects were asked to evaluate the perception of their effort by using the Borg CR-100 Scale with the 0-value meaning "nothing at all" and the 100-value meaning "maximal" (Borg & Kaijser, 2006).

Figure 5 Panel a report an in-scale representation of the running course (m). In panels b and c, uneven and even running course are presented.

2.3.4 Data analysis

For each condition, data were collected continuously, and the final two minutes of exercise were analysed. The EMG signals were band-pass filtered (20–450 Hz; 20 dB/oct) to remove noise and were full wave rectified. A linear envelope of the EMG signal was obtained through a 20 Hz Butterworth 4th-order low-pass digital filter, to overlap the EMG signals relative to all the running cycles over a time-normalized period and to discard eventual running cycles in which the EMG signal was out of 95 % confidence interval. For each running condition, the level of muscle involvement was considered as the averaged rectified value (ARV) of the valid running cycles (Zoppirolli et al., 2017) over the entire cycle time; the coefficient of variation (CV) of each ARV also was calculated (CV = SD/mean). All cardio-metabolic data resulted from the average of the final two minutes of exercise. Moreover, we calculated the energy cost of running (Cr = metabolic power / speed) in both running conditions (Di Prampero et al., 1993).

2.4 Results

Subjects ran both the courses with an average step frequency of 1.97 ± 0.19 Hz and an average speed of 1.71 ± 0.17 m/s.

2.4.1 Surface electromyography

During UE-T, an elevated EMG activation was observed in the muscles involved in ankle joint stabilization compared to E-T. Specifically, a significant difference was found in TAn, with mean activation increasing from 0.048 ± 0.013 mV in E-T to 0.058 ± 0.012 mV in UE-T (+22 %; $p = 0.03$; $d = 0.82$) and PLo, increasing from 0.055 ± 0.017 in E-T to 0.060 ± 0.015 in UE-T (+10 %; $p = 0.02$; $d = 0.87$), while GMe showed a 24 % increase between conditions, although this difference was not statistically significant (+24 %, $p = 0.08$, $d = 0.62$). On the contrary, no differences were found in VLa, Sol and BFe muscles ($P > 0.05$). (Fig. 6). CV analysis did not show any statistical difference in the level of muscle activation variability between the two running conditions (Table 2).

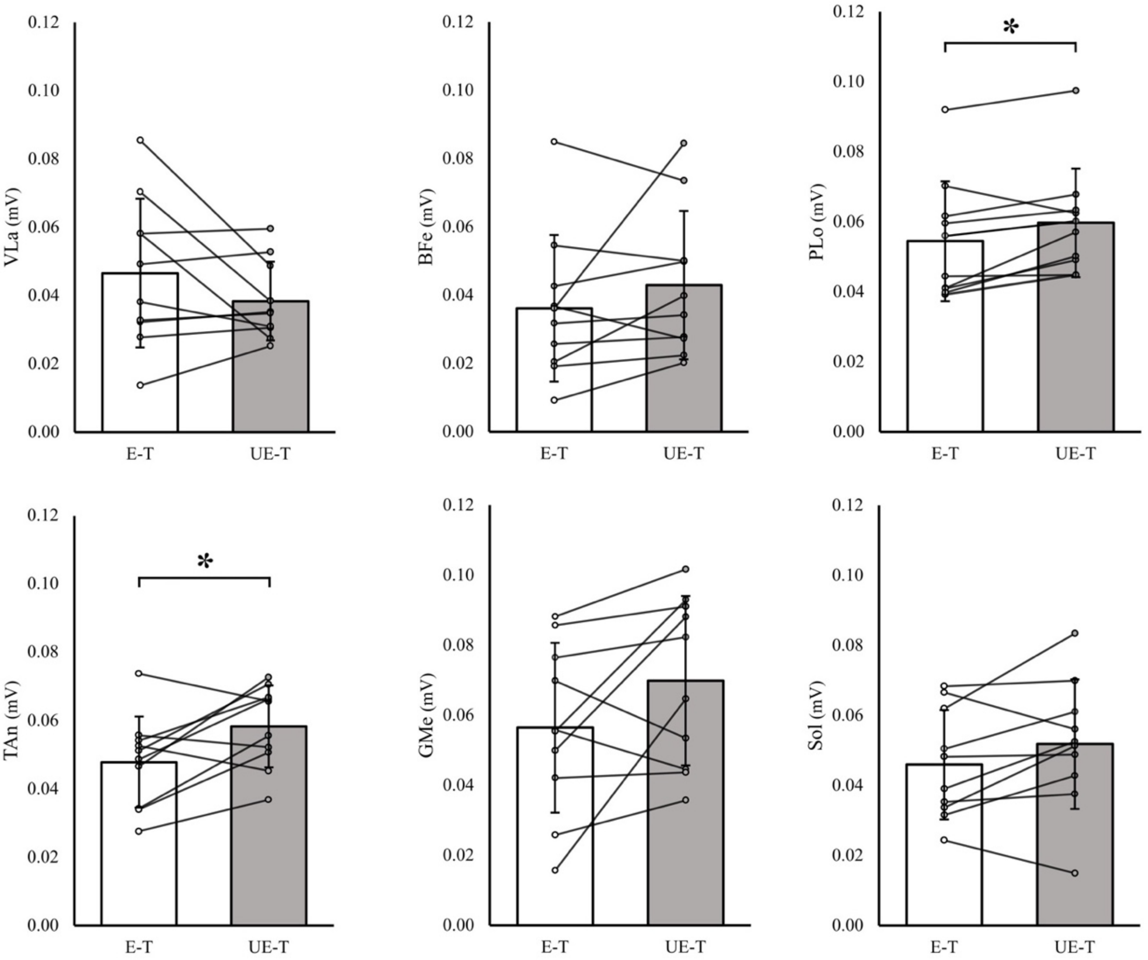


*Figure 6 EMG mean activation of muscles, in both running conditions (E-T: even terrain, UE-T: uneven terrain). Dots and lines represent individual responses. VLa: vastus lateralis, BFe: Biceps femoris, Tan: Tibialis anterior, PLo: peroneus longus, GMe: gastus mediali , Sol: soleus. Student t-test results are reported: * = p<0.05*

CV	VLa	BFe	TAn	PLo	GMe	Sol
E-T	2.79 ± 1.46	2.21 ± 0.92	1.37 ± 0.16	1.56 ± 0.17	1.95 ± 0.28	1.66 ± 0.21
UE-T	2.34 ± 1.40	2.31 ± 1.13	1.44 ± 0.13	1.55 ± 0.18	1.92 ± 0.12	1.70 ± 0.12

Table 2 Coefficient of variation (CV) of muscle activation in even (E-T) and uneven (UE-T) running conditions. VLa: vastus lateralis, BFe: Biceps femoris, Tan: Tibialis anterior, PLo: peroneus longus, GMe: gastus medialis, Sol: soleus. Data are expressed as mean ± standard deviation

2.4.2 Cardiopulmonary measurements and rate of perceived exertion

During UE-T, respiratory parameters and cost of running were all significantly higher ($p < 0.05$) with respect to E-T. In specific, we measured an 18 % increase in VO_2, from 1767.94 ± 359.78 mL/min in E-T to 2085.22 ± 192.66 mL/min in UE-T ($p = 0.01$; $d = 1$) together witha23%increaseinCr(p=0.02;d=0.9),a7%increaseinRf(p= 0.02; d = 0.85), a 20 % increase in VE ($p = 0.006$; $d = 1.1$) and a 10 % increase in heart rate (HR), from 140.8 ± 29.9 bpm in E-T to 155 ± 27.1 bpm in UE-T ($p = 0.02$; $d = 0.94$). A significant difference between conditions for RPE was also observed, with a 50 % increase in UE-T compared to E-T running ($p < 0.001$) (Fig. 7).

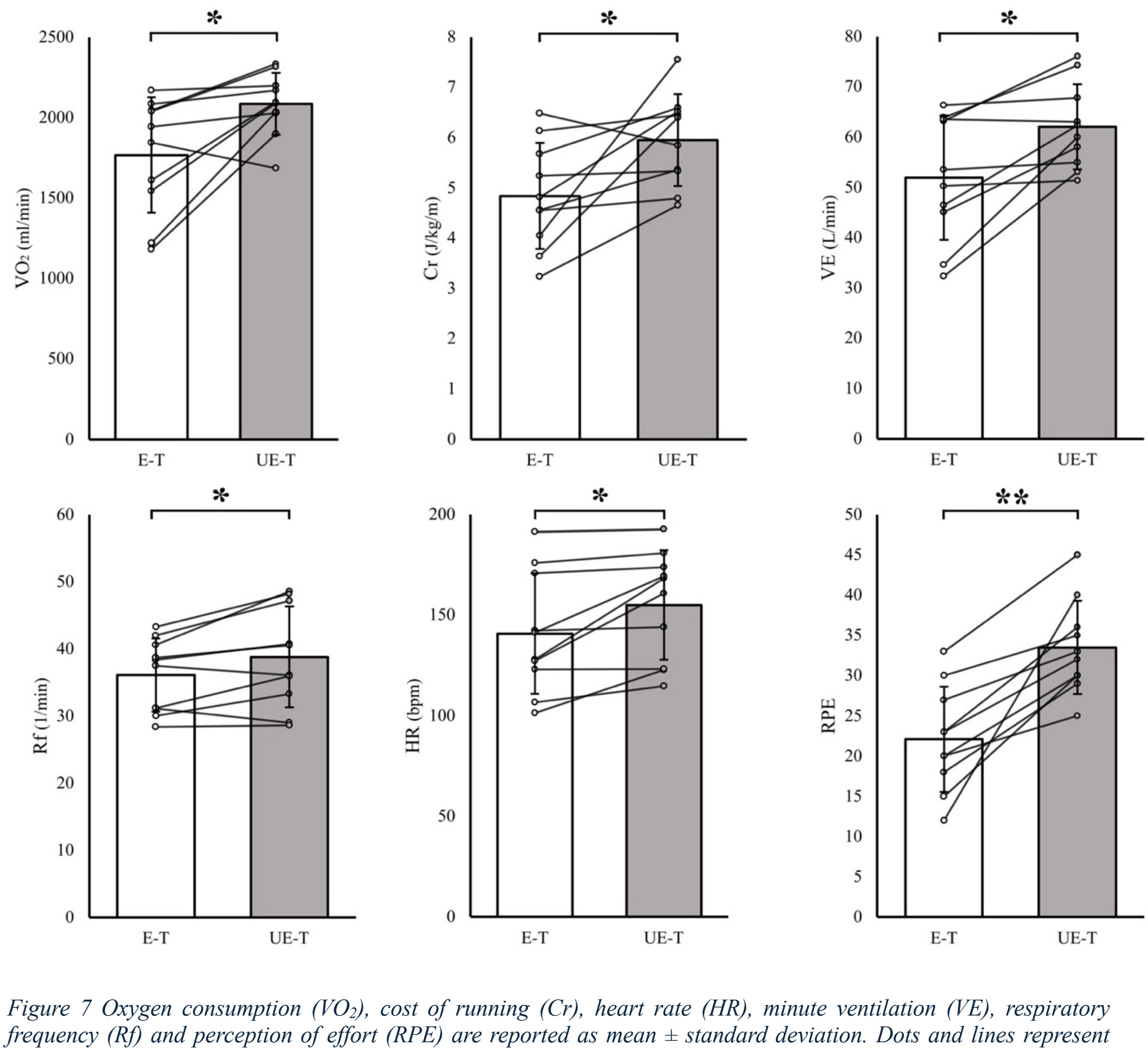


*Figure 7 Oxygen consumption (VO_2), cost of running (Cr), heart rate (HR), minute ventilation (VE), respiratory frequency (Rf) and perception of effort (RPE) are reported as mean ± standard deviation. Dots and lines represent individual responses. Student t-test results are reported: * = $p<0.05$; ** = $p<0.001$*

2.5 Discussion

This study aimed to explore the energetic and neuromuscular effects of running on both even (E-T) and uneven (UE-T) terrains, using standardized smooth and rough iterative running paths with consistent characteristics in terms of step positioning, running velocity, and step frequency. This allowed us to analyse the effect of different surface conditions on energy consumption and muscle activation. By maintaining consistency in the timing and positioning of foot placement between the two conditions, we could isolate the specific impact of the uneven surface, reducing the variability that would have been introduced if foot positioning during the stance phase had been different and inconsistent between the two conditions. The major findings of this study were that: i) EMG activation of the muscles involved in the ankle joint stabilization was increased when running on uneven terrain, despite step sequence being the same as in the even terrain condition ii) cost of running and all the cardio-metabolic values were elevated in UE- T, as well as the rate of perceived exertion; iii) maintaining a comparable running pattern in the two conditions, the variability of muscle activation was not different between UE-T and E-T.

This ensured consistency in the timing and positioning of foot placement between the two conditions.

2.5.1 Neuromuscular impact

We found that two key muscles responsible for ankle joint stabilization exhibited increased EMG activation during running on uneven terrain compared to the even condition. Specifically, the tibialis anterior and peroneus longus showed enhanced activity by 22 % and 10 %, respectively. The gastrocnemius medialis also exhibited a 24 % increase in activity, although this difference did not reach statistical significance. No differences were found in the thigh muscles monitored in this study or in the other leg muscles. Furthermore, the lack of significant differences in the CV for any of the monitored muscles suggests that, overall, the magnitude and variability of muscle activity patterns remained largely stable across conditions, particularly for the larger leg muscles. Taken together, these findings suggest a greater neuromuscular involvement of the leg muscles controlling the inversion-eversion movements of the foot, and to a lesser extent flexion–extension, when maintaining the same running pattern on rough terrains compared to smooth terrains. These results aligned with the findings highlighted by Nicot et al. (Nicot et al., 2022), who reported elevated mediolateral acceleration variability when running on trail terrain, indicating an increased mechanical effort in moving or controlling the feet along the mediolateral direction (Nicot et al., 2022).

A different involvement of the ankle joint during running on uneven surfaces, compared to smooth surfaces, has previously been shown by different authors (Gantz & Derrick, 2018; Schrøder Jakobsen et al., 2022; Voloshina & Ferris, 2015). For instance, Gantz (2018) reported that runners on uneven terrain exhibited a reduction in inversion at foot contact, possibly as a protective mechanism to avoid ankle sprains, while also increasing knee flexion to compensate for the loss of shock attenuation (Gantz & Derrick, 2018). Voloshina and Ferris (2015) observed that running on un- even terrain led to slightly decreased ankle range of motion, suggesting runners adapted by landing with flatter feet, likely to reduce ankle instability (Voloshina & Ferris, 2015). Jakobsen et al. (2022) also noted a reduction in ankle plantarflexion/dorsiflexion ROM and plantar flexor moments on unstable surfaces, which might be a protective strategy against ankle injuries (Schrøder Jakobsen et al., 2022). However, our EMG results are in contrast with the findings of the other studies that tried to recreate standardized laboratory setups to analyse running dynamics on irregular terrain. For example, Voloshina and Ferris did not find any significant difference in lower leg muscle activation during running on a modified uneven surface treadmill (Voloshina & Ferris, 2015), but they found increased EMG activity in thigh muscles. These differences could be explained by the fact that in our protocol, we imposed a running pattern to isolate the effect of the running surface from other factors.

The CV of muscle activation did not show any differences between running conditions. These results are in contrast with previous studies that showed differences in this parameter (Schrøder Jakobsen et al., 2022; Skroce et al., 2023; Voloshina & Ferris, 2015) as well as in the CV of joint accelerations (Nicot et al., 2022). In Voloshina and Ferris (2015), all the muscles analysed showed an increased EMG variability during uneven running (Voloshina & Ferris, 2015), while Skroce et al. (2023) found greater EMG variability only in tibialis anterior and soleus muscle during running on a treadmill with unexpected lateral oscillations (Skroce et al., 2023). The discrepancies with our results may again be attributable to the different experimental setups. Indeed, in the previous studies, athletes could choose their preferred running pattern in terms of foot positioning (ground contact), thus introducing possible biases during the EMG analysis. With our protocol, we ensured a consistent step pattern across conditions, for the first time in the specific scientific literature, altering the characteristics of the contact surface only. We can state that, only certain lower leg muscles show increased activation, when the running surface is uneven compared to when running on smooth surfaces, and that there are no significant differences in the variability of muscle activation between the two conditions.

The integration of all these new findings together with the previous observations indicated that a greater involvement of the muscles proximal to the ankle joint without an increased variability in muscle activation is a specific neuro-muscular feature related to the influence of the running surface only (type of foot support) on movement control.

These results indicated an elevated neuro-muscular engagement to control foot support during uneven running with a fast adaptation of the central nervous system in supplying an adequate and consistent level of muscle control during foot support under uneven conditions. In this view, our neuro-muscular results can be considered as a basic step in the comprehension of neuro-muscular control during trail running.

2.5.2 Cardio-metabolic parameters

The collected data underscored significant differences in cardio-metabolic parameters between running on even or uneven terrains. Notably, there was an increase in respiratory frequency, oxygen consumption, heart rate, and perceived exertion during UE-T compared to E-T conditions, even if the run was performed at the same standardized velocity and at the same standardized step length and frequency. As previously discussed, the augmented need for stabilization during ground contact and the subsequent increase in EMG activation could explain the different metabolic responses observed here, as also found by other authors (Seki et al., 2020). Our results showed an approximate 18 % increase in oxygen consumption when running on uneven terrain compared to even terrain. This aligns with prior studies showing heightened physiological demands during off-road running in contrast to treadmill running at similar speeds and gradients (Jensen et al., 1999; Nicot et al., 2022). Nicot et al. reported a 10 % increase in the oxygen cost of trail running compared to treadmill running, with the energy expenditure escalating further with augmented trail technicity (Nicot et al., 2022).

In indoor settings, literature presents conflicting results. Voloshina and Ferris (Voloshina & Ferris, 2015) tested subjects on a treadmill under both even and uneven conditions, observing a 5 % increase in energy demand when running on uneven terrain. Gantz et al. found similar results with a 10 % increase in oxygen consumption while running on a modified irregular surface treadmill (Gantz & Derrick, 2018). However, Dhawale and Venkadesan (Dhawale & Venkadesan, 2023) noted a 5 % rise in metabolic power consumption when running on a custom-made path of both even and uneven surfaces, although this difference wasn't statistically significant. The magnitude of the increase in the cost of running was greater in our study compared to the mentioned studies, but the disparities observed in the results could be attributed to the diverse setups and the different absolute intensities employed by the various research groups. As discussed above, the uneven conditions induce biomechanical and neuro-muscular alterations, thought to contribute to the increase in energy expenditure. Therefore, as the magnitude of these perturbations amplifies, the muscle activation increases, leading to an augmented cost of running. In our research, we forced the same running velocity and an equal running pattern in both even and uneven running conditions. This task did not let the athlete naturally adapt to the surface for a more economical and easy running pattern, suggesting that when running with the same pattern, the effect of surface condition on running economy is even greater than what can be measured in the ecological trail environments.

Although the activation pattern of the lower leg muscles remained largely consistent between conditions, with only increased activity observed in the ankle stabilizers, this localized neuromuscular adaptation likely contributes to the increased oxygen consumption observed during uneven terrain running. However, it is likely that other factors, such as the activity of stabilizing muscles in the trunk and upper body, also play a role in the rise in metabolic cost. As this was not directly assessed in our study, further research is needed to explore the involvement of these additional muscle groups and gain a more comprehensive understanding of the physiological demands of running on uneven terrain.

This study has several limitations that should be acknowledged. Firstly, the focus was primarily on the muscles of the lower body, without a comprehensive analysis of core and upper body muscles, which may play a crucial role in stabilizing locomotion and influencing energy expenditure. Secondly, plantar forces were not measured, which could have provided valuable insights into pressure distribution and its relationship with stability. Thirdly, we analysed only one submaximal speed for both running conditions.

Additionally, the 8-shaped course used in the study, while carefully designed to emulate specific characteristics of trail running in a controlled laboratory setting, included curvilinear running and a relatively short length that may not fully replicate the dynamics of outdoor trail running. The turns, although moderate, could have influenced the participants' biomechanics and should be considered when interpreting the results. Furthermore, even and uneven surfaces could have had different stiffness, partially influencing the data. This aspect should also be interpreted in light of the characteristics of real off-road running environments. Off-road running is performed on natural terrain, and surface description is considered an important element for the interpretation of both training and competition demands (Scheer et al., 2020). Accordingly, outdoor trail surfaces may vary substantially in composition and mechanical properties across sections, rather than being represented only by fixed rigid irregularities. In ecological settings, runners may therefore encounter under-foot conditions that differ from those reproduced in the present protocol, potentially eliciting different foot-ground interactions and stabilization strategies. In addition, the fixed positioning of the irregular elements in the present setup intentionally reduced one source of step-to-step variability. While this choice improved standardization and strengthened the isolation of surface effects, it may not fully reflect the range of locomotor adjustments reported when running on irregular surfaces (Gantz & Derrick, 2018; Voloshina & Ferris, 2015). Therefore, the present model should be interpreted primarily as a controlled representation of one specific component of uneven terrain, rather than as a full reproduction of the mechanical variability encountered during outdoor trail running. This could be taken into consideration for future studies, where surface stiffness should also be assessed, and athletes who habitually run on uneven surfaces, such as trail runners, should be involved.

2.6 Conclusions

In conclusion, our protocol was designed to ensure similar gait pat- terns across different surface conditions by providing foot placement targets and using a metronome to standardize step lengths and step frequencies. This approach aimed to isolate the influence of the terrain on running performance, minimizing the effects of step positioning during ground contact on both even and uneven surfaces. Running on UE-T elicits elevated neuromuscular activation of the ankle’s stabilizer muscles and elevated metabolic responses compared to E-T. The findings obtained have contributed to elucidating the impact of terrain variability in trail running on physiological and neuromuscular features. This study underlines the importance of considering the unique characteristics of outdoor terrains where off-road running competitions are held, including their unevenness and variability. Understanding how surface terrain influences physiological and neuromuscular responses can aid in developing more effective injury prevention strategies and optimizing performance.

Chapter 3 – Kinematics and performance on uphill and downhill trail running in elite and well-trained athletes

3.1 Abstract

This chapter is based on the Accepted Manuscript of the following article:

Bettega, S., Pellegrini, B., Viscioni, G., Fornasiero, A., Bortolan, L., Schena, F., & Zoppirolli, C. (2025). Kinematics and performance on uphill and downhill trail running in elite and well-trained athletes. International Journal of Sports Science & Coaching. https://doi.org/10.1177/17479541251352176

Trail running performance is strongly shaped by the biomechanical demands of varying slopes, where athletes must continuously adapt their stride patterns to manage energy cost, fatigue, and control. Uphill running requires greater muscular effort and energy expenditure, while downhill running poses unique challenges due to increased braking forces and the need to optimize stride mechanics. To examine how these slope-specific adaptations influence performance, we investigated the impact of uphill (UH) and downhill (DH) sections and their relative running gross kinematics in elite and well-trained athletes.

Data were collected during the 2023 Dolomyths Skyrace (22km, ±1750m elevation gain/loss). Split times for UH and DH sections were analysed for the top100 male finishers. Running kinematics (stride time, contact time, stride frequency, and stride length) was assessed via video analysis on four-course segments (two UH, two DH). Athletes were grouped by performance level, and ANCOVA was used to analyse kinematic differences.

Athletes spent more time on UH (62.5±1.3%) than DH (37.5±1.3%), making UH the strongest predictor of overall time ($p<0.001$). However, percentage time differences among runners were greater in DH, where time loss from the best split was higher (21.6±0.3%) than in UH (18.5±0.3%, $p<0.001$). The relationship between time loss and performance varied between sections ($p<0.001$). Across race sections, speed decreased from elite to well-trained athletes, accompanied by longer CT and SD and lower SF and SL in both UH and DH ($p<0.001$). After adjusting for speed, no significant group differences in kinematics remained ($p>0.05$). In DH, SL and SD increased in the second

segment. SF explained 38-39% of speed variability in UH, while SL explained 66-73% in DH.

Between-group differences in DH performance exceed those in UH, significantly impacting trail-running outcomes and highlighting the need for targeted descent training. Kinematic variations were mainly driven by speed, with stride frequency driving ascent speed and stride length facilitating faster descents.

3.2 Introduction

Trail running has surged in popularity over the past two decades (Hoffman et al., 2010). Defined as a foot race set in natural environments—such as mountains, deserts, forests, and plains—trail running covers varied terrains like dirt roads, forest trails, single tracks, and beach sand, with paved sections comprising no more than 20–25% of the course (Scheer et al., 2020). Unlike road races, trail running occurs in unpredictable, complex settings, requiring runners to adapt biomechanically and physiologically. Sky-running, a high-altitude form of trail running, often involves technical mountain terrain, including glaciers, moraines, and scrambling sections with ropes (Scheer et al., 2020). Additionally, trail running and sky-running races are characterized by significant elevation changes, with uphill and downhill sections of varying lengths that further challenge athletes. These challenging conditions demand adjustments in running patterns, impacting neuromuscular requirements and energy expenditure (Nicot et al., 2022).

Due to all these characteristics, researchers have tried in recent years to develop performance models that could better describe trail running and sky-running performance compared to classical endurance performance models (Balducci et al., 2017; Ehrström et al., 2018; Lemire, Hureau, et al., 2021). As proposed by Ehrström and co-authors, the predictive power of the classic endurance running model is enhanced by introducing elements specific to trail running, such as local muscle endurance and running economy on a positive slope (Ehrström et al., 2018). These elements address the unique demands of trail running, distinguishing it from traditional endurance running on level surfaces. Theoretically, trail running model could be further refined by identifying the predictive factors for uphill and downhill performance separately, as demonstrated by Lemire and colleagues (Lemire, Hureau, et al., 2021).

Running velocity at VO_2max (vVO_2max) appears to be the major performance factor in both UH and DH conditions in short trail running, with BMI and maximal strength playing an essential role in completing the UH predictive model (Lemire, Hureau, et al., 2021). VO_2max (Fornasiero et al., 2022), high vVO_2max, and low BMI (Lemire, Hureau, et al., 2021) are crucial for UH performance outcomes. In contrast, the prediction of performance for DH trail running appears to be more complex, with vVO_2max, maximal strength and musculotendinous leg stiffness in the lower limbs playing a critical role in

DH performance outcomes (Lemire, Hureau, et al., 2021). However, the physiological model proposed by Lemire and co-authors has 84% of predictive power, indicating that some factors are still missing for a comprehensive analysis of running downhill on trails (Lemire, Hureau, et al., 2021). Downhill sections occur in challenging environmental conditions, such as uneven terrain, steep slopes and unpredictable surfaces, and it was speculated that individual DH running technical abilities and the impact on running biomechanics (Vermand et al., 2022) could be crucial for overall performance outcomes, although these factors are difficult to assess.

In such demanding conditions and prolonged efforts, running biomechanics continuously adapt (Vermand et al., 2022). These adaptations can be indicative of the onset of fatigue (Lemire, Remetter, et al., 2021), serve as protective mechanisms against injuries (Giandolini et al., 2017; Lemire, Remetter, et al., 2021), and influence the energy cost of running (Lemire, Hureau, et al., 2021), ultimately affecting performance. Research on running kinematics in trail running highlights the impact of varying terrains and slopes on stride patterns and biomechanical adjustments. Uphill running typically demands greater muscular effort and energy expenditure, with less pronounced kinematic differences between performance levels, as observed by Genitrini et al. (Genitrini et al., 2023) and Besson et al. (Besson et al., 2023). However, when accounting for running speed, variations in stride length and frequency may emerge (Vermand et al., 2022). Downhill running, by contrast, presents unique biomechanical challenges, including greater braking forces (Ehrström et al., 2018; Lemire, Hureau, et al., 2021) and the need to optimize stride length and contact time to maintain control and minimize energy loss (Björklund et al., 2019; Townshend et al., 2010). These adaptations are crucial for managing fatigue and preventing injuries, particularly in prolonged efforts and steep descents (Giandolini et al., 2017; Lemire, Hureau, et al., 2021). Furthermore, Genitrini et al. (Genitrini et al., 2023) suggest that efficient downhill running involves enhanced swing leg mechanics to optimize propulsion, while Lemire et al. (Lemire, Hureau, et al., 2021) emphasize the protective role of reduced stride length and contact time in minimizing mechanical stress over extended downhill sections. Collectively, these findings underscore the importance of terrain-specific biomechanical adjustments and their direct influence on fatigue, injury prevention, and overall performance outcomes in trail running.

Therefore, the aim of this study was twofold: i) to investigate how uphill (UH) and downhill (DH) performances affect overall trail running race results; ii) to analyse running kinematics during UH and DH segments in athletes of different performance levels, while considering the potential effects of fatigue as the race progresses. These objectives were addressed in the context of a Skyrunning competition with a specific elevation profile, characterized by a continuous 10 km uphill section followed by a 12 km downhill section.

We hypothesized that i) the downhill section could be the most decisive factor influencing the race outcome, and ii) better-performing athletes would demonstrate better maintenance of elevated speed throughout the race, longer cycle lengths, and a reduced duty cycle.

3.3 Material and Methods

3.3.1 Experimental setup

The data were collected during the 2023 Dolomyths Skyrace, a 22 km Skyrunning race with a total elevation gain and loss of 1750 m. Starting and finishing at 1450 m, the race reaches its highest point at 3152 m. The first 10 km consists of a continuous uphill section, followed by a 12 km downhill section that leads to the finish line.

The race took place in July in the Italian Alps under sunny and dry conditions, which allowed athletes to perform at their best without the influence of adverse weather or terrain.

The race featured 825 ranked participants (694 male and 131 female athletes) representing different performance levels. The first athlete finished the race in 02:04:39, while the last athlete finished in 05:44:11 (hh:min:ss).

We collected split times for each athlete from the official timing system, both for the uphill and downhill sections. In addition, we filmed the athletes on four different segments of the course during the race: two on the UH section and two on the DH section. All segments were chosen in advance during a route reconnaissance carried out by the research team one week before the event and were characterized by comparable inclines (about ±35% incline). All the participants were recorded during the race on the four segments of interest. Segments were selected to be representative of the overall uphill and downhill sections, while avoiding overly technical terrain that could introduce excessive variability in running patterns. Similar slopes and surface conditions were prioritized to ensure comparability across segments. Their distribution along the course also allowed us to explore potential fatigue-related changes in running mechanics. The permission to record videos was obtained from the organizing committee, as the participants had previously granted their consent to the committee, which extended this permission to third parties.

3.3.2 Split time analysis

For each participant, overall race time, uphill and downhill split times were collected from the official timing system and analysed. To determine whether the uphill or downhill section was the primary determinant of overall race performance, we focused on the top 100 male finishers to obtain a more complete analysis of elite and well-trained athletes. For each athlete, we calculated the percentage time loss in both the uphill and downhill sections relative to the best split in each section to explore the linear relationship between section-specific performance and overall race outcome.

3.3.3 Running kinematic analysis

For the kinematic analysis of running, four segments were selected, as previously described. These segments were chosen to have similar slopes (±35%), with two located in the uphill section and two in the downhill section of the race, at approximately 3.5 km (UH1), 9 km (UH2), 10.5 km (DH1), and 18 km (DH2) of the racecourse. Additionally, all selected segments featured comparable surface types, ensuring that the technical demands across the segments were consistent. For each of the selected segments, a 12 to 15-meter was precisely measured, with the start and end points clearly marked on both sides of the path using four coloured cones.

To capture the athletes' horizontal displacement during both uphill and downhill running, a high-frequency camera (GH5S LUMIX, Panasonic Corp., Osaka, Japan) was employed, set to a 100 Hz acquisition frame rate and FHD recording quality. The camera was positioned laterally in each segment to ensure visibility of the cones and allow clear observation of the athletes' foot positions.

Additionally, At the beginning of each segment, a smartphone mounted on a tripod recorded the athletes from a frontal view, allowing investigators to identify their bib numbers.

Although the analysed segments were relatively short (12–15 meters), this choice allowed for precise kinematic assessment under controlled conditions in a mountain environment. These segments were selected to reflect the typical characteristics of the uphill and downhill sections, while minimizing variability due to extreme terrain. Nonetheless, we acknowledge that this may only partially reflect the full range of technique variability over the entire race course (Pellegrini et al., 2021).

3.3.4 Participants/athletes selection

Male athletes were categorized into different performance groups, and a performance coefficient (PC) was calculated for each athlete by dividing their final race time by the winner's time. This coefficient ranged from 0 to 1, with 1 representing the winning performance (Fornasiero et al., 2022). Performance groups were created at every 5% decrease in the performance coefficient. For the kinematic analysis, we included the top ten athletes from the first five performance groups who: i) were identified in all four segments and ii) completed at least three visible steps within each segment. This allowed us to analyze five groups of runners with progressively lower performance levels, each composed of 10 participants. To better assess the performance level of each group, we collected the individual ITRA score of each participant (ITRA). Given the selection criteria, the athletes involved in this study are primarily elite, sub-elite, and well-trained runners, with all participants finishing within the top 90 positions in the race (race time of the last athlete included in the analysis: 02:41:20). Table 3 reports the ITRA score, age, race time, and uphill (UH) and downhill (DH) running time for the five groups of runners investigated.

		Performance Groups				
Mean ± SD		1	2	3	4	5
PC	---	1 - 0.95	0.95 – 0.90	0.90 – 0.85	0.85 – 0.80	0.80 – 0.75
ITRA Score	---	905.4 ± 20.5	871.7 ± 26.8	861.4 ± 26.7	779.1 ± 26.8	754.8 ± 58.1
Age	years	29.5 ± 4.6	28.5 ± 4.7	30.2 ± 6.2	28.6 ± 4.8	28.9 ± 4.4
Race time	min	126.7 ± 1.4	136.5 ± 2.3	142.4 ± 2.0	149.1 ± 0.9	158.8 ± 1.4
Uphill time	min	79.9 ± 1.2	85.9 ± 1.4	89.6 ± 2.5	92.7 ± 2.2	98.8 ± 2.2
Downhill time	min	46.9 ± 0.9	50.6 ± 1.7	52.8 ± 2.0	56.4 ± 2.3	60.0 ± 2.5

Table 3 Groups characteristics – The table reports the characteristics of the performance groups (1-5). ITRA score, age, overall race time, and split times for uphill and downhill are reported. Data are expressed as mean ± standard deviation (SD).

3.3.5 Tracking procedures and analysis

All selected athletes were analysed using Kinovea software (v.2023.1.2) in each segment. The time taken by the athletes to travel each selected segment was calculated through video analysis, measuring the duration between the athletes crossing a virtual line perpendicular to the path, connecting the first two cones and the final two cones. Mean speed was then calculated as the distance between the cones divided by the time taken to cross that segment. Foot ground contact and toe-off frames of each stride recorded from the selected athletes were visually identified and used to calculate the following metrics: mean stride time (ST: the time between consecutive ground contacts of the same foot), mean contact time (CT: the time between ground contact and toe-off of the same foot), mean duty cycle (percentage of time the foot spends in contact with the ground during a stride), mean stride frequency (SF = 1*stride time^{-1}) and mean stride length (SL = mean speed*stride frequency^{-1}).

All gait events were identified manually by two experienced operators, who followed a standardized set of visual criteria to determine ground contact and toe-off frames. The operators were aligned on definitions and worked independently but in agreement on event detection guidelines, minimizing potential subjectivity. Based on the video frame rate (100 Hz), the potential error in event identification was limited to ±1 frame (0.01 s), ensuring a high level of temporal accuracy.

3.3.6 Statistical analysis

Data distribution was assessed with the Shapiro-Wilk test and presented as mean ± standard deviation (SD). Where necessary, data were log-transformed to meet the assumptions of normality. An ANCOVA was performed on the top 100 participants for the time percentage difference data, with overall race time as a covariate and section (uphill vs downhill) as a factor. The interaction between race time and section was also included to determine if the relationship between race time and percentage difference varied between uphill and downhill sections. A significance level of $p < 0.05$ was used.

For the kinematic data, two separate analyses were conducted for uphill and downhill segments. A two-way ANOVA was used to assess the effects of group and segment, as well as their interaction, on the kinematic parameters. Since speed was significantly different between groups, an ANCOVA was also performed, including speed as a covariate, to control for its influence on the kinematic variables. Bonferroni correction was applied for post-hoc tests to compare specific groups. Effect sizes were reported using eta squared (η^2), with 0.01, 0.06, and 0.14 representing small, medium, and large effects, respectively. A stepwise multiple linear regression was used to explore the relationship between stride frequency, stride length, and running speed variability, with $p < 0.05$ indicating statistical significance.

3.4 Results

3.4.1 Split time analysis

Although the total race time is primarily influenced by the time spent on the uphill (UH) section (R2=0.90), which accounts for a greater percentage of the overall race time (62.5 ± 1.3 %) compared to the downhill (DH) section (37.5 ± 1.3 %), the analysis of the percentage difference from the best split time in both uphill and downhill running revealed a significant effect of overall race time ($p < 0.001$, $\eta^2 = 0.882$) and section (uphill vs downhill) ($p < 0.001$, $\eta^2 = 0.008$) on the percentage difference, referring to the variation in an athlete's split time relative to the best time recorded for each section. Additionally, a significant interaction between race time and section was observed ($p < 0.001$, $\eta^2 = 0.010$) (Fig.8), indicating that the relationship between race time and the percentage difference differed between the UH and DH sections. The marginal means showed that the percentage difference was higher in the DH section (21.572 ± 0.325) compared to the UH section (18.533 ± 0.325).

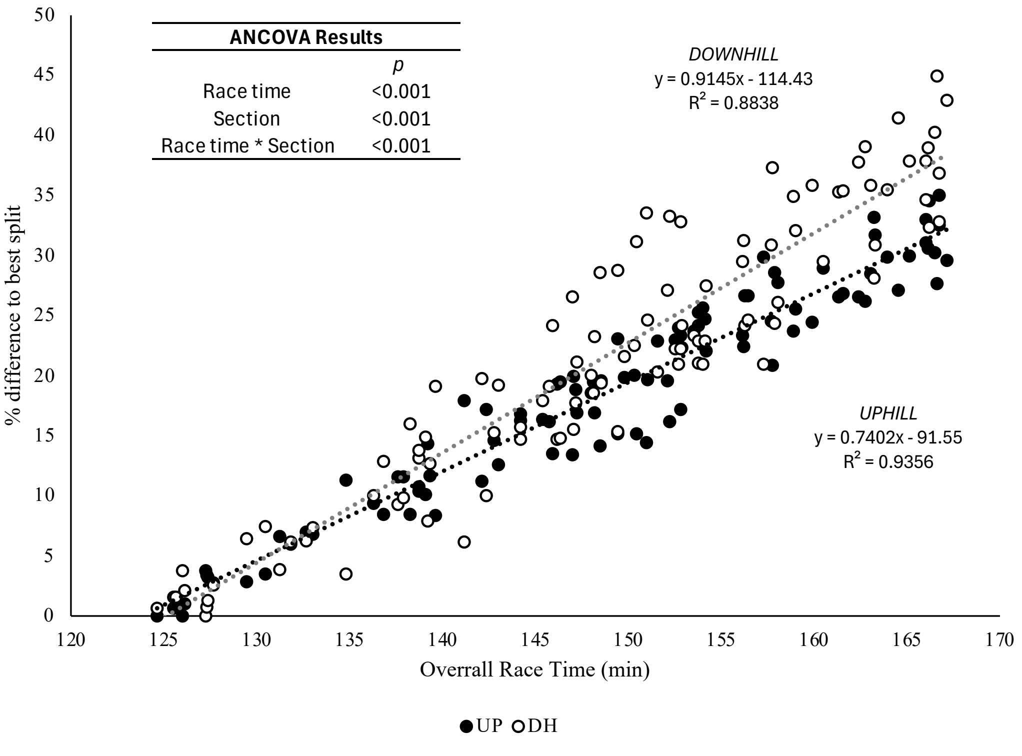


Figure 8 Relationship between overall race time (min) and percentage difference to the best split time for both uphill (UP) and downhill (DH) sections. The dotted lines represent linear trends for each section. ANCOVA results show significant effects of race time.

3.4.2 Kinematic analysis

3.4.2.1 Uphill running

The kinematic analysis of uphill running demonstrated significant differences across performance groups for several parameters, including speed, CT, SD, SF, and SL. Specifically, speed significantly decreased from Group 1 to Group 5 ($p < 0.001$, $\eta^2 = 0.601$). All these parameters showed clear trends corresponding to the runners' performance group ($p < 0.001$ for most parameters). No significant segment*group interactions were found, indicating that these effects were consistent across both uphill segments. However, ANCOVA results, with tract-specific speed added as a covariate, revealed no significant differences in any kinematic parameters across groups after adjustments ($p > 0.05$). No significant differences were found between the two uphill segments (Fig.9).

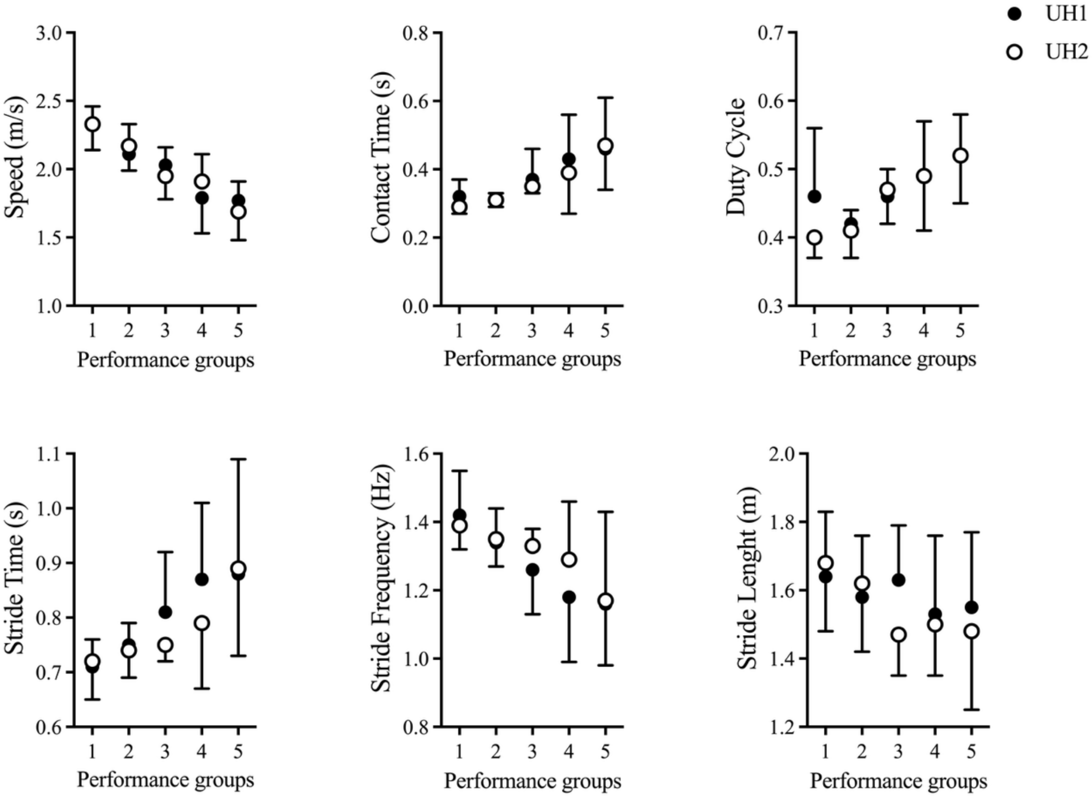


Figure 9 Kinematic parameters (speed, contact time, duty cycle, stride time, stride frequency, and stride length) across performance groups (1 to 5) for two uphill segments (UH1 and UH2). Filled circles represent values for UH1, and open circles represent values for UH2. Data are presented as means ± standard deviation.

Uphill - ANOVA Results

	Speed	Contact Time	Stride Time	Stride Frequency	Stride Lenght	Duty Cycle
	p	*p*	*p*	*p*	*p*	*p*
Segment	0.778	0.348	0.238	0.183	0.238	0.566
Group	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Segment*Group	0.352	0.944	0.730	0.781	0.491	0.872
Uphill - ANCOVA Results						
Segment	-	0.344	0.240	0.180	0.183	0.619
Group	-	0.327	0.738	0.854	0.890	0.164
Segment*Group	-	0.841	0.649	0.703	0.654	0.698

Table 4: Statistical results for kinematic parameters (speed, contact time, duty cycle, stride time, stride frequency, and stride length) during uphill running. Results from two-way ANOVA are reported for the effects of group (performance level), segment (UH1 vs. UH2), and their interaction. ANCOVA results, adjusted for running speed as a covariate, are also presented.

3.4.2.2 Downhill running

The kinematic analysis of downhill running revealed significant differences across performance groups in several parameters. Speed decreased progressively from Group 1 to Group 5 ($p < 0.001$, $\eta^2 = 0.704$), while CT, duty cycle, and SD increased with lower performance groups ($p < 0.001$ for each parameter). SF and SL both showed a decreasing trend across groups. The absence of significant segment*group interactions indicates that these trends were consistent across both downhill segments. When controlling for speed in the ANCOVA, no significant differences were found in any of the kinematic parameters across groups ($p > 0.05$) and no significant interaction segment*group was found ($p > 0.05$). However, significant differences between the two segments remained. Specifically, SD and SL increased in the second sector. CT and duty cycle both showed a tendency toward a significant effect of the segment ($p = 0.065$ and $p = 0.066$, respectively), with both parameters decreasing in the second segment (Fig.10).

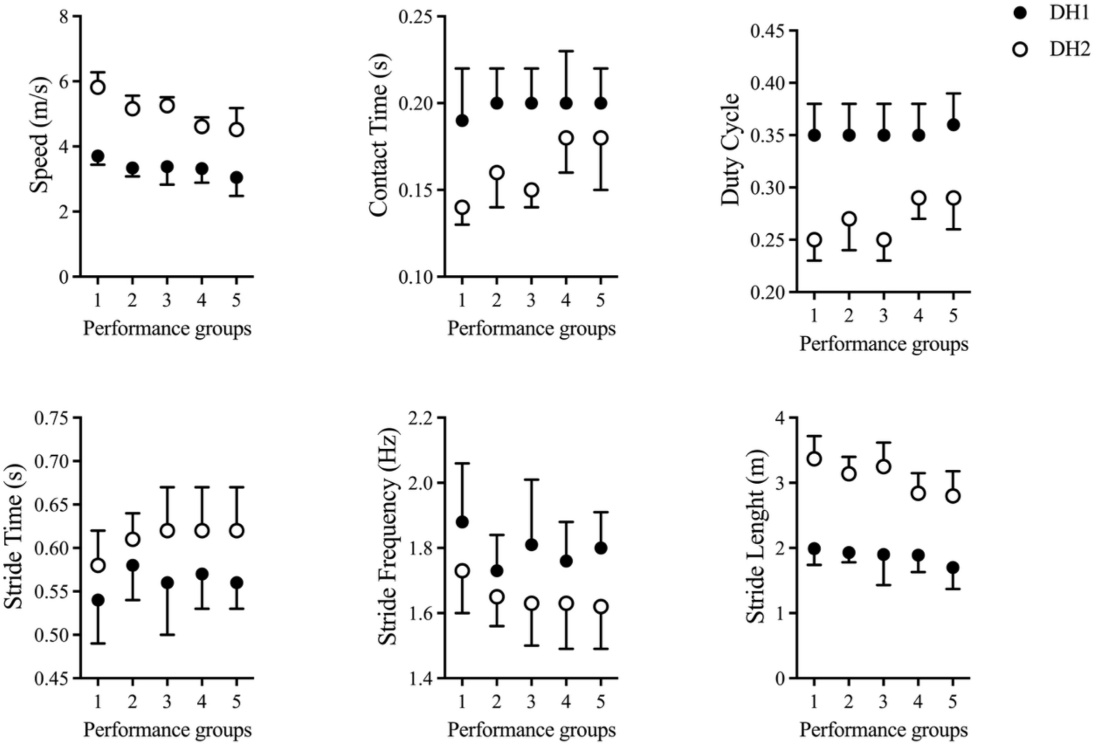


Figure 10 Kinematic parameters (speed, contact time, duty cycle, stride time, stride frequency, and stride length) across performance groups (1 to 5) for two Downhill segments (DH1 and DH2). Filled circles represent values for DH1, and open circles represent values for DH2. Data are presented as means ± standard deviation.

Downhill - ANOVA Results						
	Speed	Contact Time	Stride Time	Stride Frequency	Stride Lenght	Duty Cycle
	p	*p*	*p*	*p*	*p*	*p*
Segment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Group	<0.001	0.002	0.096	0.050	<0.001	0.020
Segment*Group	0.029	0.506	0.770	0.777	0.196	0.077
Downhill - ANCOVA Results						
Segment	-	0.065	0.027	0.032	0.032	0.066
Group	-	0.739	0.209	0.126	0.153	0.293
Segment*Group	-	0.933	0.773	0.781	0.858	0.470

Table 5 Statistical results for kinematic parameters (speed, contact time, duty cycle, stride time, stride frequency, and stride length) during downhill running. Results from two-way ANOVA are reported for the effects of group (performance level), segment (DH1 vs. DH2), and their interaction. ANCOVA results, adjusted for running speed as a covariate, are also presented.

3.4.2.3 Stepwise linear regression analysis

The stepwise multiple linear regression analysis (Fig.11) revealed that SL and SF contribute differently to the variation in speed during uphill and downhill running, with adjusted R^2 values of 1 in all segments ($p < 0.001$). In uphill running, SF alone accounted for 38-39% of the variance in speed, while the addition of SL increased the explained variance to 100%. In downhill running, SL was a stronger individual predictor, explaining 66-73% of the variance in speed.

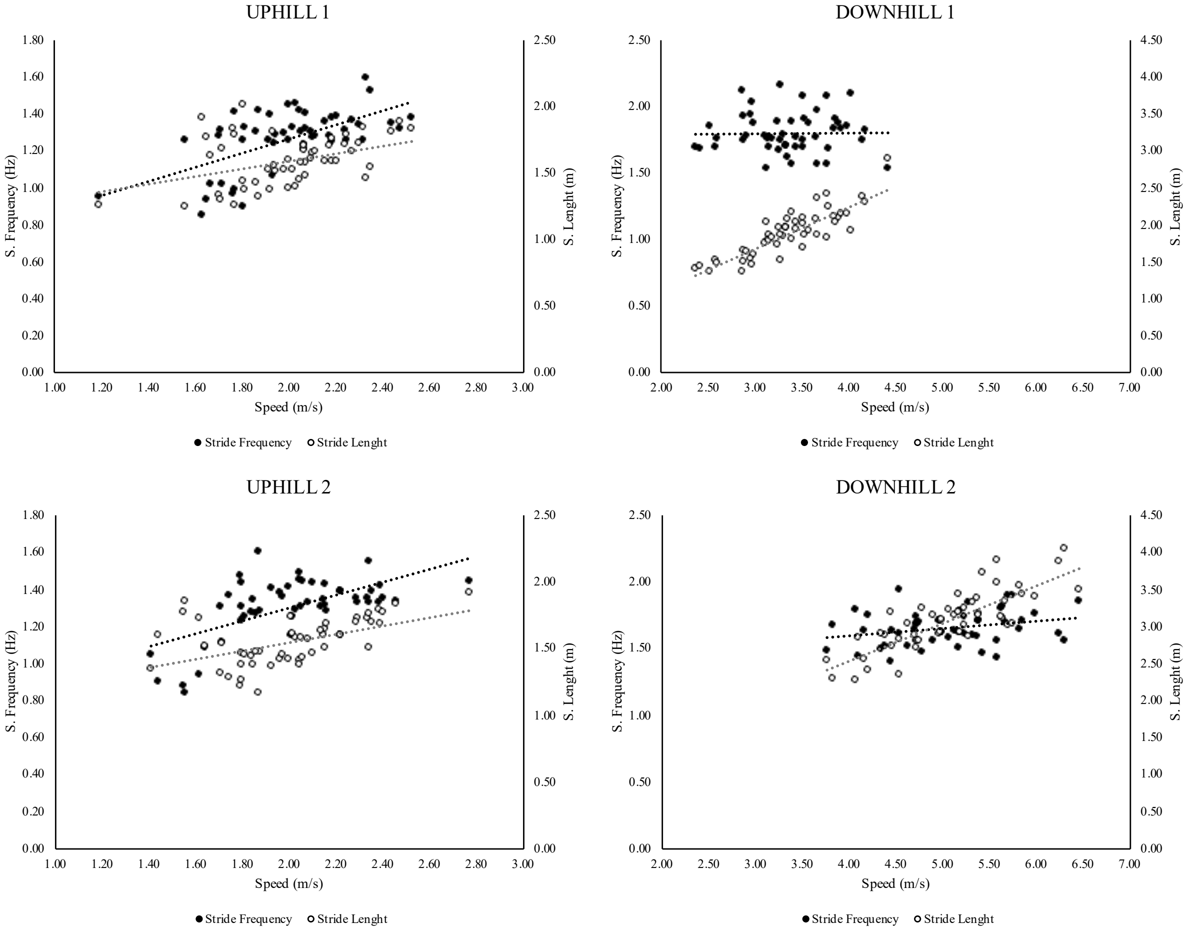


Figure 11 Figure reports the relationships between stride frequency and stride length with speed for all the segments analyzed (UH1, UH2, DH1, and DH2).

Stepwise multiple linear regression analysis

Section	Dependent Variable	Predictors	R	R^2	Adjusted R^2	R^2 change	p Change	Linear relation
Uphill 1	Speed	SF	0.62	0.38	0.37	0.39	**<.001**	*Speed* = 2.161**SF* + 0.241
Uphill 2	Speed	SF	0.63	0.39	0.58	0.39	**<.001**	*Speed* = 2.399*SF + 0.014
Downhill 1	Speed	SL	0.85	0.73	0.72	0.73	**<.001**	*Speed* = 1.282*SL + 0.950
Downhill 2	Speed	SL	0.81	0.66	0.66	0.66	**<.001**	*Speed* = 1.308*SL + 1.046

Table 6table shows stepwise multiple linear regression results showing the contribution of stride SF and stride SL to speed in both uphill and downhill segments

3.5 Discussion

Our study aimed to address two main objectives within the context of a Skyrunning race. Before presenting the main findings, it is important to acknowledge that the analysis was conducted on a selected sample of high-performing male athletes (top 100 finishers). This choice was motivated by the aim of exploring performance and kinematic differences within a competitive field, to better understand where elite athletes are able to make the difference compared to those finishing immediately behind. Female athletes were excluded solely due to the limited sample size available, which would not have allowed for a robust statistical comparison.

First, to investigate whether uphill or downhill running performance plays a different role in determining the overall trail running performance. Second, to analyse running kinematics during the UP and DH segments across athletes of different performance levels, while considering how fatigue may influence kinematic patterns as the race progresses. The results showed that: i) despite the uphill section has the most important relative weight in determining the overall race outcome, as performance level decreases, athletes tend to lose a greater percentage of time in the downhill section compared to the uphill section, indicating a larger margin for improvement in their downhill performance; and ii) kinematic analysis reveals that differences between performance groups (from elite to well-trained athletes) are mainly driven by speed, with subsequent kinematic differences being primarily related to speed variations. Interestingly, in the uphill sections, stride frequency appears to be the primary factor driving the increase in ascent speed, while in the downhill sections, faster descent speeds are mainly achieved through an increase in stride length. Running kinematics was not significantly affected by fatigue in the uphill segment, while specific kinematic changes in the downhill section, such as an increased stride length and a decreased stride frequency from DH1 to DH2, may reflect either accumulated fatigue from the ascent or a strategic adjustment to manage technical race demands.

3.5.1 Split time analysis

While total race time is mainly determined by the time spent uphill, which also represents a larger portion of the overall race duration, our analysis showed that downhill performance significantly impacted the final outcome. Larger percentage split time differences were observed in downhill sections, increasing as performance level decreased. As race time increased, the percentage difference from the best split grew more in downhill, suggesting its greater influence on overall race time, particularly for slower runners. Higher marginal means in downhill further support this, indicating greater time loss relative to the best split, especially in lower-performing athletes.

Elite athletes maintained higher speeds throughout the entire race, which may be attributed to a combination of higher metabolic capacities, superior downhill running skills, as well as other factors such as strength, leg stiffness, and reduced exercise-induced muscle damage, which are likely to mitigate the impact of fatigue on performance (Ehrström et al., 2018; Lemire, Hureau, et al., 2021).

In this first analysis, we included the top 100 finishers, because including only the athletes selected for the kinematic analysis would have been limiting and resulted in data distribution gaps; however, the data distribution for the subset of athletes included in the kinematic analysis showed a similar trend, with comparable linear relationships observed in both sections (slope values for uphill: 0.915 vs. 0.912, slope values for downhill: 0.740 vs. 0.742, for the top 100 and the kinematic subgroup respectively).

This insight into uphill and downhill performance in trail running is relatively novel, as comprehensive analyses are still lacking, with only a few studies addressing these aspects in short-distance races (Björklund et al., 2019; Ehrström et al., 2018; Lemire, Hureau, et al., 2021). Ehrström et al. reported that finish time was more strongly correlated to split times in uphill than in downhill sections during a short trail running race (27 km) (Ehrström et al., 2018), while Björklund et al. found the greatest time loss in uphill sections of a 7 km trail running trial (Björklund et al., 2019). Both studies, however, considered split times in absolute terms, which inherently reflects the greater amount of time spent in uphill sections compared to downhill. Moreover, the races analysed in those studies featured varied elevation profiles, with multiple uphill and downhill sections, unlike the continuous 10 km uphill and 12 km downhill sections present in the race analysed in our study. Recent research has focused on pacing strategies in ultra-trail races (>50 km) (Corbí-Santamaría et al., 2023; Genitrini et al., 2022), where faster finishers show higher speeds in downhill sections compared to slower competitors (Genitrini et al., 2022). Our results align with this trend, indicating that top athletes lose less time downhill, likely due to superior descent skills (Kay, 2014) and less muscle damage from frequent downhill exposure (Bontemps et al., 2020). The present study offers a new approach by analysing the percentage difference relative to the best split time, which minimizes the influence of absolute time differences between the uphill and downhill sections. Our findings suggest that downhill performance is more influential on race outcomes. This highlights the importance of the chosen analytical method and race profile, as these factors can shape how uphill and downhill performance contributions are interpreted. Further, focusing on elite athletes in short-distance races with varying elevation profiles can provide deeper insights into how pacing strategies affect overall performance.

3.5.2 Running kinematics

To our knowledge, this study remains one of the few that investigated running kinematics in trail runners under ecological conditions, focusing on a large cohort of elite and well-trained athletes. Many other previous studies have compared different levels of athletes using treadmill running tests at the same speed and incline (Besson et al., 2023; García-Pinillos et al., 2019; Lemire et al., 2023; Padulo et al., 2012). In contrast, fewer studies have explored performance differences under ecological conditions (Genitrini et al., 2023; Vermand et al., 2022). When comparing these works, caution is necessary, as running speed differences between athletes in ecological setups can introduce a significant bias that must be considered when analysing kinematic differences. While treadmill running controls this variable, it limits the athlete’s ability to self-regulate their running speed, which is a typical feature of overground running and competition (Townshend et al., 2010; Vernillo et al., 2017).

In our analysis of the kinematic data, some interesting findings emerged. We initially hypothesized that higher-performing athletes would maintain higher speeds, have longer stride lengths, exhibit a reduced duty cycle, and possibly experience less fatigue in both final segments. The best-performing athletes consistently showed higher speeds throughout the course. Kinematic differences occurred between different performance levels; however, these kinematic differences appeared to be attributed to variations in running speed rather than differences inherent to the athletes' performance levels. While fatigue had minimal impact on uphill running mechanics, the kinematic adjustments observed in the downhill segments may reflect strategic adaptations or the cumulative effects of the preceding uphill effort.

3.5.2.1 Uphill running

Our findings on uphill running kinematics emphasize the critical role of speed in influencing kinematic characteristics across groups of different performance levels. The significant decrease in speed from higher- to lower-performing groups, together with increases in CT and changes in stride parameters, suggests that higher-performing runners sustain greater speeds by minimizing CT and optimizing stride mechanics. These results align with previous literature, which shows that as speed increases, athletes adapt their running kinematics (Padulo et al., 2012; Vernillo et al., 2020).

The absence of significant differences after adjusting for speed indicates that the observed variations in kinematic parameters are primarily attributable to differences in running speed itself. This suggests that interventions aimed at improving uphill running performance in high-level athletes should focus more on strategies to enhance speed, as technique-related factors do not appear to distinguish between performance levels. Additionally, the lack of significant differences between the two analysed uphill segments suggests that all athletes maintained a relatively steady pace throughout the climb, reinforcing the idea that the key differentiator in uphill performance is the ability to sustain a higher running speed rather than changes in running mechanics over time. This aligns with previous research highlighting the importance of athlete's metabolic profile in uphill trail running performance (Fornasiero et al., 2022).

Similar kinematics results have been found in previous studies. Genitrini et al. reported no significant differences in running kinematics between more proficient and less proficient trail runners during uphill trail running (Genitrini et al., 2023). Vermand et al. observed kinematic differences during uphill running in a 40 km trail race, with these differences being attributed to variations in running speed (Vermand et al., 2022). Additionally, Garcia-Pinillos et al. found no differences in running kinematics between groups of amateur and elite runners when running at the same speed (García-Pinillos et al., 2019). Besson et al. also found no biomechanical differences between elite and experienced trail runners during treadmill uphill running, although the elite runners demonstrated a lower cost of running at the same speed (Besson et al., 2023).

Additionally, we observed in the present study that SF appears to play a critical role in uphill running, as runners tend to increase their cadence to maintain speed despite the limited SL imposed by the incline. This suggests that SF is a compensatory mechanism for overcoming the physical constraints of uphill movement, which is consistent with previous literature showing significant differences in SF as speed or gradient increased (Vernillo et al., 2020).

3.5.2.2 Downhill running

Regarding downhill running, we observed differences in running speed and kinematic parameters between performance levels, in accordance with previous literature (Genitrini et al., 2023), but our results emphasize the critical role of speed in influencing kinematic characteristics. After adjusting for speed, there were no differences between performance groups for the parameters analysed. However, clear distinctions emerged between the two downhill segments in the downhill section. The changes between segments could be attributed to various factors, such as a deliberate shift in strategy to manage the technical terrain or a response to accumulated fatigue due to the previous uphill portion of the course, or the progressive exposure to downhill running.

The tendency towards reductions in contact time and duty cycle in the second segment suggests that runners adapt their running kinematics not only due to speed but also to possibly reduce braking forces or maintain momentum. While downhill running involves greater braking forces compared to uphill running (Ehrström et al., 2018; Lemire, Hureau, et al., 2021), uphill running requires greater propulsive forces, primarily concentric in nature, whereas downhill running is dominated by eccentric muscle actions with differing metabolic efficiencies. Reducing the duty cycle may, therefore, represent a way to minimize the force impulse during contact, helping runners better tolerate the mechanical demands of downhill running.

We also observed that in downhill running, stride length becomes a dominant factor in explaining speed differences in maintaining speed, as the slope enables runners to extend their stride more freely. Townshend et al. found that stride length when running downhill increased by 16.2% compared to level running (Townshend et al., 2010), further supporting the idea that stride length plays a critical role in regulating speed during downhill segments. This observation is also in line with findings from Genitrini et al.

(Genitrini et al., 2023), outlined previously. Adjustments in stride frequency and stride length allow runners to accommodate the technical demands of varied terrain while managing the forces involved in the descent. These adaptations suggest that athletes adjust their running patterns in a consistent manner as they progress through the course. This consistency among high-level athletes may indicate that runners at this performance level have developed similar strategies to cope with the demands of the race and downhill sections.

3.6 Conclusion

Our study contributes to the limited research on trail running performance and kinematics under ecological conditions, focusing on a large cohort of elite and well-trained athletes. The findings may be of practical relevance for coaches and athletes aiming to optimize performance through a better understanding of kinematic strategies in real-world conditions. In particular, our results highlight the critical role of downhill sections in Skyrunning competitions, where greater time losses in the descent—compared to the uphill—had a stronger impact on race outcomes. This underscores the importance of prioritizing downhill running in training, as it plays a substantial role in determining final rankings. Frequent exposure to downhill running can enhance technique, help athletes manage braking forces, and adapt to the mechanical demands of these sections.

When kinematic parameters were normalized for speed, no significant differences emerged between athlete levels, suggesting that metabolic factors, along with specific neuromuscular and technical abilities, play a primary role in differentiating performance in both uphill and downhill sections.

Regarding uphill running, our results show that speed is the main factor driving differences in running kinematics, as there were no significant differences across groups after controlling for speed.

In the downhill section, all athletes, regardless of performance level, adjusted their running patterns similarly, notably increasing stride length and reducing stride frequency as the descent progressed. These changes likely reflect a combination of factors, including the accumulation of fatigue from the prior uphill section, adaptation to the technical demands of the terrain, and shifts in technique to manage the steep descent effectively.

3.7 Limitation & perspectives

This study has some limitations that should be acknowledged. First, sample size is limited to elite and well trained athletes and while this approach provides insight into performance determinants among well-trained and elite male athletes, it limits the generalizability of the findings to female and recreational runners. This limitation should be considered when interpreting the results.

while physiological or neuromuscular tests before or after the race could have offered deeper insights into athletes' capacities and performance, these were not included in the protocol. Our primary focus was to analyse a large number of athletes in a real race scenario, prioritizing kinematic analysis. Additionally, it was not possible to predict which athletes would provide high-quality video data for analysis, making such additional measurements impractical. Second, the kinematic analysis was limited to short segment of around 15 meters, which may not fully capture variability in running patterns across the course. Third, potential sources of error in the video-based kinematic analysis should be acknowledged. Although efforts were made to standardize video acquisition and minimize measurement error, minor inaccuracies in stride parameter detection cannot be entirely excluded. Lastly, while multiple linear regression was used to explore the contribution of stride length and stride frequency to running speed, we acknowledge that this approach assumes linear relationships between variables. Although this assumption is supported by previous treadmill-based studies (Padulo et al., 2012), real-world trail running conditions may involve more complex and non-linear interactions, particularly due to terrain variability and individual adaptation strategies (Vernillo et al., 2020). Future research could focus on analysing the relationship between stride frequency and stride length in real-world conditions across a range of slopes and speeds, potentially using non-linear or mixed-model approaches to better reflect the biomechanical complexity of trail running.

3.7.1 Perspectives

Future research should explore trail running performance in real-world conditions by integrating physiological, biomechanical, and neuromuscular factors. Special focus should be placed on downhill running, where greater time losses suggest potential for improvement. Increased exposure to downhill terrain may enhance adaptation and optimize performance in high-level athletes.

In particular, the inclusion of physiological markers (e.g., VO_2max, lactate, heart rate) and neuromuscular assessments (e.g., strength, fatigue indices) would provide a more comprehensive understanding of the mechanisms underlying performance differences. Combining these data with kinematic analysis could help clarify how physiological capacity and neuromuscular control contribute to uphill and downhill running efficiency.

This work has been published in the International Journal of Sports Science & Coaching. The version included in this dissertation is the Accepted Manuscript and may differ from the Final Published PDF in formatting or editorial corrections.

Chapter 4 – How sex differences shape performance and running kinematics in competitive trail running

4.1 Abstract

Sex-related differences in endurance performance have been extensively studied in road and track running, yet limited evidence exists regarding how these differences manifest in trail running, where athletes face highly variable terrain and elevation changes. This study examined sex differences in trail running performance and kinematics by comparing elite female athletes (F) to males with similar relative (M-R: time relative to the first man/woman) or absolute (M-A: similar racing time) performance. Data were collected during the Dolomyths Skyrace (22 km, 1750 m D±). Total and split performance times (uphill, UH; downhill, DH) were analysed, and video-based kinematic analysis (contact time, duty factor, stride length and frequency) was conducted across four course segments in the three subgroups (F, M-R, M-A; n=15 each).

The M-A group, although matched for overall race time, distributed effort differently, spending a higher percentage of total race time downhill compared to F ($p = 0.03$). M-R, who ran at higher speed, also showed longer strides, shorter contact times, and lower duty cycles ($p < 0.05$). After adjusting for speed, most between-group differences were no longer significant, except for a lower uphill duty factor in F compared to M-A ($p = 0.016$). All groups exhibited similar segment-related adaptations, particularly reduced stride length and increased frequency in the second uphill compared to the first.

These findings indicate that while most kinematic differences between sexes were explained by running speed, females showed a lower uphill duty factor than M-A, which may reflect more favourable muscular characteristics for propulsion at comparable speeds. Across all groups, consistent within-race adjustments in stride parameters were observed, reflecting common pacing and fatigue management strategies in competitive trail running.

4.2 Introduction

Participation in trail running has grown significantly in recent years, with a notable increase in female involvement, rising from 18% in 2013 to 26% in 2019 (ITRA). However, male athletes still represent the majority of competitors, and a consistent performance gap persists. Elite male athletes typically outperform females by 8–12% in endurance running (Besson et al., 2022; Cheuvront et al., 2005; Coast et al., 2004), mainly due to higher maximal oxygen uptake (VO_2max) values (Bassett, 2000), attributed to lower body fat and higher haematocrit levels in males (Besson et al., 2022; Sparling & Cureton, 1983). The gap appears to be even more pronounced in uphill events, where physiological constraints, including lower maximal oxygen uptake relative to body mass, higher fat-to-lean mass ratio, reduced anaerobic power associated with lower muscle mass, and a smaller proportion of fast-twitch fibers, place female athletes at a greater disadvantage (G. P. Millet et al., 2025). However, little is known about how running mechanics differ between male and female athletes with comparable performance levels, particularly in uphill running. Spatiotemporal parameters, such as contact time, step length, and step frequency, are influenced by body size and proportions more than by biological sex itself. For instance, shorter individuals tend to exhibit shorter contact times, step lengths, and higher step frequencies, so as lighter individuals generally have shorter contact times compared to heavier ones (Roche-Seruendo et al., 2019).

Understanding these biomechanical differences is particularly relevant in trail running, a sport where athletes encounter both uphill and downhill sections while navigating diverse terrains (Scheer et al., 2020). These races take place in natural environments with varying elevation, where paved surfaces are typically limited to 20–25% of the course (Scheer et al., 2020). Unlike road races, trail races lack standardization, as they involve continuous variations in terrain, technical difficulty, elevation gain, and total race distance. If and to which extent these varying race conditions affect male and female athletes differently remains unclear (Besson et al., 2022). While previous research has found no significant sex differences in running economy (Besson et al., 2022), some biomechanical variations have been observed, especially when examining running mechanics across a large range of running velocities and slopes (Almonroeder & Benson, 2016; Besson et al., 2022; Chumanov et al., 2008; García-Pinillos et al., 2020).

Additionally, in prolonged efforts in natural environments, such as trail running, athletes are known to adapt their running biomechanics over the course of the race (Vermand et al., 2022). These adaptations may indicate the onset of fatigue (Lemire, Remetter, et al., 2021) or act as protective mechanisms to prevent injuries (Giandolini et al., 2017; Lemire, Remetter, et al., 2021), ultimately influencing performance. Interestingly, sex differences in performance appear to diminish with increasing distance. For example, men's speed has been shown to decrease by 4.02% for every 10 km-effort increase, whereas the decrease in women's speed is only 3.25%, suggesting superior fatigue resistance in female athletes. Several studies have also highlighted that female runners tend to adopt a more even pacing strategy, from short-distance events such as 5 km races up to the marathon (Besson et al., 2022). These observations align with evidence indicating that women generally exhibit better endurance capacities than men, particularly in ultra-endurance events (Le Mat et al., 2023).

Research on trail running kinematics reveals how varying terrains and slopes influence stride mechanics. Uphill running demands more muscular effort and metabolic energy but shows fewer kinematic differences across performance levels (Bettega, Pellegrini, et al., 2025), though stride length and frequency may vary significantly when not normalized for running speed (Besson et al., 2023; Genitrini et al., 2023; Vermand et al., 2022). Downhill running, in contrast, presents greater biomechanical challenges, such as higher braking forces, requiring adjustments in stride length and contact time to maintain control and minimize energy loss (Björklund et al., 2019; Ehrström et al., 2018; Lemire, Hureau, et al., 2021). These adjustments are crucial for managing fatigue and preventing injuries, particularly on steep descents (Giandolini et al., 2017; Lemire, Hureau, et al., 2021). Efficient downhill running involves optimized swing leg mechanics (Genitrini et al., 2023), while reducing stride length and contact time helps minimize mechanical stress (Lemire, Hureau, et al., 2021).

Although there is a considerable amount of research on biomechanical differences between sexes, much of it has been conducted in controlled indoor settings, such as treadmill running. These studies may not fully capture the complexities of running in natural environments. Furthermore, few studies have focused on elite athletes in outdoor settings, particularly in trail running, where diverse terrains and environmental conditions play a significant role in performance. Therefore, this study analyses differences in trail running between sexes by matching female athletes with male counterparts who exhibited similar absolute and relative performance levels, with the aim to assess sex differences in running kinematics, considering both relative and absolute performance, while examining how uphill and downhill kinematic variables evolve throughout the race due to the potential effects of fatigue.

4.3 Material and Methods

4.3.1 Study design

Data collection was conducted during the Dolomyths Skyrace, a sky running competition in Italy. The race covers a 22 km course with 1,750 m of elevation gain, reaching a maximum altitude of 3,152 m. The first section of the race consists of a continuous 10 km uphill climb, followed by a 12 km descent to the finish line. The event took place in summer under dry and sunny conditions in the Italian Alps. A total of 825 athletes participated (694 men and 131 women). The winning time was 2:04:39 in the men's class and 2:24:40 in the women's class. Race times for all athletes were collected from the official timing system, including split times for the uphill and downhill sections. For the kinematic analysis, four ~15 m segments (±35 % incline/decline) were selected along the racecourse, two in the uphill section and two in the downhill section, ensuring comparable surface conditions and gradients. The choice of two segments, along the course, of both the uphill and downhill sections, aimed to capture potential kinematic variations resulting from fatigue over the course of the race. Researchers identified these segments during a prior course inspection, and athletes were video recorded as they passed through them. The study was conducted according to the ethical standards of the Helsinki Declaration. Data used for the performance analysis are publicly available, and permission to record videos was obtained from the organizing committee, as the participants had previously granted their consent to the committee, which extended this permission to third parties. Given that the data collection was observational and did not interfere with the athletes' performance or well-being, no additional ethical approval was required.

4.3.2 Subjects

Inclusion criteria for data analysis required athletes to be clearly visible in all four recording segments, to be running in each segment, and to have at least three consecutive stride cycles captured on video. All the characteristics of the groups and race times are reported in Table 7. Based on the final ranking, three groups were created. The first group included female athletes, selected using a performance index ranging from 1 to 0.85 within their gender category (all eligible female athletes - n = 15). The performance index was calculated as the ratio between an athlete's race time and the winner's race time within the same gender category, with a value of 1 corresponding to the winner's time (Fornasiero et al., 2022). This index was computed separately for both female and male categories. To compare differences in performance between males and females, two male groups were formed. The first male group was matched for relative performance (M-R) and included male athletes whose performance index range matched that of the selected female athletes. The second male group was matched for absolute performance (M-A) and consisted of male athletes with total race times closest to those of the females. Since the number of male participants was much higher, 15 athletes were selected from each male group by considering the age, to ensure an equal sample size for all the groups.

		Groups			
Mean ± SD		M-R	F	M-A	ANOVA p
ITRA Score	---	860.7 ± 36.3 *	749.3 ± 29.0	759.5 ± 66.5	<0.001
Age	years	30.5 ± 4.9	30.0 ± 4.4	29.1 ± 5.6	1.000
Race time	min	138.4 ± 7.1 *	160.1 ± 8.6	160.0 ± 8.6	<0.001
Uphill time	min	87.3 ± 4.9 *	100.4 ± 5.0	97.7 ± 5.3	<0.001
Downhill time	min	51.0 ± 2.8 *	59.7 ± 4.3	62.3 ± 6.0	<0.001

*Table 7 The table reports the characteristics of the groups for female (F), men with same relative (M-R) and absolute performance (M-A). ITRA score, age, overall race time, and split times for uphill and downhill are reported. Data are expressed as mean ± stand (SD). * significantly different from both F and M-A.*

4.3.3 Kinematics analysis

Two segments were selected in the uphill section and two in the downhill section, located at about 3.5 km (UH1), 9 km (UH2), 10.5 km (DH1), and 18 km (DH2). The selection of two uphill and two downhill segments was intended to assess potential kinematic variations due to fatigue. Each segment was between 12 to 15 meters long, with the start and end clearly marked by colored cones. To capture athletes' horizontal movement, a high-frequency camera (GH5S LUMIX, Panasonic Corp., Osaka, Japan) was positioned laterally at each segment, recording at 100 Hz and FHD quality. A smartphone mounted on a tripod recorded the frontal view of each segment, enabling identification of the athletes by their bib numbers. The following kinematic variables were measured for each segment: segment velocity (speed), stride length (SL), stride time (ST), stride frequency (SF), ground contact time (CT) and duty factor (DF = SD/CT). Kinematic variables were analyzed through frame-by-frame tracking procedures using Kinovea software (v.2023.1.2) for each segment. The foot-ground contact and toe-off frames for each stride were visually identified and used to calculate the relevant metrics. Stride length was calculated from segment velocity and stride time.

4.3.4 Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test. A one-way ANOVA was conducted to assess differences among the three groups in several parameters, including ITRA points, age, overall race time, uphill time, downhill time, as well as the percentage of time spent in the uphill and downhill sections. When significant main effects were found, post-hoc comparisons were performed to identify pairwise differences between groups. Effect sizes were calculated using eta squared (η^2) for the ANOVA and Cohen's d (d) for the post-hoc comparisons.

For the kinematic analysis, separate linear mixed models were conducted for the uphill and downhill sections. Each model was executed for every kinematic variable, with 'group' (M-R, F, and M-A) and 'segment' (segment 1 vs. segment 2) as fixed factors, and 'subject' included as a cluster variable (random effect) to account for repeated measures. When significant main effects or interactions were identified, post-hoc pairwise comparisons were performed with Bonferroni correction. Effect sizes were calculated using Cohen's d for post-hoc comparisons.

Initially, all models were run without covariates. Subsequently, when a significant group effect in running speed was observed, speed was added as a covariate to assess its influence on the kinematic variables and to control for potential confounding effects.

For all statistical analyses, a significance level of $p < 0.05$ was established. Cohen's d values of 0.2, 0.5, and 0.8 were considered to indicate small, medium, and large effects, respectively. Effect sizes for the ANOVA were evaluated using eta squared, with thresholds of 0.01, 0.06, and 0.14 interpreted as small, medium, and large effects, respectively.

4.4 Results

4.4.1 Performance analysis

Overall race time was significantly different between group ($p < 0.001$; $\eta^2 = 0.63$) and lower in M-R (138.4 ± 7.1 min) compared to F (160.1 ± 8.6 min) and M-A (160.0 ± 8.6 min). UH times were 87.3 ± 4.9 min for M-R, 100.4 ± 5.0 min for F, and 97.7 ± 5.3 min for M-A ($p < 0.001$; $\eta^2 = 0.57$), while DH times were 51.0 ± 2.8 min, 59.7 ± 4.3 min, and 62.3 ± 6.0 min for M-R, F, and M-A respectively ($p < 0.001$; $\eta^2 = 0.54$). M-R were faster than F (UH: $p < 0.001$; $d = 2.6$ – DH: $p < 0.001$; $d = 1.9$) and M-A (UH: $p < 0.001$; $d = 2.1$ – DH: $p < 0.001$; $d = 2.5$) in both UH and DH sections, while no statistical differences between M-A and F were found in uphill or downhill time ($p > 0.05$). In Figure 12b, the performance index of individual subjects was plotted.

The percentage of time spent in UH and DH was different between groups ($p = 0.005$; $\eta^2 = 0.23$). Percentage times were similar between M-R and F (63.1% vs. 62.7% for UH and 36.9% vs. 37.3% for DH, respectively). In contrast, a significant difference was found between F and M-A, with M-A spending a lower percentage of time during UH (61.1% vs 62.7%, $p = 0.03$; $d = 0.96$) and then a higher percentage during downhill (38.9% vs 37.3%, $p = 0.03$; $d = 0.96$) with respect to F (Fig 12a).

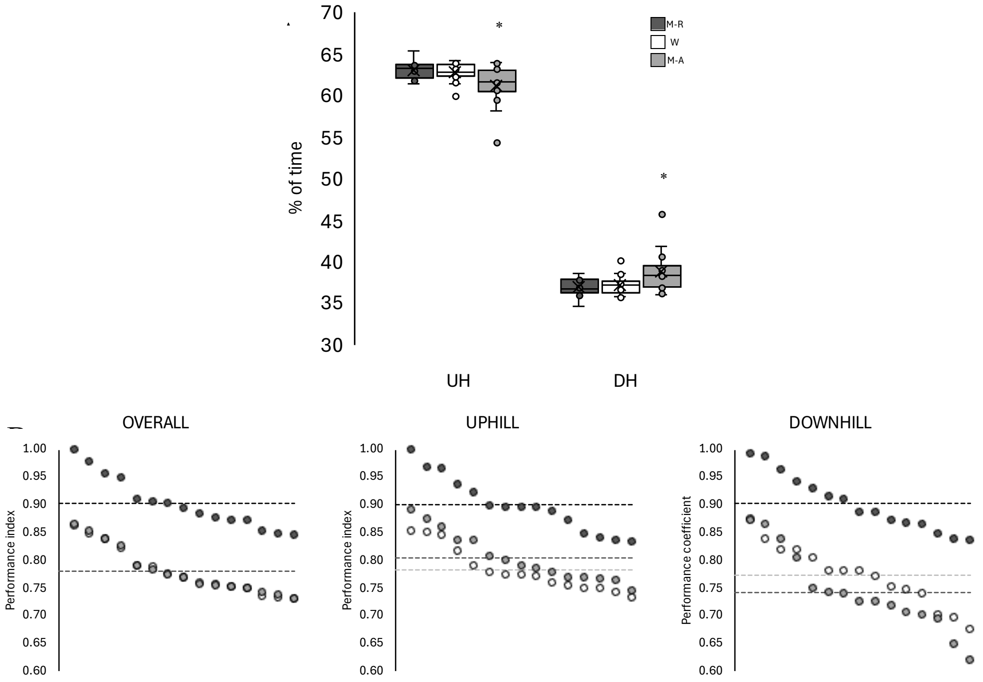


Figure 12 (a) Box plot showing the distribution of individual percentage time spent in UH and DH for each group. (b) The performance index (with 1 indicating the best overall time) of individual subjects was plotted for overall, uphill (UH), and downhill (DH) times. *$p < 0.05$.

4.4.2 Uphill kinematics

Significant group differences were identified for running speed ($p < 0.001$), with M-R running faster than both F and M-A (both $p < 0.001$). Regarding contact time, a group effect was found ($p = 0.008$), with shorter contact times in M-R compared to M-A ($p = 0.003$) and F ($p = 0.021$). Stride time showed a significant segment effect ($p = 0.003$), with shorter durations in UH2 compared to UH1 ($p = 0.003$). Stride frequency increased significantly between segments ($p < 0.001$), with higher values in UH2 ($p < 0.001$). Stride length revealed both a group ($p < 0.001$) and segment effect ($p < 0.001$). Post-hoc comparisons showed longer stride length in M-R compared to both F and M-A (both $p < 0.001$), and a reduction from UH1 to UH2 across all groups ($p < 0.001$). Duty factor exhibited main effects of group ($p < 0.001$) and segment ($p = 0.007$), as well as a group-by-segment interaction ($p = 0.036$). Post-hoc analysis showed a higher duty factor in F and M-A compared to M-R ($p = 0.010$ and $p < 0.001$, respectively), and significant reductions from UH1 to UH2 for M-R ($p = 0.010$) and F ($p = 0.008$), while no significant differences were found in M-A between the segments ($p = 0.582$).

Given the significant differences in running speed among groups, speed was subsequently included as a covariate in the statistical models to account for its influence. The covariate had a significant effect on all kinematic variables ($p < 0.001$), and its inclusion attenuated or eliminated some of the previously observed group differences (Tab. 8).

After controlling for speed, no significant effects remained for contact time. Stride time and stride frequency still differed significantly between segments (both $p < 0.001$), with lower stride time and higher stride frequency in UH2. Stride length remained significantly affected by segment ($p < 0.001$), with reduced values in UH2. Duty factor showed a group effect ($p = 0.036$) and a segment effect ($p = 0.031$). Post-hoc comparisons indicated a significant difference between F and M-A ($p = 0.016$) in this parameter, and a reduction in duty factor between UH1 and UH2 ($p = 0.031$). Box plots of uphill running kinematics results are presented in Fig 13.

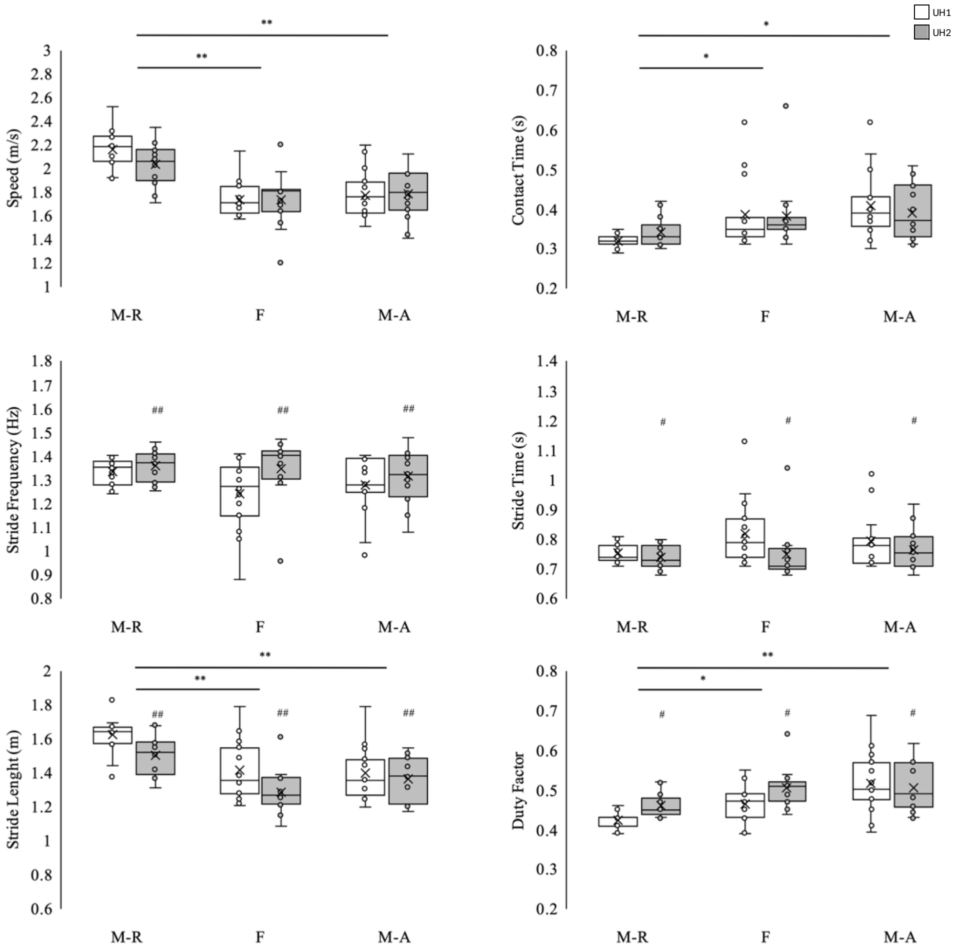


*Figure 13 Box plots representing uphill running kinematics results for all measured parameters (speed, CT, SL, SF, ST, and duty factor) across all analyzed groups (W, M-A, and M-R) for both UH segments (first segment: white plot; second segment: grey plot). # (p<0.05) and ## (p<0.001) represent differences between segments, * (p<0.05) and ** (p<0.001) represent post hoc results of differences between groups.*

Kinematic Variable	Fixed Effect	F (df1, df2)	p-value
Contact Time	Group	F (2, 47.9) = 1.069	0.351
	Segment	F (1, 42.7) = 0.850	0.362
	Group * Segment	F (2, 42.7) = 0.271	0.764
	Speed (covariate)	F (1, 79.2) = 33.417	<0.001
Stride Time	Group	F (2, 48.2) = 0.381	0.381
	Segment	F (1, 43.0) = 17.126	<0.001
	Group * Segment	F (2, 43.1) = 1.651	0.204
	Speed (covariate)	F (1, 23.425) = 23.425	<0.001
Stride Frequency	Group	F (2, 48.7) = 1.220	0.304
	Segment	F (1, 43.0) = 23.250	<0.001
	Group * Segment	F (2, 43.0) = 2.310	0.111
	Speed (covariate)	F (1, 81.7) = 26.270	<0.001
Stride Length	Group	F (2, 48.2) = 0.780	0.464
	Segment	F (1, 42.9) = 20.252	<0.001
	Group * Segment	F (2, 43.0) = 2.235	0.119
	Speed (covariate)	F (1, 80.0) = 44.886	<0.001
Duty Factor	Group	F (2, 46.4) = 3.580	0.036
	Segment	F (1, 42.4) = 4.980	0.031
	Group * Segment	F (2, 42.5) = 2.740	0.076
	Speed (covariate)	F (1, 71.3) = 22.270	<0.001

Table 8 Results of linear mixed models for uphill running kinematics with running speed as covariate.

4.4.3 Downhill kinematics

Significant effects of group ($p < 0.001$) and segment ($p < 0.001$) were found for running speed. Post-hoc comparisons indicated that M-R ran faster than both F and M-A (both $p < 0.001$), and all groups increased their speed in DH2 compared to DH1 ($p < 0.001$). Stride time and stride frequency showed significant segment effects (both $p < 0.001$). Stride time increased in DH2 ($p < 0.001$), while stride frequency decreased ($p < 0.001$) across all groups. Stride length was significantly affected by both groups ($p < 0.001$) and segment ($p < 0.001$). M-R exhibited longer stride length than both F and M-A (both $p < 0.001$), and all groups increased their stride length in DH2 compared to DH1 ($p < 0.001$). Duty factor showed main effects of group ($p = 0.003$) and segment ($p < 0.001$). Post-hoc analysis revealed lower duty factor in M-R compared to M-A ($p = 0.001$) and F ($p = 0.050$), and a reduction from DH1 to DH2 across all groups ($p < 0.001$). No significant effects were found for contact time (all $p > 0.05$).

Given the significant differences in running speed among groups, speed was subsequently included as a covariate in the statistical models to account for its influence. The covariate showed a significant effect for stride length ($p < 0.001$), contact time ($p = 0.014$), and duty factor ($p < 0.001$), while it did not reach significance for stride time ($p = 0.102$) and stride frequency ($p = 0.120$) (Tab. 9). After controlling for speed, no significant effects remained for contact time, stride time, or stride frequency. Stride length remained significantly associated with the covariate but showed no longer group or segment effects. Duty factor maintained a significant segment effect ($p = 0.013$), with lower values in DH2 compared to DH1 ($p = 0.014$), while group and interaction effects were no longer significant.

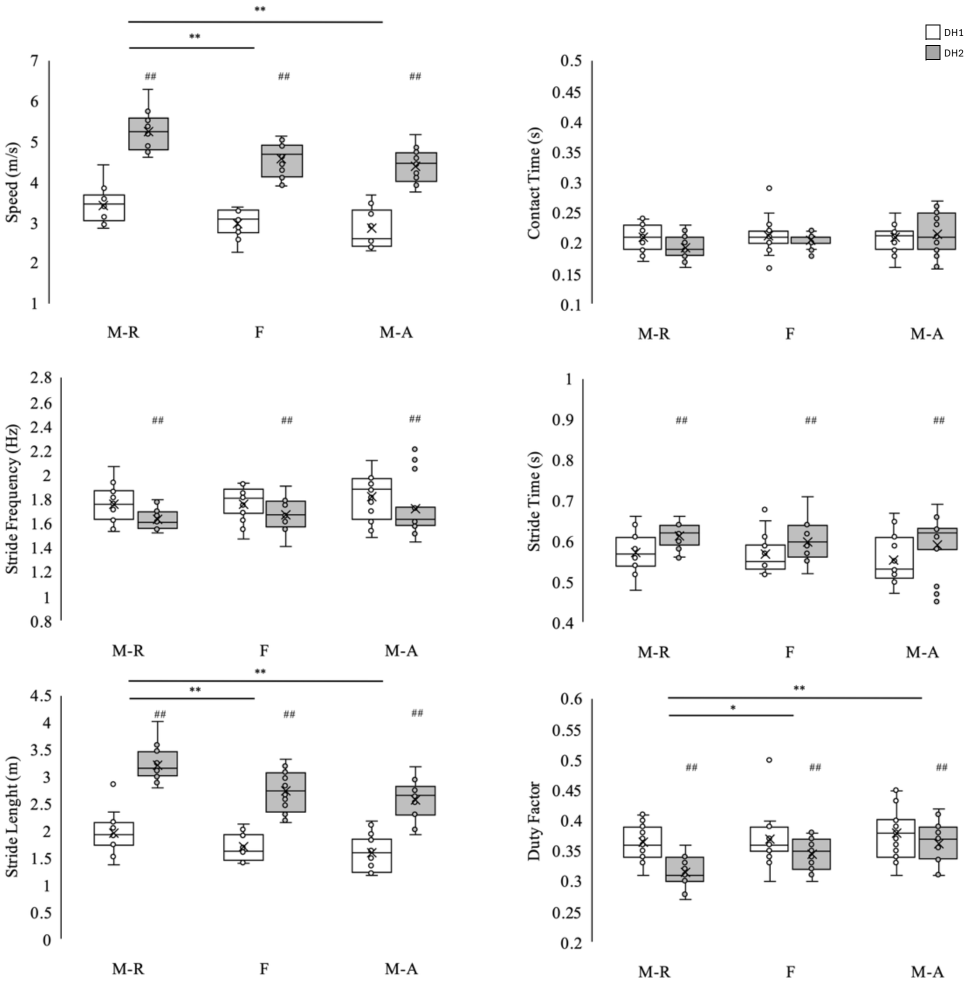


*Figure 14 Box plots represent downhill running kinematics results for all measured parameters (speed, CT, SL, SF, ST, and duty factor) across all analyzed groups (W, M-A, and M-R) for both DH segments. # (p<0.05) and ## (p<0.001) represent differences between segments, * (p<0.05) and ** (p<0.001) represent post hoc results of differences between groups.*

Kinematic Variable	**Fixed Effect**	**F (df1, df2)**	**p-value**
Contact Time	Group	F (2, 46.5) = 0.037	0.964
	Segment	F (1, 82.9) = 3.075	0.083
	Group * Segment	F (2, 41.5) = 1.025	0.368
	Speed (covariate)	F (1, 77.8) = 6.281	0.014
Stride Time	Group	F (2, 48.2) = 0.381	0.826
	Segment	F (1, 43.0) = 17.126	0.997
	Group * Segment	F (2, 43.1) = 1.651	0.934
	Speed (covariate)	F (1, 23.425) = 23.425	0.102
Stride Frequency	Group	F (2, 48.6) = 0.355	0.703
	Segment	F (1, 78.5) = 0.000	0.997
	Group * Segment	F (2, 42.2) = 0.085	0.919
	Speed (covariate)	F (1, 81.7) = 2.475	0.120
Stride Length	Group	F (2, 48.4) = 0.178	0.838
	Segment	F (1, 80.1) = 0.021	0.883
	Group * Segment	F (2, 42.1) = 0.398	0.674
	Speed (covariate)	F (1, 82.7) = 162.836	<0.001
Duty Factor	Group	F (2, 83.0) = 0.445	0.643
	Segment	F (1, 83.0) = 6.501	0.013
	Group * Segment	F (2, 83.0) = 1.169	0.316
	Speed (covariate)	F (1, 83.0) = 26.560	<0.001

Table 9 Results of linear mixed models for downhill running kinematics with running speed as covariate.

4.5 Discussion

This study aimed to analyse trail running performance differences between females and males matched for the same absolute or relative performance, with a specific focus on running kinematics in uphill and downhill sections. Performance analysis showed that men matched for relative performance completed the race faster, performing better in both uphill and downhill sections. In contrast, when matching was based on absolute performance, women allocated a greater percentage of their race time to the uphill segment, as they were slower in this section, but compensated with faster downhill running.

Kinematic analysis revealed that in uphill running, men matched for relative performance ran at a faster speed, which was associated with longer stride length, shorter contact time, and a lower duty factor. All groups adjusted running kinematics similarly across the two uphill segments, with a reduction in stride length and an increase in stride frequency in the second segment. When controlling for running speed, most group differences were attenuated or disappeared, suggesting that speed was a major determinant of the observed kinematic patterns. Segment-related changes, however, remained consistent. In downhill running, males matched for relative performance, who were then faster, with higher speed driving differences in stride length and duty factor. Across both downhill segments, all groups increased speed by lengthening their stride and reducing stride frequency, while contact-related variables showed smaller or no changes. When speed was included as a covariate, group differences in kinematics were no longer significant, confirming the predominant role of running speed in shaping downhill running mechanics. Finally, when men and women were matched for absolute performance, they exhibited similar kinematic patterns and segment-related adaptations in both uphill and downhill running, with no significant sex differences.

4.5.1 Performance analysis

Performance in trail running requires further investigation, as environmental conditions and varying race profiles complicate the analysis, particularly regarding sex differences. Millet et al. found sex differences in uphill performance ranging from 18% to 28% (G. P. Millet et al., 2025), contrasting with the 8% to 12% gap observed in flat endurance running (Besson et al., 2022). Our results show a 15.7% performance gap between elite male and female athletes (W vs. M-R) for overall race time, with a 15% difference in the uphill and 17% in the downhill sections. As the M-R group was matched to the female group in relative performance, the performance gap was even larger when comparing females with the top 15 male athletes, reaching 24.7% for overall race time, 24% for the uphill, and 25.7% for the downhill section. These findings align with Millet et al.'s, with slightly lower differences (15% for uphill and 17% for downhill), confirming Millet's theory of higher sex differences in performance gap for uphill rather than flat endurance performance.

Of note, although F and M-A were matched for similar overall race performance, the latter showed a lower %time in the uphill and a higher percentage in the downhill segments. Millet et al. suggested that gender differences in uphill performance may be due to the lower lean-to-fat mass ratio and lower percentage of fast-twitch fibers in women (G. P. Millet et al., 2025). This could explain why M-A outperforms women uphill, where neuromuscular and maximal strength are crucial (Lemire, Hureau, et al., 2021). In contrast, women run faster in the downhill sections. It is reasonable that greater training volume in F compared to M-A, especially in downhill conditions, may be key to improving downhill performance, as previous studies have indicated (Kay, 2014).

4.5.2 Uphill running kinematic

The comparison between F and M-R revealed significant differences in three of the analysed kinematic variables, with SF being the only exception ($p > 0.05$). The greater SL in M-R during the uphill segments (+12.8 % in UH1 and +14.5 % in UH2 compared to F) is likely attributed to higher propulsive forces generated by men, a result of their greater muscle mass both in absolute terms and relative to body weight (A. J. Bassett et al., 2020; S. K. Hunter et al., 2023). Given that running speed is the product of SF and SL, and SF did not differ significantly between sexes, the higher speed observed in M-R (+19.6% in UH1 and +15% in UH2 compared to F) is directly linked to their longer SL. Additionally, females exhibited a higher duty factor (F: 0.46 ± 0.05 and 0.50 ± 0.05; M-R: 0.42 ± 0.02 and 0.46 ± 0.03 for UH1 and UH2, respectively), likely due to longer ground CT.

When speed was included as a covariate, its effect on running kinematics was seen to be substantial ($p<0.001$), and the previously observed significant differences between sexes disappeared. This finding suggests that the observed sex differences in stride length, contact time, and duty factor are largely explained by variations in running speed rather than intrinsic biomechanical disparities in trail running.

Uphill running requires greater mechanical work from the lower limbs (Vernillo et al., 2017), and female athletes may compensate lower overall muscle mass (A. J. Bassett et al., 2020; S. K. Hunter et al., 2023) by increasing ground contact time to generate the necessary propulsion. This difference in duty factor further highlights the advantage of higher muscle mass in men, enabling them to apply force more quickly and produce more powerful movements.

No significant differences were found between F and M-A. Their comparable running speed resulted in similar kinematics, despite potential differences in lean-to-fat mass ratio between male and female athletes. Interestingly, females showed a slightly lower duty factor compared to M-A, meaning they spent less relative time in ground contact at the same speed. This may suggest more favourable muscular characteristics (e.g., greater reliance on fast-twitch fibers or a more similar lean-to-fat mass ratio to higher-level male athletes), allowing them to achieve the required propulsion with shorter contact times. This finding further highlights the primary influence of sustainable speed, rather than sex, on uphill running kinematics (Padulo et al., 2012; Vernillo et al., 2020). Previous studies support the idea that speed plays a crucial role in determining running kinematics rather than sex alone. Garcia-Pinillos et al. reported no significant kinematic differences between amateur and elite runners when running at the same speed (García-Pinillos et al., 2019). Similarly, Besson et al. found no biomechanical differences between elite and experienced trail runners during uphill treadmill running, although elite athletes exhibited a lower energy cost at the same speed (Besson et al., 2023). These findings align with our results, reinforcing the notion that when male and female athletes run at comparable speeds, their running mechanics are also similar, despite potential physiological differences. Overall, this suggests that running speed, rather than sex, is the primary determinant of uphill running kinematics.

In examining how kinematic variables evolve throughout the uphill segments due to the potential effects of fatigue, we observed that all athlete groups exhibited a significant increase in SF, along with a reduction in SL and duty factor from UH1 to UH2. These changes likely indicate accumulated fatigue as the uphill race progressed (García-Pinillos et al., 2020).

Given that running speed remained relatively unchanged between the two uphill segments, it can be assumed that both male and female athletes adopted a similar strategy, compensating for fatigue by increasing stride frequency to sustain their pace. This adaptive response is consistent with previous studies showing that fatigue during running leads to spatiotemporal adjustments such as increased step frequency and reduced stride length, rather than changes in joint kinematics (Degache et al., 2016; Giovanelli et al., 2016; Trowell et al., 2024). As highlighted by Ehrström et al., these findings emphasize the critical role of muscle strength and endurance in sustaining efficient movement patterns during prolonged uphill efforts (Ehrström et al., 2018). Consequently, targeted lower-limb strength and fatigue-resistance training may be beneficial for improving uphill trail running performance, regardless of sex.

4.5.3 Downhill running kinematic

As observed in the uphill segments, differences between F and M-R were also evident during downhill running, with M-R displaying higher speeds, longer SL, and lower duty factor than F. The greater SL in M-R can be attributed to their higher muscle mass (A. J. Bassett et al., 2020; S. K. Hunter et al., 2023), which likely contributes to greater lower limb strength. Strength, along with musculotendinous stiffness, plays a crucial role in generating both negative and positive mechanical work during the stance phase, enabling athletes to sustain longer strides and higher speeds (Lemire, Remetter, et al., 2021). Since SF did not differ between sexes, the longer SL observed in M-R directly translated into higher running speeds. As for the uphill analysis, when speed was included as a covariate, the previously observed differences in SL and duty factor between sexes disappeared, showing that variations in kinematic parameters between sexes were primarily driven by running speed, rather than sex, also in the downhill section (Besson et al., 2022; García-Pinillos et al., 2020). Between F and M-A, no significant differences were found in running kinematics during DH segments, despite potential differences in body composition and muscle characteristics between sexes.

The analysis of downhill running kinematics throughout the race revealed significant changes in all analysed variables between DH1 and DH2 for both F vs. M-R and F vs. M-

A, all following the same trend. As the race progressed, running speed and SL increased in the second segment, while SF and duty factor decreased. These adaptations may reflect either the constraining effect of accumulated fatigue from the preceding uphill, which could have limited downhill mechanics in DH1, or a deliberate strategy to increase speed in the final part of the race (DH2) (Genitrini et al., 2022), or a combination of both. The higher SF and duty factor observed in DH1 support the idea of greater influence from prior fatigue, whereas their reduction in DH2 may indicate partial recovery and a transition towards a more efficient DH running pattern.

When running at the same speed (F vs. M-A), no significant differences were observed in uphill or downhill kinematic parameters. The only exception was the duty factor, which was slightly lower in females, suggesting shorter relative ground contact times at the same speed. This may indicate more favourable muscular characteristics to achieve propulsion in uphill running. Differences between F and M-R, on the other hand, were primarily due to variations in running speed rather than intrinsic biomechanical factors, as previously shown in high-level male trail running athletes (Bettega, Pellegrini, et al., 2025). Moreover, all groups adjusted their running patterns in a similar manner throughout the race, suggesting that changes in kinematics were largely dictated by pacing strategy and fatigue management rather than sex-related differences. These findings are consistent with those reported by Nardello et al., who observed that both men and women adjusted their step kinematics in a similar way across different slopes (±7 %) during a half-marathon on the road (Nardello et al., 2021). But in contrast to previous studies on sex differences in road half marathon (Nardello et al., 2021), sprinting (Montalvo et al., 2025) or on treadmill running (Besson et al., 2023), which reported significant differences in running kinematics between men and women, we did not observe any such differences in our trail running context. This suggests that sex-based performance differences in trail running may not be driven by kinematic factors, but rather by the inability to sustain a given running speed due to other well-established physiological differences, such as maximal oxygen-carrying capacity or body fat/lean mass ratio. Therefore, further studies accounting for these physiological determinants are warranted to verify and expand on these findings. The hypothetical greater fatigue resistance in women (A. J. Bassett et al., 2020; Joyner, 2017; Le Mat et al., 2023) does not seem to be reflected in different running kinematics during a trail running race of this distance, whether men are similarly trained or less trained. However, slower male athletes tend to be slower in the downhill sections, suggesting that other factors, not addressed in this study, may support the theory of reduced fatigue in women with similar overall performance time, even in races of this length.

4.6 Conclusions

This study highlights significant differences between male and female athletes in performance and running kinematics. When matched for relative performance, males outperformed females in terms of race time, completing the race faster. However, both groups exhibited the same time distribution between uphill and downhill sections. When matched for absolute performance, despite similar overall race times, the time distribution differed between genders. Overall, males achieved faster times in the uphill section, suggesting an advantage in sustaining higher speeds. However, in the uphill segments analysed, running speed was comparable between females and M-A, and females exhibited a slightly lower duty factor. This indicates shorter relative ground contact times at the same speed, which may reflect favourable muscular characteristics for propulsion. Taken together, these findings suggest that while male athletes display a performance advantage in the uphill section as a whole, female athletes may rely on efficient muscular strategies when running at comparable speeds, whereas downhill abilities and greater exposure to downhill running could be more decisive for their performance in the descent.

Kinematic differences were observed between F and M-R, with males faster in both UH and DH. In UH, males showed longer SL, shorter CT, and a shorter duty factor. In DH, they were faster with differences in SL and duty factor, with these differences driven by actual speed performed by athletes, in both uphill and downhill segments. Both sexes demonstrated similar kinematic adjustments throughout the race: in the uphill sections, there was an increase in stride frequency and a decrease in stride length, while in the downhill sections, speed and stride length increased, accompanied by a reduction in stride frequency. These changes in kinematics were likely influenced by factors such as fatigue and/or pacing strategies.

4.7 Limitation & perspectives

This study has some limitations. The small sample size limits the generalizability of the findings, partly due to the lower female participation in such races, which led to the exclusion of many male athletes to ensure balanced groups. Analysing only one race further restricts the applicability of results, as course characteristics may influence strategy and performance. Additionally, the study focused solely on spatiotemporal parameters. While integrating joint kinematics, physiological and anthropometric data could provide deeper insights, this choice was intentional to maintain a practical and race-specific approach, ensuring the analysis remained applicable to real-world conditions and the performance of high-level international athletes. Future research should include multiple races, biomechanical and physiological analyses, and explore how sex differences evolve in longer races, considering potential female advantages in fatigue resistance and energy efficiency.

4.7.1 Perspectives

This study investigated sex differences in trail running performance and kinematics in a real-world race setting, comparing elite female athletes with males matched for either relative or absolute performance. By analysing uphill and downhill running across multiple course segments, the study aimed to provide an understanding of how sex and performance level influence movement patterns and effort distribution in competitive trail running. Future research should build on these findings by integrating physiological, neuromuscular, and biomechanical assessments to better understand the mechanisms underpinning these differences. Inclusion of markers such as VO_2max, and muscle strength, along with fatigue indices, could clarify how muscular characteristics interact with kinematic patterns to influence performance in both uphill and downhill sections.

Moreover, examining how athletes of different sexes and performance levels adapt pacing strategies across varied terrain may reveal potential approaches for optimizing training. In particular, targeted interventions aimed at improving muscular strength or enhancing downhill performance could help reduce sector-specific time losses. Combining real-world race analyses with laboratory-based assessments would allow a more comprehensive understanding of the interplay between sex, muscular characteristics, and kinematics, ultimately informing tailored training programs for competitive trail runners.

Chapter 5 – Effects of downhill stride frequency on neuromuscular fatigue, uphill performance, and durability in trail runners

5.1 Abstract

Trail running unfolds over continuously changing terrain, where athletes must adapt their biomechanics, pacing, and physiological responses to alternating uphill and downhill gradients. Uphill sections challenge aerobic capacity and local muscular endurance, whereas steep descents impose substantial eccentric loading, increasing neuromuscular strain and potentially compromising performance in subsequent climbs. Despite the relevance of downhill-induced fatigue for race outcomes, little is known about how specific mechanical strategies, such as stride-frequency modulation, affect fatigue development, neuromuscular function, and durability in trail runners.

This project examined the combined effects of prolonged uphill (UH) and downhill (DH) running on neuromuscular, biomechanical, and metabolic responses, with a focus on whether increasing stride frequency during downhill running could mitigate muscle damage or preserve subsequent uphill performance.

Twelve well-trained and elite male trail runners completed a controlled laboratory protocol including an uphill incremental test, a fresh 2.5-km uphill time trial (15% incline - TT_{fresh}), and two experimental sessions consisting of a 20-min UH run (15%) within the heavy-intensity domain (at the running speed corresponding to 50% of the difference between speed at VT_1 and speed at VT_2), a 15-min −20% DH bout at speed corresponding to 50% of VO_2max, and a final uphill TT (TT_{PSF} and TT_{ISF}). DH running was performed either at preferred stride frequency (PSF) or with a +10% increase (ISF). Throughout all sessions, oxygen consumption, ventilation, plantar forces, step characteristics and surface electromyography were recorded. Neuromuscular function (MVC and RTD) was assessed before the session, immediately after the downhill bout, and at the end of each experimental trial. Muscle damage markers were measured before and 24 h after each trial.

ISF reduced impact forces and loading rate but substantially increased metabolic cost (+5.5%) during the DH section. Despite these differences in mechanical and physiological load, ISF and PSF elicited similar reductions in maximal voluntary torque (pre vs postDH: ~11–12%), overall rapid force production capacity, and EMG-derived indicators of fatigue, with no differences in exercise-induced muscle damage. However, UH performance was more impaired after ISF than PSF (+13.6% vs +9.9%; $p = 0.026$),

accompanied by higher ventilatory and cardiovascular cost per unit of speed (increased VE/speed, Rf/speed, and HR/speed). Blood lactate concentrations were markedly lower in both TT_{PSF} and TT_{ISF} compared to TT_{fresh} (-32.7%, $p < 0.001$), suggesting reduced glycolytic contribution due to the preceding eccentric load.

TT_{fresh} performance was strongly predicted by VO_2max and local knee-extensor endurance, while peak rapid force development further improved the predictive model. Whereas none of the traditional physiological or neuromuscular markers explained individual differences in fatigue-induced performance decline. Instead, cardiometabolic decoupling, captured through HR-, VE-, and Rf-to-speed ratios, emerged as the strongest correlate of durability, supporting the interpretation of durability as an independent performance trait not fully captured by standard laboratory assessments.

In summary, 10% increased stride frequency during steep DH running alters the balance between mechanical impact and energetic cost but it seemed not a protection against muscle damage or neuromuscular fatigue and ultimately worsens subsequent UH performance. These findings highlight the importance of managing internal workload during prolonged descents and point toward cardiometabolic decoupling as a practical and sensitive indicator of fatigue resilience in trail runners.

5.2 Introduction

Over the past two decades, trail running has grown rapidly, with competitions typically held on natural, off-road terrain and characterized by substantial positive and negative elevation gain. According to the International Trail Running Association, events are classified based on distance and elevation profile, but most races share an alternating sequence of uphill and downhill sections that impose distinct physiological and biomechanical demands on the athlete (Hoffman et al., 2010; ITRA; Scheer et al., 2020).

Among the multiple factors influencing trail running performance, downhill running represents one of the most demanding components due to its high biomechanical stress (Bontemps et al., 2020; Brancaccio et al., 2007; Buford et al., 2009; Eston et al., 1996; Peake et al., 2005). During downhill running, particularly high impact forces are generated (Giandolini, Horvais, et al., 2016; Giandolini, Vernillo, et al., 2016), which can lead to substantial eccentric loading of the lower limbs, especially the quadriceps. This eccentric stress contributes to muscle damage, neuromuscular fatigue, and impairments in maximal voluntary force production and rate of force development (RFD) (Coratella et al., 2024; Pokora et al., 2014; Varesco et al., 2022). The adoption of longer stride lengths, commonly used to maintain speed during descents (Bettega, Pellegrini, et al., 2025), further increases these impact forces compared with shorter strides at the same velocity (Baggaley et al., 2020; Eston et al., 2000; Rowlands et al., 2001).

Previous research investigating fatigue following downhill running has primarily focused on the decline in maximal voluntary isometric contraction (MVC) force (Bontemps et al., 2020; Giandolini, Horvais, et al., 2016). However, growing evidence suggests that the ability to generate force rapidly, quantified as RFD, represents an equally relevant functional marker of neuromuscular fatigue (D’Emanuele et al., 2021; Maffiuletti et al., 2016). Downhill running has been shown to impair both maximal voluntary force and the late phase of RFD, reflecting the combined influence of structural muscle damage and altered neural drive (Aagaard et al., 2002; Boccia et al., 2017; Coratella et al., 2024; Varesco et al., 2022).

In longer events, such as ultra trail races, this muscle damage has been identified as one of the main limiting factors of performance (Tiller & Millet, 2025). Moreover, many racecourses feature a downhill segment immediately followed by an uphill section, making the subsequent effort even more demanding, increasing both physiological and neuromuscular strain tremendously. To date, only a few studies have examined the effects of stride frequency modulation during downhill running (Eston et al., 2000; Giandolini, Vernillo, et al., 2016; Rowlands et al., 2001; Schubert et al., 2014; Vincent et al., 2019), reporting contrasting results and often using intermittent protocols that are not fully representative of real race conditions. Furthermore, no study has yet adopted an integrated approach considering both the prior uphill-induced fatigue and the subsequent uphill performance following a continuous downhill bout, two key elements for understanding the true impact of downhill running on trail running performance.

Therefore, the main aim of this study was to investigate whether manipulating running biomechanics during downhill running, specifically through stride frequency modulation, can reduce muscle damage and enhance subsequent uphill performance in well-trained and elite trail runners. A secondary aim was to identify the physiological and neuromuscular factors associated with greater durability, defined as a smaller decline in performance (Δ time) during the uphill time trial following a preceding bout of uphill and downhill running.

5.3 Material and Methods

5.3.1 Participants

We enrolled 12 experienced male trail runners (age: 28.9 ± 5.6 years; body mass: 69.0 ± 6.4 kg; height: 177.6 ± 4.5 cm), ranging from well-trained to elite level. All participants had prior experience with downhill running to minimize potential adaptation effects between session 3 and session 4. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the University of Verona (protocol #25/2025). Group characteristics are presented in Table 10.

n = 12		Mean		SD
Age	*years*	28.92	±	5.58
Weight	*kg*	68.98	±	6.40
Height	*cm*	177.58	±	4.52
BMI	*kg/m^2*	21.84	±	1.42
$FAT_{\%}$	*%*	12.76	±	1.95
VO2max	*mL/min/Kg*	71.59	±	4.49
TR years	*years*	6.58	±	3.82
Training volume	*h/week*	10.33	±	3.26
Weekly D+	*m*	2016.67	±	1750.76

Table 10 Descriptive characteristics of the participants (n = 12). Values are presented as mean ± SD. TR years, years of trail-running experience; Weekly D+, average weekly positive elevation gain.

5.3.2 Experimental design

Participants completed four laboratory sessions over a period of approximately 2.5 weeks. Each session was separated by sufficient recovery to prevent residual fatigue (≥48 h between sessions 1–2 and 2–3, and 7 days between sessions 3 and 4). All testing was conducted under standardized laboratory conditions (temperature 19 ± 0.5 °C).

The study consisted of one baseline assessment, one performance test, and two experimental trials performed under distinct downhill cadence conditions (preferred (PSF) vs. +10% (ISF)) (Eston et al., 1996, 2000; Rowlands et al., 2001; Vincent et al., 2019).

Session 1 – Baseline assessment and familiarization

During the first visit, participants received a detailed explanation of the study procedures and completed anthropometric and body composition assessments.

Subsequently, after a standardized warm-up comprising 10 min of uphill running and a brief set of progressive submaximal isometric contractions of the right knee extensors (described in detail below), participants performed isometric contractions of the right knee extensors to determine maximal voluntary contraction (MVC), rate of force development (RFD), and local endurance (LE).

Participants then completed an uphill incremental test (15% incline) performed until volitional exhaustion to determine maximal oxygen consumption (VO_2max) and ventilatory thresholds (VT_1 and VT_2).

The test began with 3 min at 4 $km \cdot h^{-1}$, after which the speed increased by 0.5 $km \cdot h^{-1}$ every minute until exhaustion. The running speed corresponding to 50% ($s_{50\%}$) of the difference between speed at VT_1 (sVT_1) and speed at VT_2 (sVT_2) was subsequently used for the submaximal uphill bouts in the experimental trials.

After adequate recovery, participants were familiarized with the self-paced treadmill system used in the following sessions. The treadmill (Rodby RL3500Ex2500, Rodby Innovation AB, Vänge, Sweden) was equipped with a stereoscopic motion sensor (RealSense D415, Intel Corporation, Santa Clara, USA) that adjusted belt speed according to the runner's position, allowing intuitive speed regulation during the time-trial protocols. The front-mounted camera continuously tracked the runner's position along the belt, and when the runner moved forward or backward relative to a predefined central area, the treadmill automatically accelerated or decelerated to maintain natural pacing behaviour, thereby mimicking outdoor running conditions.

Session 2 – Uphill time trial (TT_{fresh}) and downhill intensity determination

Following a standardized 15-min warm-up, participants completed a 2.5-km uphill time trial (TT) at a 15% incline. During the TT, runners could freely regulate their pace through the optical system previously described.

To enhance pacing control and ecological validity, real-time visual feedback was projected on the wall in front of the participants. The projection displayed:

- A live side-view video of the runner's position on the treadmill, including two horizontal lines marking the central "neutral zone," where treadmill speed remained constant, and the forward/backward zones that triggered gradual speed increases or decreases.
- A dynamic elevation profile of the 2.5-km course, showing the distance covered, elevation gain, and a moving marker progressing along the profile in real time according to the runner's instantaneous speed.

This visual setup allowed participants to self-pace effectively and optimize their performance while maintaining full awareness of their progress throughout the trial.

After recovery, participants performed three 3-min downhill runs at a −20% grade and at different velocities (ranging from 12 to 20 $km \cdot h^{-1}$, depending on individual performance level). From the linear relationship between running velocity and oxygen uptake, the individual downhill velocity corresponding to 50% of the previously measured VO_2max was determined (s_{DH}). Participants then ran for 2 min at this individualized downhill velocity, during which their preferred step frequency (PSF) was calculated as the average step frequency recorded during the final minute of running.

Sessions 3 and 4 – Experimental trials

Both experimental sessions followed the same structure, differing only in the prescribed step cadence during the downhill bout: PSF (as measured in Session 2) or +10% (ISF). The order of the two cadence conditions was counterbalanced across participants.

Each session consisted of the following sequence:

- Resting blood sampling for analysis of markers of exercise-induced muscle damage (EIMD).
- Standardized warm-up: 10 min total, including 3 min at 5 $km \cdot h^{-1}$ (10% incline), 3 min at 6 $km \cdot h^{-1}$ (10%), and 4 min at 7 $km \cdot h^{-1}$ (15%).
- Pre-exercise neuromuscular assessment, RFD and MVC, separated by 1 min of recovery.
- Uphill run: 20 min at 15% grade, at $s_{50\%}$.
- Downhill run: 15 min at −20% grade, at s_{DH}, with running cadence controlled through synchronized visual and auditory cues according to the experimental condition.
- Post-downhill neuromuscular test, RFD and one MVC contraction, performed immediately after the running bout, separated by 1min of recovery. Within 6 min after the end of the downhill bout, athletes were back on the treadmill ready for TT.
- Uphill time-trial (TT): 2.5 km at 15% grade, performed at self-selected speed as described in Session 2.
- Post-TT neuromuscular test, RFD and one MVC contraction (1 min recovery).
- Delayed blood sampling 24 h post-exercise for EIMD assessment.

5.3.3 Measurements and procedures

Throughout all the sessions, several physiological and biomechanical variables were continuously or periodically monitored: blood lactate, cardio-metabolic responses, surface EMG, in-shoe pressure and rate of perceived exertion (RPE).

Anthropometric and body composition assessment

Participants underwent a dual-energy X-ray absorptiometry (DEXA) scan to determine body mass, height, and body mass index (BMI). The scan also provided total and regional body composition measurements, including fat mass, fat mass percentage, lean mass, and bone mineral content. All scans were performed by the same trained operator and analysed using the manufacturer's standard software to ensure consistency and accuracy.

Neuromuscular assessment

Isometric contractions of the right knee extensors were used to assess maximal voluntary contraction (MVC), rate of torque development (RTD), and local endurance (LE). All contractions were performed on a custom-built dynamometric chair, with the hip and knee angles fixed at 90°. A load cell (PSD-S1, Pushton Electronic Equipment, Zhengzhou, China; 500 Hz sampling) was attached to the distal tibia, approximately 3 cm above the medial malleolus, and the pelvis was stabilized with a belt to minimize upper-body movement. The position of the load cell and all mechanical adjustments were recorded during the first session and reproduced identically in all subsequent sessions to ensure consistency across measurements. A standardized warm-up consisting of 20 × 2 s submaximal contractions separated by 10s rest was performed, during which participants were instructed to progressively increase the force until reaching their maximal effort (Coratella et al., 2024).

RFD was assessed through 10 isometric contractions in which participants were instructed to "push as fast and as hard as possible," with 5 s recovery between attempts (Varesco et al., 2022).

After 1 min of rest, participants performed two MVC separated by 1 min of recovery (F. Brown et al., 2023; F. C. W. Brown et al., 2022; Engel et al., 2016; Goto & Morishima, 2014). If the difference between the two peak forces exceeded 5%, a third trial was conducted. The highest value was used for subsequent analysis (Coratella et al., 2024).

Finally, isometric local endurance was evaluated by asking participants to sustain an isometric contraction at 60% of their individual MVC (target range: 55–65%) as long as possible (Persson et al., 2000). Real-time visual feedback of force output was provided, and participants received standardized verbal encouragement to maintain the target force. The test was terminated when force output dropped below 50% of MVC for more than 3 consecutive seconds (Allaire, 2004).

The lever arm was measured as the distance from the lateral femoral epicondyle to the point of force application on the anterior tibia, aligned with the load cell. Torque was then calculated as the product of force and lever arm length.

For each MVC, the peak torque (τMVC) was determined as the highest 2-s average recorded during the contraction. For the explosive isometric contractions, the RTD was analysed using the torque–time signal. From all valid trials the five contractions exhibiting the highest peak RFD were retained and averaged in the subsequent analysis. The onset of contraction was automatically detected as the last local minimum before the rising edge of the contraction (Maffiuletti et al., 2016) and was subsequently verified through visual inspection by an experienced investigator blinded to the experimental condition. Force data were then extracted at 25, 50, 100, and 200 ms following onset. RTD was computed over specific time intervals: 0–50 ms ($RTD_{0\text{-}50}$), 50–100 ms ($RTD_{50\text{-}100}$), and 100–200 ms ($RTD_{100\text{-}200}$), as the mean linear slope of the torque–time relationship within each window (Aagaard et al., 2002; Coratella et al., 2020; Varesco et al., 2022). In addition, a global RTD value ($RTD_{0\text{-}200}$) was calculated from contraction onset to 200 ms to represent the overall rapid force production capacity. Peak RTD (RTD_{peak}) was also calculated, the torque–time signal using a Butterworth 4th-order zero-lag low-pass filter with a cutoff of 5 Hz, and identified as the highest slope of the signal between two consecutive time points.

Cardio-metabolic measurements

During all laboratory visits, oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER), and heart rate (HR; Polar H10, Polar Electro, Kempele, Finland) were continuously recorded using a breath-by-breath metabolic cart (CPET, Cosmed, Rome, Italy). The system was calibrated before each test according to the manufacturer's instructions.

Following session 1, ventilatory thresholds (VT_1 and VT_2) were independently identified by three blinded investigators based on the standard ventilatory equivalents and V-slope methods (Wasserman et al., 1973). VO_{2max} was defined as the highest 20-s average VO_2 obtained from a rolling average.

For the experimental sessions, breath-by-breath data were time-averaged over 30-s intervals for analysis. Specifically, metabolic variables were extracted every 5 min during the UH bout, every 3 min during the DH section, and at 10% increments of total distance during the TTs. Metabolic cost of running (CE) was then calculated using oxygen-equivalent values (Di Prampero et al., 1986). During the experimental trials, cardiometabolic variables (VO_2, HR, VE, and Rf) were normalized to running speed to account for differences in external workload. To quantify the decoupling between internal and external load, a decoupling ratio was computed for each variable based on mean values over the entire time trial. For example, HR decoupling was calculated as:

$$\text{HR decoupling ratio} = (\text{HR\_TT}_{\text{PSF}} / \text{speed\_TT}_{\text{PSF}}) / (\text{HR\_TT}_{\text{fresh}} / \text{speed\_TT}_{\text{fresh}})$$

Higher values indicate a greater increase in internal cost per unit of running speed under fatigued conditions (Smyth et al., 2022).

Lactate samples (Biosen C-line, EKF Diagnostics, Barleben, Germany) from the ear lobe were collected immediately after each major phase (uphill, downhill, TT).

EIMD markers and blood lactate

Venous blood samples were collected from the antecubital vein before (PRE) and 24 h after (POST-24h) each experimental session (sessions 3 and 4). Samples were collected into standard serum tubes and immediately transported to an accredited medical laboratory for analysis on the same day. Serum concentrations of Creatine Kinase (CK), Lactate Dehydrogenase (LDH) (Joo, 2015; Leite et al., 2023; Wiewelhove et al., 2016), and C-reactive Protein (CRP) (Milias et al., 2005; G. Y. Millet et al., 2011) were determined using standardized procedures.

Surface electromyography

During the experimental sessions (3 and 4), surface EMG activity was recorded from the *vastus lateralis* (VLa) and *vastus medialis* (VMe) muscles of the right (DUE Pro,OT

Bioelettronica, Torino, Italy). Bipolar electrodes (24 × 24 mm; CDE-C, OT Bioelettronica, Torino, Italy) were placed with an inter-electrode distance of 2 cm in accordance with the SENIAM guidelines (Hermens et al., 2000). This placement minimized crosstalk and geometric artifacts, even during dynamic contractions (Rainoldi et al., 2000). Before electrode placement, the skin was shaved, lightly abraded, and cleaned with alcohol to reduce impedance. Surface EMG signals were sampled at 2048 Hz and recorded continuously during DH and TT, and for 1 min every 5 min during the UH bout.

Surface EMG signals from the vastus lateralis and vastus medialis of the right leg were processed offline using custom routines developed in MATLAB (The MathWorks Inc., Natick, MA, USA). Raw EMG data were band-pass filtered using a zero-lag 4th-order Butterworth filter with a frequency range of 20-500Hz. The filtered signals were full-wave rectified, and a linear envelope was obtained using a moving RMS window of 125ms (Konrad, 2005). These filtering parameters were applied consistently to all recordings (MVC, UH, DH, and TT). For the MVC trials (pre, post-downhill, and post-TT), the maximal EMG amplitude was computed as the highest 0.5s moving average of the envelope within the contraction.

For the same MVC trials, frequency-domain variables were also extracted. The EMG power spectral density (PSD) was estimated using Welch’s method. A 1-s Hanning window (50% overlap) was applied, and the PSD was computed between 15 Hz and the upper bound 450 Hz. These includes the mean (MNF) and median (MDF) frequency and the relative power in low- (15-45 Hz), mid- (45-95 Hz), and high-frequency bands (95-450 Hz), also normalized to the total power of the spectrum, together with their ratios and the slope of the spectrum, to characterize potential neuromuscular fatigue–related shifts in the spectrum (Allison & Fujiwara, 2002). During the running bouts, EMG was analysed in predefined time windows. For the uphill bout, 1-min segments were analysed at the start (excluding the first 10 s of transition), and at fixed intermediate time points (5, 10, 15 and 20 min). For the downhill bout, the six 30-s windows before fixed intermediate time points (1, 3, 6, 9, 12 and 15 min) were analysed, while during the time-trial, 30-s EMG windows were analysed at predefined time points corresponding to every 10% of TT completion. Within each window, the EMG envelope was used to derive time-domain variables. Contraction onset and offset were identified using a threshold set at 10% of the mean peak amplitude of the contractions (Carvalho et al., 2023).

Hence, for each contraction, time-domain variables like the average muscle activation (mean_ON), peak activation (peak), and contraction duration (CD) were calculated, then averaged within the window.

All EMG amplitude variables were normalized to the pre-exercise MVC EMG amplitude of the corresponding muscle, so that activation during running could be expressed as a fraction of the individual maximal EMG. In addition, the frequency-domain variables previously reported where calculated on the raw EMG corresponding to the same windows to provide additional information on fatigue-related changes.

Insoles

During the experimental trials, plantar force data were collected using wireless in-shoe pressure insoles (Loadsol®, Novel GmbH, Munich, Germany), sampling at 100 Hz. Each insole contains three capacitive pressure sensors: one posterior sensor positioned under the heel to capture impact forces, and two sensors located medially and laterally in the forefoot region. The insoles were inserted into the participants' own running shoes and connected to the recording device via Bluetooth. Prior to each session, the system was calibrated according to the manufacturer's guidelines to ensure consistent and accurate measurements across trials. Ground reaction forces were continuously recorded during specific time windows: for the uphill bout, 1 min of acquisition was performed every 5 min; for the downhill segment, 1 min every 3 min; and during the time trial, 1 min of data collection was conducted every 500 m. All force signals were processed offline using custom MATLAB routines (The MathWorks Inc., Natick, MA, USA). The total vertical ground reaction force (vGRF) was obtained by summing all active sensors within each insole From the force–time curve, Foot Contact (FC) and Foot Off (FO) events where automatically identified respectively as the last local minimum before the rising edge and the first local minimum after the falling edge. Step-by-step analysis was performed on the total GRF and on regional components (posterior, anterior-medial, anterior-lateral, and anterior), obtained by grouping the corresponding sensors. For each step, the impact peak (IP) was identified as the local maximum in the vGRF prior to the peak vGRF, defined as the active peak (AP). When an IP is not present during the stance, IP location was estimated to occur at 30.79% of stance time to AP (Schmida et al., 2022). Then, for each step, the following parameters were computed: mean force during stance (F_{mean}), peak force (F_{peak}), contact time (CT), time to impact peak (IP_{time}), time to active peak (APtime), averaged loading rate from 20–80% of the vGRF magnitude at IP (LR_{avg}), vertical impulse (VI), step frequency (SF), step time (ST), and duty factor (Df). All force-related variables were computed separately for the left and right limbs, averaged across all valid steps within the analysed window, and expressed relative to body weight (%BW). For regional analysis, mean and peak forces were also computed for the posterior (heel), anterior (forefoot), anterior-medial and anterior-lateral regions of the insole, allowing the estimation of the distribution of load throughout stance.

Ratings of Perceived Exertion (RPE)

The Borg CR100 scale (0–100) was used for all RPE assessments (Borg & Kaijser, 2006). During the experimental sessions, RPE was collected after 10 min and at the end of the uphill segment, every 5 min during the downhill run (i.e., at 5, 10, and 15 min), and immediately after the TT. At each time point, participants provided two separate ratings: one referring specifically to local exertion in the leg muscles (RPE_{legs}), and one representing the overall perceived effort (RPE).

5.3.4 Statistical analysis

To provide an initial overall comparison of uphill time-trial performance across conditions, a one-way repeated-measures ANOVA was conducted with condition (TT_{fresh}, TT_{PSF}, TT_{ISF}) as the within-subject factor. This analysis was applied to the primary performance outcomes (completion time and average speed), as well as to peak and mean cardiorespiratory variables recorded during the test. When a significant main effect of condition was detected, pairwise post-hoc comparisons were performed using Holm correction to control for familywise error. Effect sizes for ANOVA main effects were expressed as partial eta squared (η^2p), while post-hoc contrasts were expressed as Cohen's d.

To evaluate exercise-induced muscle damage (EIMD), percentage changes from baseline ($\Delta\%$ = POST-24h − PRE) were calculated for each condition and compared using paired t-tests. Effect sizes for these comparisons were expressed as Cohen's d.

To examine differences in pacing strategy, neuromuscular fatigue, plantar loading, metabolic responses, and electromyographic activity across conditions, multiple linear mixed models (LMMs) were applied. For each variable, 'condition' and 'time' were included as fixed effects, and 'subject' was modelled as a random effect to account for repeated measures. Variables collected during the downhill bout (metabolic, plantar loading, EMG) were analysed with two-level models including PSF and ISF. For the uphill time-trial analyses, TT_{fresh} was included as an additional condition to provide a non-fatigued performance reference; pacing and metabolic variables were therefore analysed with LMMs including TT_{fresh}, TT_{PSF}, and $TT_{ISF,}$ and repeated time points within each trial. When significant fixed effects or interactions were detected, Bonferroni-adjusted post-hoc pairwise comparisons were conducted.

Effect sizes for paired comparisons (t-tests) and LMM post-hoc contrasts were expressed as Cohen's d, interpreted as 0.2 (small), 0.5 (medium), and 0.8 (large). Effect sizes for ANOVA and LMM main effects and interactions were expressed as partial eta squared (η^2p), with thresholds of 0.01 (small), 0.06 (medium), and 0.14 (large). Statistical significance was set at $p < 0.05$.

To address the secondary aim of the study, identifying the physiological and neuromuscular factors associated with greater durability (i.e., smaller performance decrement during the uphill time-trial following prior downhill running), a two-step analytical approach was performed.

First, the determinants of uphill time-trial performance in the TT_{fresh} were examined. A correlation matrix including metabolic, biomechanical, and neuromuscular variables was computed using Pearson's linear correlations to identify candidate predictors significantly associated with TT_{fresh} completion time. Variables showing significant or near-significant correlations were retained for subsequent modelling.

Second, a multiple linear regression model was constructed with TT_{fresh} time as the dependent variable and the selected physiological and neuromuscular variables as predictors. This provided a baseline performance model under non-fatigued conditions, enabling the identification of key determinants of uphill running performance.

Subsequently, durability was quantified as the performance decline between the fatigued and fresh conditions (Δ time = $TT_{fatigued} - TT_{fresh}$). A second multiple linear regression model was then performed with Δ time as the dependent variable and the same set of candidate predictors entered as independent variables. This model was used to identify the physiological and neuromuscular characteristics associated with smaller performance deterioration following prior downhill running.

Model assumptions (normality, homoscedasticity, and absence of multicollinearity) were verified prior to interpretation. Standardised regression coefficients (β), p-values, and model R^2 were reported to quantify the contribution and relative importance of each predictor.

5.4 Results

5.4.1 Uphill performance test

A repeated-measures ANOVA revealed a significant main effect of condition on time-trial completion time ($F(2,22) = 48.9$, $p < 0.001$, $\eta^2p = 0.817$). Post-hoc analyses showed that athletes were fastest in $TT_{fresh,}$ slower in TT_{PSF} ($\Delta = +1.53$ min, $p < 0.001$, $d = 2.81$), and slowest in TT_{ISF} ($\Delta = +2.10$ min vs TT_{fresh}, $p < 0.001$, $d = 2.27$; $\Delta = +0.57$ min vs TT_{PSF}, $p = 0.026$, $d = 0.74$).

Average speed also showed a robust condition effect ($F(2,22) = 68.0$, $p < 0.001$, $\eta^2p = 0.861$). Post-hoc comparisons indicated higher speeds in TT_{fresh} compared with both TT_{PSF} ($\Delta = +0.87$ km·h^{-1}, $p < 0.001$, $d = 3.21$) and TT_{ISF} ($\Delta = +1.18$ km·h^{-1}, $p < 0.001$, $d = 2.80$). The difference between TT_{PSF} and TT_{ISF} was also statistically significant ($\Delta = +0.29$ km·h^{-1}, $p = 0.022$, $d = 0.77$), confirming a consistent performance decrement post ISF downhill condition.

Together, these results indicate a progressive and large-to-very-large decline in uphill running performance from TT_{fresh} to TT_{PSF} and TT_{ISF}, with ISF producing the most significant reductions in both completion time and average speed. Condition-dependent differences in cardiorespiratory responses (peak and mean values) are reported in Table 11, while detailed analyses of pacing strategy and metabolic responses during the time trial are presented in the following sections.

Descriptive Statistics								Post-hoc Comparisons – Repeated-Measures Factor								
	Condition	**N**	**Mean**	**SD**	**SE**	**p**	**n^2p**	**Condition 1**		**Condition 2**	**Mean Difference**	**SE**	**gdl**	**t**	**p_{holm}**	**Choen's d**
TT_Time	FRESH	12	15.43	1.08	0.31	<0.001	0.82	Fresh	-	PSF	-1.53	0.16	11	-9.74	<.001	2.81
	PSF	12	16.96	1.35	0.39				-	ISF	-2.10	0.27	11	-7.87	<.001	2.27
	ISF	12	17.53	1.65	0.48			PSF	-	ISF	-0.57	0.22	11	-2.58	0.026	0.74
AVGspeed	FRESH	12	9.79	0.76	0.22	<0.001	0.86	Fresh	-	PSF	0.87	0.08	11	11.13	<.001	3.21
	PSF	12	8.92	0.78	0.23				-	ISF	1.16	0.12	11	9.71	<.001	2.80
	ISF	12	8.63	0.91	0.26			PSF	-	ISF	0.29	0.11	11	2.65	0.022	0.77
Rf_peak	FRESH	12	67.05	7.67	2.22	0.826	0.02	Fresh	-	PSF	0.75	1.48	11	0.51	1.000	0.15
	PSF	12	66.30	6.85	1.98				-	ISF	0.74	1.78	11	0.41	1.000	0.12
	ISF	12	66.31	7.70	2.22			PSF	-	ISF	-0.01	0.61	11	-0.02	1.000	0.01
VE_peak	FRESH	12	176.91	15.91	4.59	0.399	0.08	Fresh	-	PSF	2.79	3.44	11	0.81	0.869	0.23
	PSF	12	174.12	17.15	4.95				-	ISF	4.73	2.96	11	1.60	0.414	0.46
	ISF	12	172.18	17.65	5.10			PSF	-	ISF	1.94	3.84	11	0.50	0.869	0.15
VO2/kg_peak	FRESH	12	69.60	4.42	1.28	0.048	0.24	Fresh	-	PSF	1.74	1.11	11	1.57	0.290	0.45
	PSF	12	67.86	4.77	1.38				-	ISF	3.64	1.29	11	2.83	0.049	0.82
	ISF	12	65.96	5.84	1.69			PSF	-	ISF	1.90	1.67	11	1.14	0.290	0.33
%VO2max_peak	FRESH	12	97.27	3.06	0.88	0.045	0.25	Fresh	-	PSF	2.45	1.58	11	1.55	0.297	0.45
	PSF	12	94.82	3.98	1.15				-	ISF	5.17	1.79	11	2.89	0.044	0.83
	ISF	12	92.10	5.10	1.47			PSF	-	ISF	2.72	2.35	11	1.16	0.297	0.33
HR_peak	FRESH	12	182.17	10.20	2.94	0.834	0.02	Fresh	-	PSF	-0.42	1.23	11	-0.34	1.000	0.10
	PSF	12	182.58	9.86	2.85				-	ISF	0.25	1.29	11	0.19	1.000	0.06
	ISF	12	181.92	10.24	2.96			PSF	-	ISF	0.67	0.73	11	0.91	1.000	0.26
VO2/HR_peak	FRESH	12	27.42	2.83	0.82	0.014	0.32	Fresh	-	PSF	1.13	0.51	11	2.19	0.101	0.63
	PSF	12	26.30	3.30	0.95				-	ISF	1.87	0.55	11	3.39	0.018	0.98
	ISF	12	25.55	3.54	1.02			PSF	-	ISF	0.75	0.67	11	1.12	0.287	0.32
Rf_mean	FRESH	12	56.26	6.71	1.94	0.564	0.05	Fresh	-	PSF	1.05	1.32	11	0.80	0.887	0.23
	PSF	12	55.20	6.82	1.97				-	ISF	0.06	1.07	11	0.06	0.954	0.02
	ISF	12	56.19	7.66	2.21			PSF	-	ISF	-0.99	0.82	11	-1.21	0.758	0.35
VE_mean	FRESH	12	155.58	13.06	3.77	0.003	0.41	Fresh	-	PSF	5.83	2.19	11	2.66	0.045	0.77
	PSF	12	149.75	16.16	4.66				-	ISF	7.52	1.45	11	5.18	<.001	1.50
	ISF	12	148.06	13.53	3.91			PSF	-	ISF	1.69	2.31	11	0.73	0.479	0.21
VO2/kg_mean	FRESH	12	65.39	3.94	1.14	0.006	0.37	Fresh	-	PSF	2.29	1.06	11	2.16	0.108	0.62
	PSF	12	63.10	4.46	1.29				-	ISF	4.11	1.15	11	3.58	0.013	1.03
	ISF	12	61.28	5.06	1.46			PSF	-	ISF	1.82	1.23	11	1.48	0.166	0.43
%VO2max_mean	FRESH	12	91.41	3.55	1.02	0.006	0.37	Fresh	-	PSF	3.26	1.54	11	2.12	0.114	0.61
	PSF	12	88.15	3.36	0.97				-	ISF	5.84	1.61	11	3.64	0.013	1.05
	ISF	12	85.58	4.24	1.23			PSF	-	ISF	2.57	1.72	11	1.50	0.162	0.43
HR_mean	FRESH	12	173.83	10.70	3.09	0.655	0.04	Fresh	-	PSF	-1.17	1.42	11	-0.82	1.000	0.24
	PSF	12	175.00	10.05	2.90				-	ISF	-0.50	1.25	11	-0.40	1.000	0.12
	ISF	12	174.33	10.95	3.16			PSF	-	ISF	0.67	1.09	11	0.61	1.000	0.18
VO2/HR_mean	FRESH	12	25.86	2.73	0.79	0.037	0.30	Fresh	-	PSF	1.00	0.56	11	1.79	0.203	0.52
	PSF	12	24.86	3.17	0.92				-	ISF	1.56	0.53	11	2.93	0.041	0.85
	ISF	12	24.30	3.66	1.06			PSF	-	ISF	0.56	0.61	11	0.92	0.380	0.26

Table 11 Descriptive statistics and results of the one-way repeated-measures ANOVA and pairwise post-hoc comparisons for performance and cardiorespiratory variables across the three uphill time-trial conditions (TT_{fresh}, TT_{PSF}, TT_{ISF}).

Pacing strategy

Running speed profiles differed markedly across conditions. In the split-based analysis, the linear mixed model revealed significant main effects of condition (F = 217.96, $p < 0.001$) and time (F = 17.06, $p < 0.001$), as well as a condition × time interaction (F = 4.25, $p < 0.001$). Post-hoc tests showed that TT_{fresh} was faster than both downhill-fatigued trials, and that TT_{PSF} remained significantly faster than TT_{ISF} (all $p < 0.001$).

Findings from the cumulative average speed analysis supported this pattern. Significant main effects of condition (F = 995.39, $p < 0.001$) and time (F = 9.27, $p < 0.001$), and a smaller but significant interaction (F = 1.85, $p = 0.020$), indicated condition-dependent pacing accumulation. TT_{fresh} remained faster than both fatigued conditions, and TT_{PSF} maintained higher cumulative speed than TT_{ISF} throughout the trial (all $p < 0.001$).

These patterns collectively suggest a consistent but non-significant tendency for ISF to exhibit a greater pacing disruption, without strong evidence for specific segment-level differences.

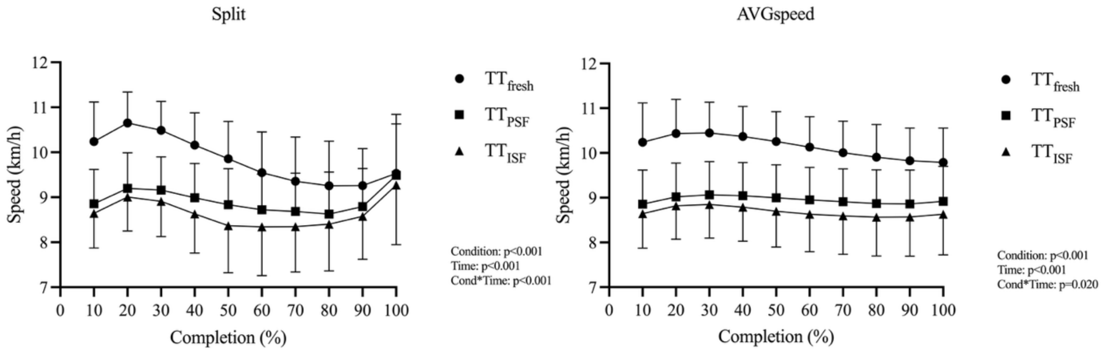


Figure 15 Split-by-split (left) and average (right) running speed during the uphill time trial performed in fresh conditions (TT_{fresh}), after the downhill bout with preferred stride frequency (TT_{PSF}) and after the downhill bout with increased stride frequency (TT_{ISF}). Speed is expressed as mean ± SD for each 10% of trial completion. Reported p-values refer to the main effects of condition, time, and their interaction (condition × time).

Metabolic responses during the time-trial

During the time-trial, %VO_2max differed significantly between conditions, with main effects of condition (F = 61.97, $p < 0.001$) and time (F = 6.02, $p < 0.001$), and a condition

× time interaction ($F = 1.75$, $p = 0.031$). Post-hoc comparisons showed higher relative oxygen consumption in TT_{fresh} compared with both fatigued conditions ($p < 0.001$), and lower $\%VO_2max$ in TT_{ISF} compared with TT_{PSF} ($p < 0.001$).

VE differed across conditions ($F = 22.01$, $p < 0.001$) and time points ($F = 70.47$, $p < 0.001$), with a significant interaction ($F = 1.79$, $p = 0.025$). TT_{fresh} showed higher VE than both fatigued conditions ($p < 0.001$), while TT_{PSF} and TT_{ISF} did not differ ($p = 0.369$). When normalized to running speed, VE/speed showed a clear condition effect ($F = 45.76$, $p < 0.001$): TT_{fresh} presented lower values than both downhill-fatigued conditions ($p < 0.001$), and TT_{ISF} showed higher VE/speed than TT_{PSF} ($p = 0.007$).

Rf showed no overall condition effect ($p = 0.132$), although a significant interaction was present ($F = 1.77$, $p = 0.027$). When expressed relative to speed, Rf/speed differed markedly between conditions ($F = 53.48$, $p < 0.001$), with higher values in TT_{ISF} compared with TT_{PSF} ($p < 0.001$), and in both fatigued conditions compared with TT_{fresh} ($p < 0.001$).

HR showed significant effects of condition ($F = 3.68$, $p = 0.026$) and time ($F = 66.69$, $p < 0.001$), with no significant interaction ($p = 0.576$). Post-hoc analyses revealed differences between TT_{fresh} and TT_{PSF} ($p = 0.023$), with no difference between TT_{PSF} and TT_{ISF} ($p = 0.295$). When normalized for speed, HR/speed showed a strong condition effect ($F = 192.43$, $p < 0.001$), with higher ratios in TT_{ISF} compared with TT_{PSF} ($p < 0.001$), and lower values in TT_{fresh} compared with both fatigued conditions ($p < 0.001$).

Finally, VO_2/HR differed significantly across conditions ($F = 50.35$, $p < 0.001$). Post-hoc comparisons showed higher VO_2/HR in TT_{fresh} compared with both fatigued conditions ($p < 0.001$), and significantly higher values in TT_{PSF} compared with TT_{ISF} ($p = 0.002$).

Peak blood lactate concentration was highest after TT_{fresh}, with lower values observed in both TT_{PSF} ($\Delta = -3.52$ $mmol \cdot L^{-1}$, $p = 0.002$, $d = 1.31$) and TT_{ISF} ($\Delta = -3.93$ $mmol \cdot L^{-1}$, $p < 0.001$, $d = 2.12$). No difference was detected between TT_{PSF} and TT_{ISF} ($\Delta = -0.40$ $mmol \cdot L^{-1}$, $p = 0.441$, $d = 0.23$).

RPE showed a similar trend, with no significant differences between TT_{fresh} and TT_{PSF} ($\Delta = +4.08$ a.u., $p = 0.244$, $d = 0.48$) nor between TT_{PSF} and TT_{ISF} ($\Delta = +3.92$ a.u., $p = 0.244$, $d = 0.48$). RPE was higher in TT_{ISF} compared with TT_{fresh} ($\Delta = +8.00$ a.u., $p = 0.138$, $d = 0.65$), although this contrast did not remain statistically significant.

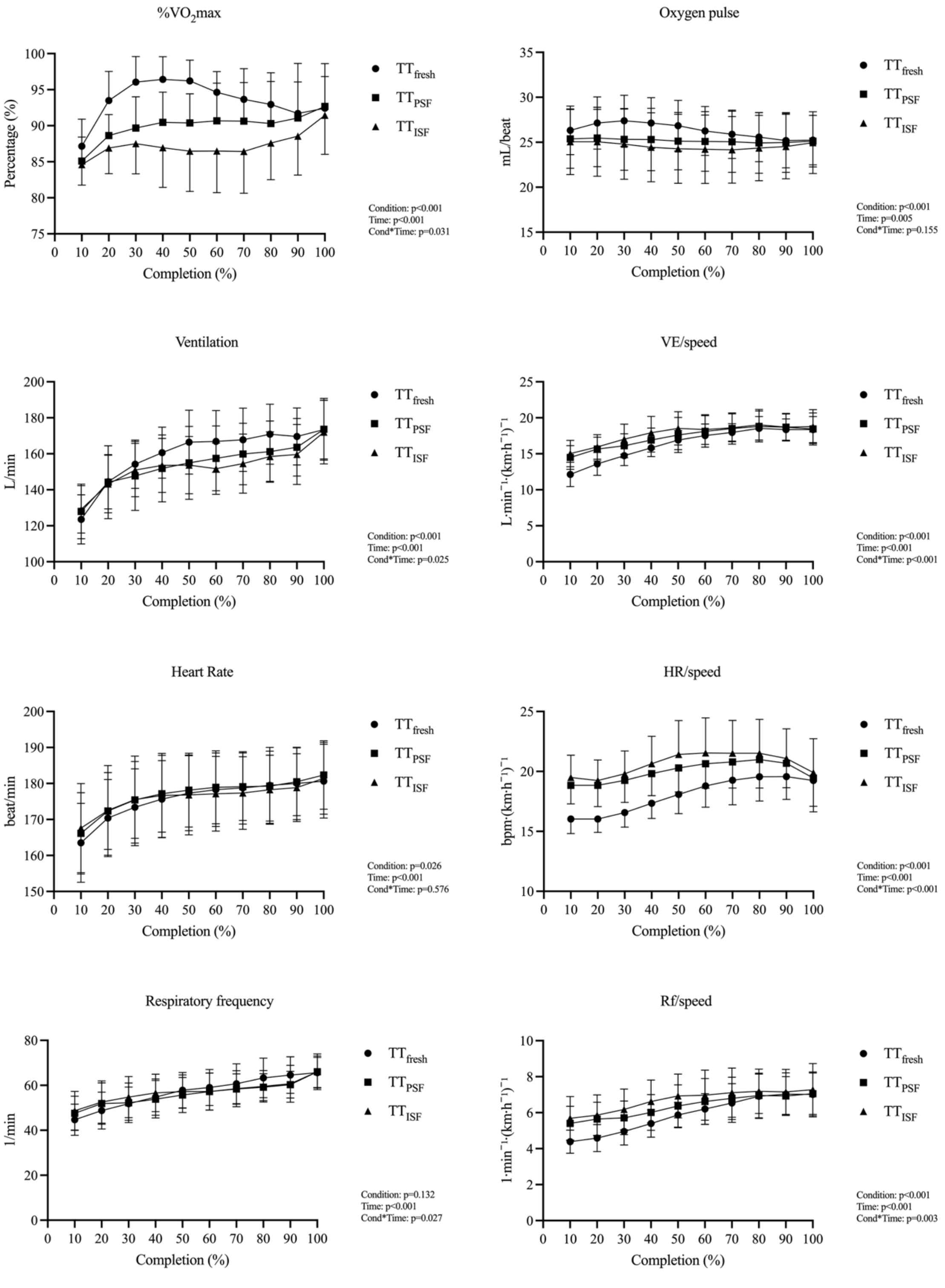


Figure 16 Cardiopulmonary responses during the uphill time trial performed in fresh conditions (TT_{fresh}), after the downhill bout with preferred stride frequency (TT_{PSF}) and after the downhill bout with increased stride frequency (TT_{ISF}). From top to bottom: percentage of VO_2max and oxygen pulse (top row), ventilation and VE/speed ratio (middle row), heart rate and HR/speed ratio (third row), respiratory frequency and Rf/speed ratio (bottom row). Data are expressed as mean ± SD for each 10% of trial completion. Reported p-values refer to the main effects of condition, time, and their interaction (condition × time).

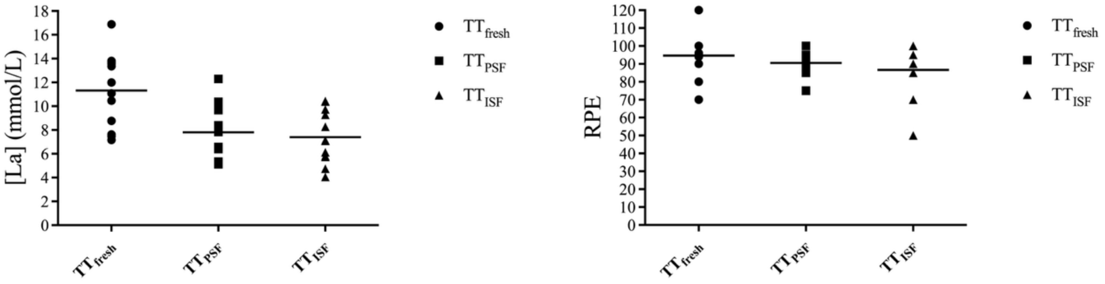


Figure 17Peak post-exercise blood lactate concentration ([La], left panel) and overall rating of perceived exertion (RPE, right panel) after the uphill time trial performed in fresh conditions (TT_{fresh}), after the downhill bout with preferred stride frequency (TT_{PSF}) and after the downhill bout with increased stride frequency (TT_{ISF}). Symbols represent individual values; horizontal lines indicate group means.

5.4.2 Downhill running section

Physiological and Perceptual Responses

Linear mixed-model analyses revealed significant differences between conditions across multiple metabolic variables during the downhill bout. Oxygen consumption relative to body mass (VO_2/kg) showed significant main effects of condition (F = 27.92, p < 0.001) and time (F = 6.65, p < 0.001); post-hoc comparisons indicated higher VO_2/kg in ISF compared with PSF (PSF - ISF difference = 1.97 $mL \cdot kg^{-1} \cdot min^{-1}$, p < 0.001). A similar pattern was observed for %VO_2max, which differed by condition (F = 27.80, p < 0.001) and time (F = 6.61, p < 0.001), with higher values in ISF (difference = 2.77%, p < 0.001). The metabolic cost of running (CE) also showed main effects of condition (F = 32.42, p < 0.001) and time (F = 6.03, p < 0.001), with CE being higher in ISF (difference = 0.171 $J \cdot kg^{-1} \cdot m^{-1}$, p < 0.001).

VE displayed significant differences between protocols (F = 55.38, p < 0.001) and across time (F = 5.67, p < 0.001); post-hoc results again showed higher VE in ISF (difference = 7.75 $L \cdot min^{-1}$, p < 0.001). Rf exhibited a smaller but significant condition effect (F = 4.38, p = 0.039), with ISF presenting higher values than PSF (difference = 1.53 $breaths \cdot min^{-1}$, p = 0.039). HR also differed significantly between conditions (F = 40.78, p < 0.001) and across time (F = 9.05, p < 0.001), with higher HR observed in ISF (difference = 6.81 bpm, p < 0.001).

In contrast, oxygen pulse (VO_2/HR) did not differ significantly between the two downhill protocols ($F = 1.92$, $p = 0.169$), with no condition effect detected in post-hoc testing (difference = 0.263 $mL \cdot beat^{-1}$, $p = 0.169$).

For Ratings of Perceived Exertion related to local muscular effort (RPE_{legs}), the LMM revealed no significant main effect of condition ($F = 1.113$, $p = 0.296$) and no condition × time interaction ($p = 0.742$). RPE_{legs} increased significantly over time ($F = 21.092$, $p < 0.001$), but values were comparable between PSF and ISF across the entire downhill protocol.

In contrast, overall perceived exertion (RPE) differed significantly between conditions. The model showed a significant main effect of condition ($F = 12.490$, $p < 0.001$) and of time ($F = 20.689$, $p < 0.001$), with no interaction ($p = 0.987$). Post-hoc comparisons confirmed higher RPE in ISF compared with PSF (difference = 6.28, $p < 0.001$), indicating that participants perceived the ISF descent as more demanding despite similar local muscular effort.

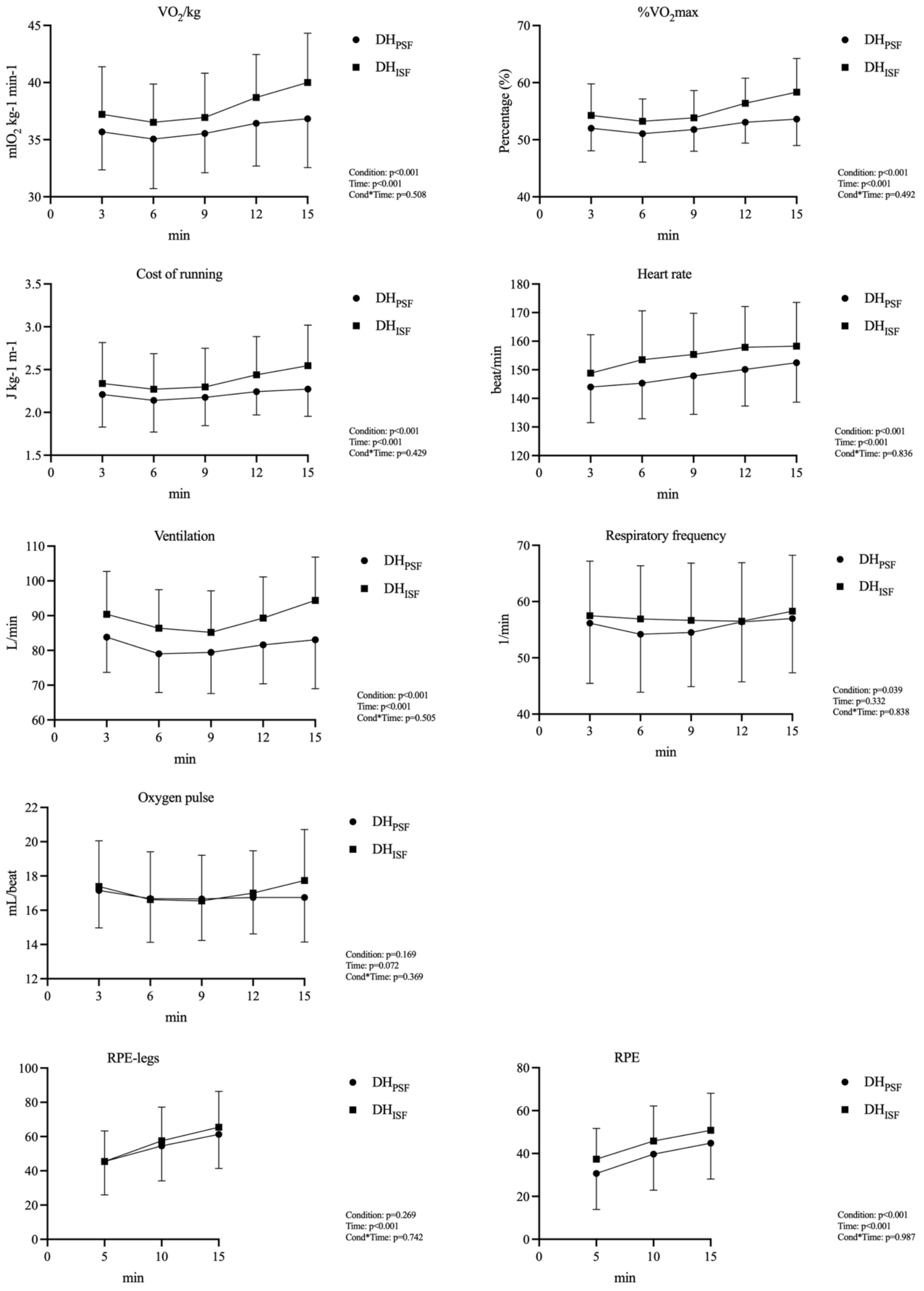


Figure 18 Cardiopulmonary, metabolic and perceptual responses during the 15-min downhill bout performed with preferred (DH_{PSF}) and increased (DH_{ISF}) stride frequency. From top to bottom and left to right: oxygen uptake (VO_2/kg), %VO_2max, cost of running, heart rate, ventilation, respiratory frequency, oxygen pulse, RPE for the legs (RPE-legs) and overall RPE. Time-course data are reported as mean ± SD at 0, 3, 6, 9, 12 and 15 min. Reported p-values refer to the main effects of condition, time and their interaction (condition × time).

Plantar Force and Impact Loading

The analysis of plantar loading variables revealed consistent condition-dependent differences between PSF and ISF across all measured parameters. F_{mean} was significantly higher in PSF compared with ISF (difference = +0.09 %BW, $p < 0.001$). F_{peak} also differed significantly between conditions, with higher values in PSF (difference = +0.329 %BW, $p < 0.001$).

Impact loading metrics showed marked differences. LR_{avg} was significantly greater in PSF compared with ISF, with between-condition difference of +40.0 $\%BW \cdot s^{-1}$ ($p < 0.001$).

VI was significantly higher in PSF than ISF (difference = +0.044 %BW·s, $p < 0.001$). Temporal gait variables also differed between conditions: CT was longer in PSF (difference = +0.015 s, $p < 0.001$), and Df was slightly but significantly increased in ISF (difference = +0.019, $p = 0.047$).

Analyses of the regional plantar loads showed consistent condition effects across both rearfoot and forefoot variables. For the rearfoot, heel_F_{mean} did not differ significantly between conditions ($p = 0.067$), whereas heel_F_{peak} showed a clear effect of condition ($F = 12.56$, $p < 0.001$), with higher peak rearfoot loading in PSF compared with ISF (difference = 0.346 BW; $p < 0.001$).

For the forefoot, significant differences were found for both average and peak force. Forefoot_F_{mean} differed across conditions ($F = 10.01$, $p = 0.002$), with higher mean forefoot forces in PSF relative to ISF (difference = 0.070 BW; $p = 0.002$). Similarly, forefoot_F_{peak} demonstrated a significant condition effect ($F = 4.72$, $p = 0.032$), with peak forefoot loading also higher in PSF (difference = 0.122 BW; $p = 0.032$).

No significant condition × tempo interactions emerged for any of the rearfoot or forefoot variables (all $p > 0.95$).

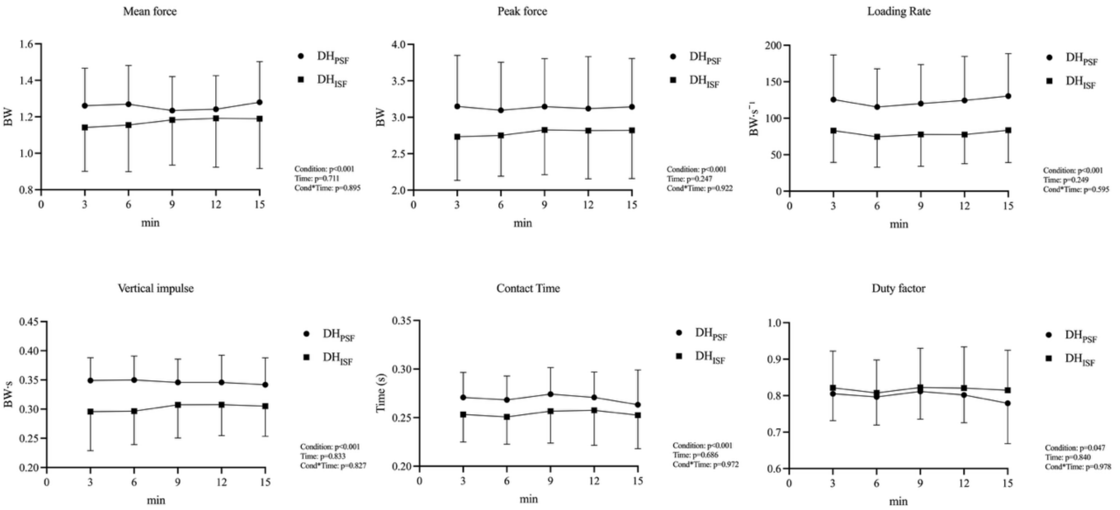


Figure 19 Vertical ground reaction force and spatiotemporal variables during the 15-min downhill bout performed with preferred (DH_{PSF}) and increased (DH_{ISF}) stride frequency. From left to right and top to bottom: mean vertical force, peak vertical force, loading rate, vertical impulse, contact time and duty factor. Data are expressed as mean ± SD at 3, 6, 9, 12 and 15 min. Reported p-values refer to the main effects of condition, time and their interaction (condition × time).

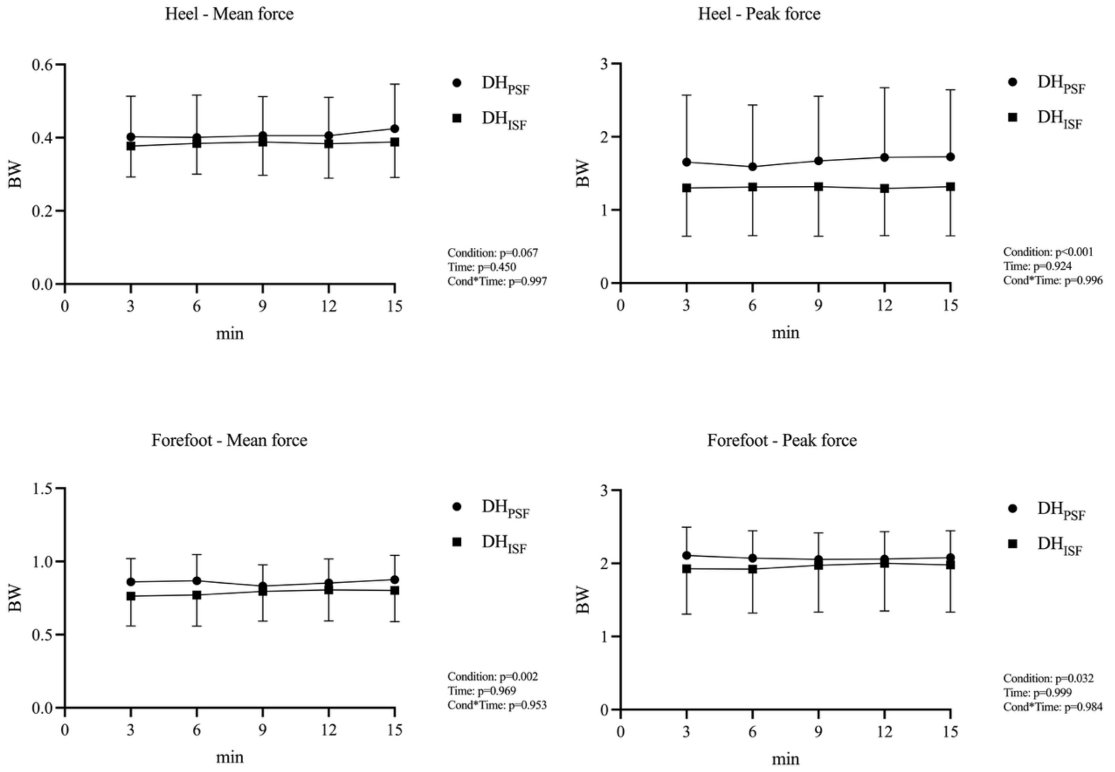


Figure 20 Heel and forefoot vertical forces during the 15-min downhill bout performed with preferred (DH_{PSF}) and increased (DH_{ISF}) stride frequency. Top panels: mean (left) and peak (right) vertical force under the heel. Bottom panels: mean (left) and peak (right) vertical force under the forefoot. Forces are expressed relative to body weight (BW) and reported as mean ± SD at 3, 6, 9, 12 and 15 min. Reported p-values refer to the main effects of condition, time and their interaction (condition × time).

Neuromuscular Activity During Downhill Running

Analyses of neuromuscular activity during downhill running showed no significant effects of condition (PSF vs ISF) and no condition × time interactions for any EMG variable. Normalized mean activation of both *vastus lateralis* and *vastus medialis* remained comparable between conditions (VL_{mean_ON}: p = 0.583; VM_{mean_ON}: p = 0.190) and did not change significantly over the course of the downhill segment (VL_{mean_ON}: p = 0.558; VM_{mean_ON}: p = 0.075).

Peak activation also did not differ between PSF and ISF (VL_{peak}: p = 0.170; VM_{peak}: p = 0.195), although a significant time effect was observed for both muscles (VL: p < 0.001; VM: p = 0.015), indicating a progressive modulation of activation throughout the descent. Contraction duration was likewise unaffected by condition (VL_{CD}: p = 0.369; VM_{CD}: p = 0.875) and showed no significant time effects (VL: p = 0.600; VM: p = 0.611).

Frequency-domain variables showed clear temporal modulation during downhill running.

For VL, both median and mean frequencies increased over time (VL_{MDF}: p < 0.001; VL_{MNF}: p < 0.001), with no condition effects (VL_{MDF}: p = 0.567; VL_{MNF}: p = 0.606).

For VM, median and mean frequencies decreased over time (VM_{MDF}: p < 0.001; VM_{MNF}: p < 0.001), and this decrease was not influenced by condition differences (VM_{MDF}: p = 0.903; VM_{MNF}: p = 0.861).

High-frequency power (HF) followed the same pattern, showing significant time effects in both VL and VM (both p < 0.001), with no condition effects (VL_{HF}: p = 0.544; VM_{HF}: p = 0.776).

5.4.3 Neuromuscular Activity During the Uphill Time-Trial

Neuromuscular activity during the uphill time-trial did not differ between conditions, with no significant effects of PSF vs ISF and no condition × time interactions for any EMG variable. Normalized mean activation remained comparable between conditions for both VL and VM (VL_{mean_ON}: p = 0.550; VM_{mean_ON}: p = 0.167), and did not show significant temporal changes across the time-trial (VL_{mean_ON}: p < 0.001; VM_{mean_ON}: p = 0.002).

Peak activation exhibited a consistent time effect (VL_{peak}: $p < 0.001$; VM_{peak}: $p < 0.001$), indicating a progressive increase in activation requirements as the climb progressed, while no condition effect was detected (VL_{peak}: $p = 0.509$; VM_{peak}: $p = 0.186$). CD showed no differences between PSF and ISF (VL_{CD}: $p = 0.474$; VM_{CD}: $p = 0.734$) and varied significantly over time, only for VL (VL_{CD}: $p < 0.001$; VM_{CD}: $p = 0.672$).

Frequency-domain parameters exhibited limited and muscle-specific temporal modulation during the uphill TT. For MDF, VL exhibited a significant time effect ($p = 0.006$), whereas VM did not ($p = 0.274$), with no condition effects for either muscle (VL: $p = 0.433$; VM: $p = 0.984$).

MNF followed a similar pattern: VL showed a significant time effect ($p = 0.025$), while VM did not ($p = 0.291$). Again, no condition differences were detected (VL: $p = 0.552$; VM: $p = 0.900$).

5.4.4 Maximal and Rapid Force Production

Results revealed no significant effect of condition on maximal voluntary contraction torque (τMVC; $F = 0.001$, $p = 0.972$), while a significant main effect of time was observed ($F = 17.94$, $p < 0.001$), with no condition × time interaction ($p = 0.980$).

For early-phase rapid force production ($RTD_{0–50}$), no significant effect of condition was detected ($F \approx 0.0001$, $p = 0.993$). A significant effect of time was present ($F = 4.25$, $p = 0.019$), with no condition × time interaction ($p = 0.613$).

Similarly, for $RTD_{50–100}$, the condition effect was not significant ($F = 0.477$, $p = 0.493$), whereas a significant effect of time was found ($F = 7.33$, $p = 0.002$). The condition × time interaction was not significant ($p = 0.130$).

For $RTD_{100–200}$, neither condition ($F = 1.41$, $p = 0.241$) nor time ($F = 2.70$, $p = 0.076$) showed significant effects, and their interaction was not significant ($p = 0.708$).

Finally, for total rapid force production capacity ($RTD_{0–200}$), no effect of condition was observed ($F = 0.0037$, $p = 0.952$), whereas a significant main effect of time emerged ($F = 12.43$, $p < 0.001$), with no condition × time interaction ($p = 0.287$).

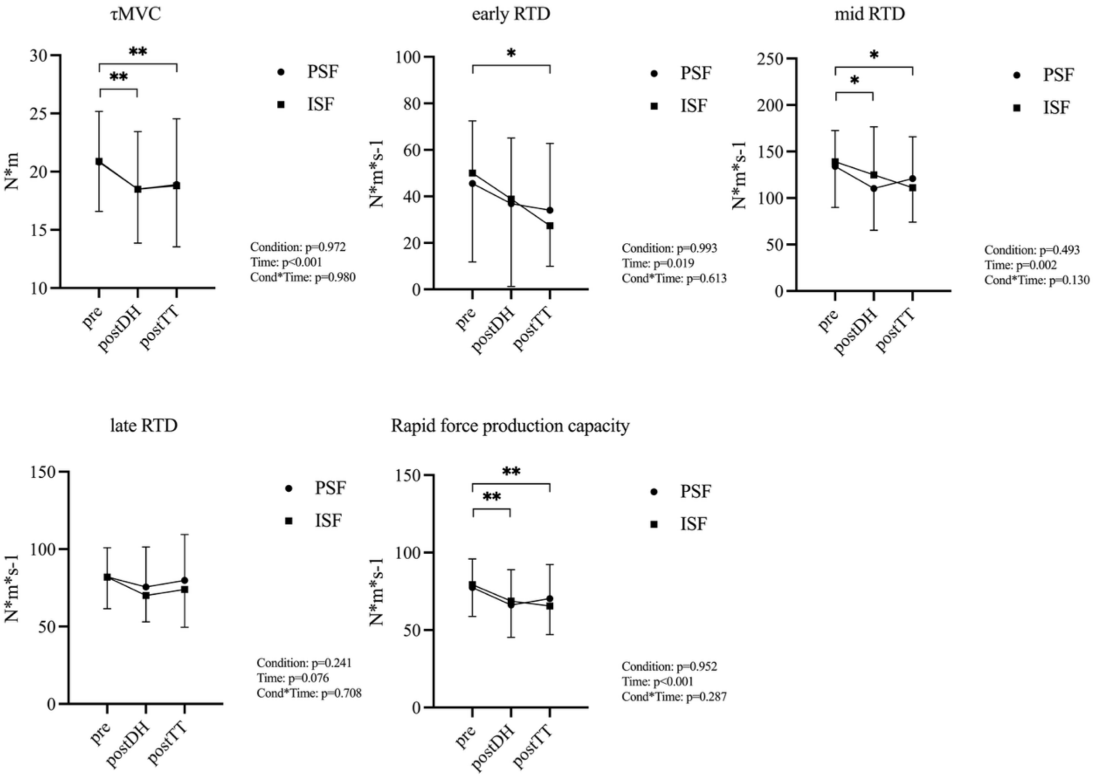


*Figure 21 Changes in knee-extensor torque and rapid force production before (pre), immediately after the downhill bout (postDH) and after the subsequent uphill time trial (postTT) in the preferred (PSF) and increased (ISF) stride-frequency conditions. Panels show maximal voluntary torque (τMVC), early RTD, mid RTD, late RTD and overall rapid force production capacity. Data are expressed as mean ± SD. Reported p-values refer to the main effects of condition, time and their interaction (condition × time). *p < 0.05, **p < 0.001.*

5.4.5 Muscle Damage Markers (EIMD)

No significant differences were observed between conditions for any of the blood-based markers of muscle damage. The percentage change from pre to 24 h postexercise did not differ between ISF and PSF for LDH (p = 0.616), CK (p = 0.338), or CRP (p = 0.592). Mean percentage changes were small and comparable between protocols, with LDH increasing by 6.58% in PSF and 9.00% in ISF, CK by 144.4% in PSF and 109.86% in ISF, and CRP by 50.41% in PSF and 32.41% in ISF.

Taken together, these findings indicate that the two downhill protocols elicited a similar magnitude of muscle damage despite the different biomechanical demands imposed during the ISF condition.

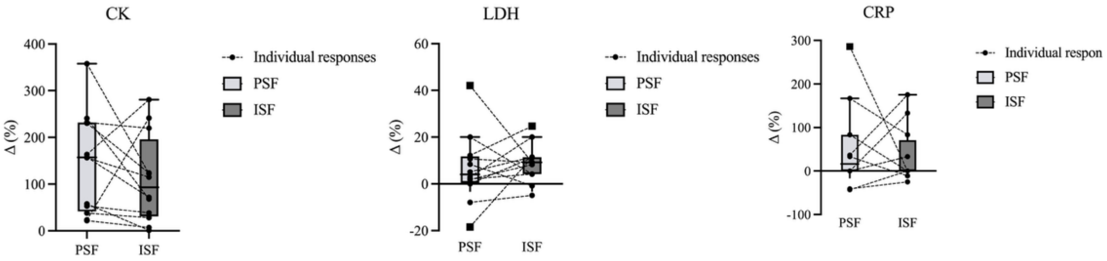


Figure 22 Percentage change (Δ%) in blood markers of muscle damage and inflammation after the preferred (PSF) and increased (ISF) stride-frequency conditions: creatine kinase (CK, left), lactate dehydrogenase (LDH, middle) and C-reactive protein (CRP, right). Boxplots represent group data, while dotted lines show individual responses.

5.4.6 Predictive Models for Uphill Performance

Fresh performance model

Linear correlations conducted on baseline (fresh) variables revealed significant associations between TT_{fresh} performance and several physiological determinants. TT_{fresh} completion time correlated strongly and negatively with VO_2max ($r = -0.868$, $p < 0.001$), peak running speed ($r = -0.764$, $p = 0.004$), VO_2 at the second ventilatory threshold ($r = -0.762$, $p = 0.004$), and VO_2 at VT_2 expressed as a percentage of VO_2max ($r = -0.792$, $p = 0.002$). A moderate negative correlation was also found between TT_{fresh} and lower-limb local endurance (LE; $r = -0.690$, $p = 0.013$). No significant correlations were observed with MVC/RTD, % body fat, BMI, or training-related variables.

A hierarchical linear regression was conducted to identify physiological and neuromuscular predictors of TT_{fresh} performance. In Model 1, VO_2max explained 75.4% of the variance in TT_{fresh} time ($R^2 = 0.754$). Adding local LE in Model 2 significantly improved model fit ($\Delta R^2 = 0.167$, $F = 19.06$, $p = 0.002$), increasing the explained variance to 92.1% ($R^2 = 0.921$). The inclusion of peak RFD torque ($tRFD_{peak}$) in Model 3 further improved the model, accounting for an additional 2.9% of the variance ($\Delta R^2 = 0.029$, $F = 4.61$, $p = 0.064$), yielding a final R^2 of 0.950 (Fig.23).

In the final model, VO_2max ($\beta = -0.173$, $p < 0.001$) and LE ($\beta = -0.022$, $p = 0.001$) were significant predictors of TT_{fresh} time, while RTD_{peak} showed a non-significant trend ($\beta = -0.007$, $p = 0.064$).

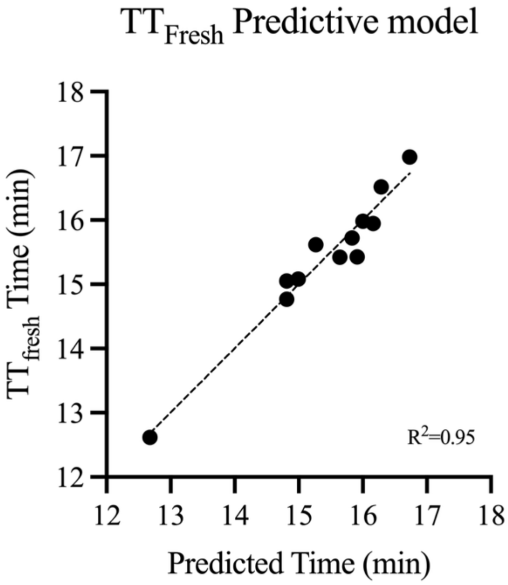


Figure 23 Predictive model for uphill time-trial performance in fresh conditions (TT_{fresh}). Relationship between observed TT_{fresh} completion time and time predicted by the multiple regression model. Each symbol represents one participant; the dashed line indicates the line of identity. The model explained 95% of the variance in TT_{fresh} time ($R^2 = 0.95$).

Decline in performance after a fatiguing task

Time-trial performance decreased in both fatigued conditions, with TT time increasing on average by +9.86% after PSF and +12.44% after ISF. Cardiometabolic decoupling, expressed as the ratio of internal load to running speed, increased consistently across variables in both conditions. In PSF, decoupling ratios were 1.11 for HR, 1.06 for VE, and 1.08 for Rf, whereas in ISF the ratios were 1.14, 1.08, and 1.14, respectively. All decoupling ratios were significantly correlated with the corresponding percentage decrement in TT performance (HR: $r = 0.810$, $p = 0.001$; VE: $r = 0.651$, $p = 0.022$; Rf: $r = 0.835$, $p < 0.001$ for PSF; HR: $r = 0.952$, $p < 0.001$; VE: $r = 0.829$, $p < 0.001$; Rf: $r = 0.812$, $p = 0.001$ for ISF).

Among the additional variables explored, body fat percentage showed a trend toward an association with the magnitude of the performance decrement (PSF: $p = 0.054$; ISF: $p = 0.090$), whereas no other physiological, neuromuscular, anthropometric, or training-related variable correlated with performance decline in either condition.

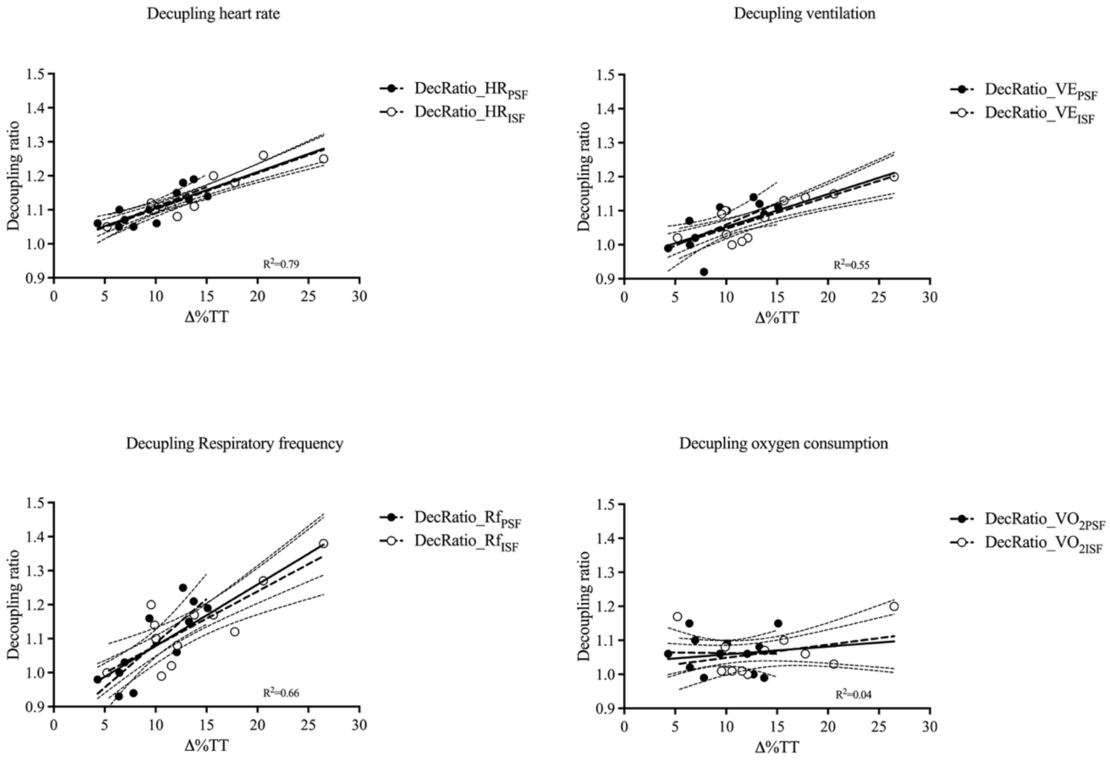


Figure 24 Relationships between cardiometabolic decoupling and uphill performance decline. Decoupling ratios for heart rate (top left), ventilation (top right), respiratory frequency (bottom left) and oxygen consumption (bottom right) plotted against the percentage change in uphill time-trial performance (Δ%TT) after the preferred (DecRatioPSFclosed symbols) and increased (DecRatioISF, open symbols) stride-frequency conditions. Solid lines represent the fitted regression models; dashed lines indicate 95% confidence intervals. Reported R² values refer to the overall goodness of fit for each model.

5.5 Discussion

This study investigated whether manipulating running biomechanics during downhill running, specifically through a 10% increase in stride frequency, could mitigate muscle damage and improve subsequent uphill performance in well-trained and elite trail runners. To date, only a limited number of studies have explored stride frequency modulation during downhill running, with inconsistent findings and protocols that rarely reflect the continuous and prolonged eccentric load characteristic of real trail-running descents. Moreover, no previous research has investigated this strategy within an experimental design that mirrors the typical sequence of trail-running terrain transitions, integrating fatigue accumulated during an uphill bout, a continuous downhill section, and the resulting effects on a subsequent uphill bout. By integrating biomechanical, neuromuscular, metabolic, and performance outcomes, the present study provides the most comprehensive evaluation to date of how downhill running strategy may influence performance in trail running.

Overall, contrary to our initial hypothesis, increasing stride frequency had clear acute biomechanical and metabolic consequences during the downhill bout but did not reduce muscle damage or improve subsequent uphill performance. Athletes showed a marked deterioration in time-trial performance after both downhill conditions, with a greater decline following the increased stride frequency condition (ISF). These differences occurred despite similar levels of exercise-induced muscle damage (CK, LDH, CRP) and comparable reductions in maximal voluntary torque and rapid force production across conditions. ISF elicited systematically higher metabolic demand, whereas PSF was associated with consistently higher impact forces. Surface EMG confirmed the presence of neuromuscular fatigue during the descent but revealed no condition-specific differences in quadriceps activation or frequency-domain indices.

Under fresh conditions, TT_{fresh} performance was strongly determined by VO_2max and local knee-extensor endurance, and to a lesser extent by rapid force production, underscoring the combined contribution of aerobic capacity and neuromuscular resilience to uphill running. Under fatigued conditions, however, none of these variables predicted the magnitude of the decline in performance. Instead, the deterioration was closely associated with cardiometabolic decoupling (HR, VE, Rf relative to speed), suggesting that reduced durability reflected a disproportionate rise in internal load for a given mechanical output rather than structural muscle damage or impaired force-generating capacity.

Collectively, these findings indicate that stride frequency modulation during downhill running substantially alters the acute mechanical and metabolic environment but does not attenuate muscle damage or preserve neuromuscular function. The greater deterioration in uphill performance following ISF highlights the importance of internal workload accumulation and decoupling responses, rather than peripheral muscle damage, in determining durability during trail running.

Downhill running and stride frequency modulation

In trail running, downhill sections represent a key determinant of overall performance and play a central role in shaping race outcomes (Bettega, Pellegrini, et al., 2025; Genitrini et al., 2022). Despite this importance, research on downhill running remains relatively limited, especially studies conducted in ecologically valid settings that capture the continuous eccentric loading and technical variability of off-road descents. A consistent body of literature has shown that downhill running induces substantial biomechanical stress (Bontemps et al., 2020; Brancaccio et al., 2007; Buford et al., 2009; Eston et al., 1996; Peake et al., 2005) and generates markedly elevated impact forces at foot strike (Giandolini, Horvais, et al., 2016; Giandolini, Vernillo, et al., 2016). These forces result in high eccentric demands on the quadriceps, contributing to neuromuscular fatigue, reductions in maximal force, and impairments in rate of force development.

Because impact loading is closely linked to stride mechanics, increasing stride frequency has been proposed as a potential strategy to mitigate downhill-induced mechanical stress. Previous studies conducted on treadmills or controlled field settings have reported that shorter strides and higher step frequencies reduce vertical loading rates, impact peaks, and braking forces compared with preferred stride patterns (Baggaley et al., 2020; Eston et al., 2000; Rowlands et al., 2001). Based on this evidence, we hypothesized that a 10% increase (Eston et al., 1996, 2000; Rowlands et al., 2001; Vincent et al., 2019) in stride frequency would lower mechanical impact and, in turn, reduce muscle damage and neuromuscular fatigue.

Our results confirm partly of this rationale. In line with previous literature, the ISF condition reduced several indices of external mechanical loading in respect relative to PSF, including peak plantar forces, loading rates, and both rearfoot and forefoot peak forces, reinforcing the mechanistic link between stride frequency and impact attenuation during downhill running.

However, this mechanical advantage was counterbalanced by a substantially greater internal physiological load. ISF was associated with systematically higher VO_2, $\%VO_2max$, VE, Rf and HR, indicating that reducing impact forces through higher stride frequency required a markedly greater metabolic cost. This pattern contrasts with earlier laboratory studies suggesting that altering stride frequency has relatively modest effects on metabolic cost during level (I. Hunter & Smith, 2007), mild-slope running (Snyder & Farley, 2011; Vincent et al., 2019).

Interestingly, surface EMG of VL and VM did not differ between conditions, suggesting that the higher metabolic cost observed in ISF was not driven by greater activation of these knee extensor muscles. This does not exclude an increased neuromuscular demand in other muscle groups, such as hip flexors–extensors, plantar flexors and trunk stabilizers, which were not monitored in the present study and are likely also involved when step frequency and leg swing increase on steep downhill terrain. Overall, these findings support the existence of a trade-off between mechanical protection from impact and energetic efficiency on steep downhill terrain. The higher internal load observed in ISF may reflect the combined effect of a greater number of steps per unit distance and increased activation of muscles involved in leg swing and trunk stabilization. From a practical standpoint, this suggests that athletes and coaches should carefully balance impact reduction and metabolic efficiency when prescribing stride-frequency strategies for prolonged downhill running.

From an applied perspective, these results suggest that an increased stride frequency should not be considered a general strategy to preserve performance during prolonged downhill running, since the higher metabolic cost observed in ISF may outweigh its mechanical benefits. Rather, its use may be more justifiable in specific situations where reducing impact and shortening step length are prioritized over energetic efficiency, such as short, steep downhill sections or highly technical and heterogeneous terrain with frequent obstacles. However, this interpretation remains speculative, as the present protocol did not include natural technical terrain or direct measures of stability, step accuracy, or fall-related risk. Therefore, any practical recommendation regarding the use of ISF in such race situations should be made cautiously and would require confirmation in more ecologically valid outdoor conditions.

Despite these divergent mechanical and metabolic responses, both downhill conditions produced similar reductions in maximal voluntary torque and rate of force development. This indicates that running for 15 min at −20% slope imposed a sufficiently high eccentric load to induce neuromuscular fatigue in the knee extensors, and that modifying stride frequency did not substantially alter the magnitude of this impairment. In our study, τMVC decreased by ~11.5% immediately after the downhill bout, in line with the magnitude reported in previous work (de Oliveira Assumpção et al., 2020; Giandolini, Horvais, et al., 2016; Giovanelli et al., 2021; Lima et al., 2021) observed a large reduction in knee-extensor isometric peak torque (−36.3 ± 26%) following 30 min of running at −15%, while Lima et al. (2021) reported a −21.8 ± 6.1% drop in MVC immediately after downhill running at ~18% that remained depressed for several days (Lima et al., 2021). Giovanelli et al. (2021), using a 30-min downhill run at −20%, found a torque loss of ~16.5% (Giovanelli et al., 2021), a value remarkably close to that observed in the present study.

Similarly, our findings for rapid force production are consistent with the literature. RTD$_{0–200}$ was significantly reduced (−16%), and mid-phase RTD$_{50–100}$ also decreased, whereas early-phase RTD$_{0–50}$ was unaffected. This pattern mirrors previous evidence showing that downhill running predominantly impairs the late phase of rapid force production, while sparing early neural-driven explosive force. Varesco et al. (2022) reported substantial reductions in RTD$_{100–200}$ (−25%) and global RTD$_{0–200}$ (~−15%), with no changes in the earliest time window (Varesco et al., 2022). Coratella et al. (2024) similarly demonstrated that downhill running selectively affects the late phase of RFD (Coratella et al., 2024), likely reflecting disruptions in muscle-tendon unit function and excitation–contraction processes rather than changes in initial neural drive.

Surface EMG analyses further support this interpretation, and clear signs of fatigue were evident, reflected in progressive modulation of activation and frequency-domain variables (Allison & Fujiwara, 2002). These EMG findings align with previous observations showing that, during prolonged downhill running, neuromuscular fatigue is primarily driven by the magnitude of the eccentric load rather than by subtle variations in running mechanics. Giandolini et al. (2016) reported that EMG activity of the vastus lateralis and gastrocnemius during a long downhill trail run correlated with subsequent neuromuscular impairments, suggesting that the fatigue stimulus arises from the sustained eccentric demand itself (Giandolini, Horvais, et al., 2016). This helps explain why no differences emerged between PSF and ISF in our study: stride-frequency manipulation was insufficient to override the dominant effect of the downhill eccentric load on muscle activation patterns.

Both conditions also led to similar responses in markers of exercise-induced muscle damage, with no significant differences between protocols. This indicates that EIMD was primarily driven by the overall eccentric load associated with steep downhill running (Chen et al., 2009; Coratella et al., 2024; Park & Lee, 2015; Pokora et al., 2014), rather than by the modest differences in impact forces observed between stride frequency conditions.

Together, these observations indicate that increasing stride frequency may reduce mechanical loading but simultaneously raise metabolic demands, ultimately producing a neuromuscular fatigue profile similar to that of preferred stride frequency. The net effect is therefore a trade-off: mechanical impact attenuation is achieved at the expense of increased cardiometabolic strain, with no apparent benefit on neuromuscular preservation or muscle damage during steep downhill running.

Uphill performance following PSF and ISF downhill running

Post ISF, uphill performance deteriorated to a greater extent than after PSF, with TT_{ISF} showing an average performance loss of 13.6%, compared with 9.9% observed after PSF. This was accompanied by a consistently slower pacing profile throughout the climb, indicating that the higher cost accumulated during the ISF descent translated into a reduced ability to sustain speed during the uphill effort.

Metabolic and ventilatory variables further highlight the greater internal load following ISF. Specifically: VE/speed was higher in ISF than PSF, indicating a greater ventilatory requirement per unit of speed and reflecting increased ventilatory strain in the fatigued condition; Rf/speed was also elevated in ISF, suggesting reduced ventilatory efficiency and a stronger reliance on high-frequency breathing patterns typically observed under heavy metabolic stress; HR/speed showed the largest divergence, with ISF eliciting markedly higher ratios than PSF, indicating a greater cardiovascular cost per unit of mechanical output and a stronger HR–velocity decoupling; VO_2/HR was lower in ISF than PSF, reflecting a reduction in cardiac efficiency and stroke volume contribution following the ISF downhill protocol.

Together, these findings indicate that stride-frequency manipulation during downhill running did not improve durability but instead exacerbated internal load accumulation, leading to a disproportionate rise in ventilatory and cardiovascular cost relative to mechanical output during the subsequent uphill TT.

Interestingly, peak blood lactate concentrations were highest when the uphill time trial was performed in a fresh state, whereas both post-fatigue trials (PSF and ISF) elicited substantially lower lactate values despite comparable or higher ratings of perceived exertion. This pattern is consistent with previous work showing that eccentric-induced muscle damage and neuromuscular fatigue limit the ability to reach high glycolytic flux during subsequent exercise bouts (Byrne et al., 2004; Chen et al., 2007). After prolonged downhill running, reduced force-generating capacity and impaired excitation–contraction coupling constrain power output and the recruitment of fast-twitch fibres (Howatson & van Someren, 2008; Proske & Morgan, 2001), thereby lowering lactate production even when perceived effort remains high.

The similar lactate responses observed in PSF and ISF further indicate that the performance differences between these conditions are unlikely to be explained by differences in systemic metabolic stress, and are instead more plausibly driven by biomechanical load, neuromuscular function, and cardiometabolic decoupling induced during the downhill bout.

Uphill performance and durability in well trained and elite trail runners

The present study offers new insights into durability in trail running by examining both fresh uphill performance and the subsequent performance decline induced by prolonged uphill–downhill fatigue. When performed in a fresh state, uphill time-trial performance was strongly determined by VO_2max, local knee-extensor endurance, and, to a lesser extent, rapid force production, confirming that aerobic capacity and neuromuscular resilience represent key performance determinants in steep uphill locomotion (Ehrström et al., 2018; Lemire, Hureau, et al., 2021). However, these same variables did not predict the magnitude of performance deterioration under fatigued conditions. This dissociation suggests that the physiological attributes governing maximal performance in fresh conditions do not necessarily translate into fatigue resilience, in line with recent endurance frameworks indicating that "durability" emerges as a distinct performance construct rather than a linear extension of classical physiological capacities (B. Hunter et al., 2025; Maunder et al., 2021).

Importantly, the present findings should be interpreted within the specific temporal and physiological framework of the experimental model. In the present study, durability was assessed as a short-term manifestation of fatigue-related performance maintenance under controlled conditions (B. Hunter et al., 2025; Maunder et al., 2021), through the performance decline observed after a standardized uphill–downhill sequence followed by an uphill time-trial. Accordingly, the observed reduction in performance reflects an acute response to accumulated exercise stress, likely influenced by downhill-induced neuromuscular fatigue and exercise-induced muscle damage, rather than the broader durability demands of prolonged trail or ultra-endurance competitions. Although this model does not reproduce the full complexity of long-duration off-road races, it provides a useful framework to examine how prior downhill load may impair subsequent uphill performance. Therefore, the present results should be interpreted primarily as evidence of short-term durability, and caution is needed when extrapolating these findings to substantially longer events (B. Hunter et al., 2025; Maunder et al., 2021).

In contrast to the lack of association with baseline physiological and neuromuscular metrics, cardiometabolic decoupling, expressed as the ratio between internal load (HR, VE, Rf) and mechanical output (Rothschild et al., 2025; Smyth et al., 2022), showed a strong and consistent relationship with the percentage decline in performance. A greater decoupling ratio reflects a disproportionate increase in physiological effort relative to speed, indicating reduced efficiency in converting internal work into mechanical propulsion Athletes exhibiting larger HR–speed, VE–speed, and Rf–speed decoupling were those who experienced the greatest performance loss. This supports the notion that durability is characterised by the ability to maintain coupling between internal physiological strain and external output despite accumulating fatigue (B. Hunter et al., 2025; Maunder et al., 2021).

HR and Rf decoupling were identified as strong correlates of the durability of the moderate-to-heavy transition, suggesting that monitoring internal–external load drift during prolonged efforts can provide a practical assessment of an athlete's fatigue resilience. Importantly, Rothschild (2025) reported that VE decoupling did not predict durability in cycling (Rothschild et al., 2025), despite VE being sensitive to multiple metabolic inputs (Nicolò et al., 2020; Tipton et al., 2017), likely because VE at VT1 remains relatively stable during prolonged moderate-intensity efforts (Nuuttila et al., 2025; Stevenson et al., 2024).

The present findings partially differ from this pattern. In our study, VE/speed showed a strong relationship with performance decline, which may be explained by the different physiological context: the uphill TT occurred well above VT_2, in the severe domain, where ventilatory drive is no longer tightly coupled to metabolic steady-state kinetics and instead reflects increasing respiratory muscle work, afferent feedback, and loss of mechanical economy.

Notably, although decoupling accurately predicted performance decline, the source of this response remained unclear: inter-individual differences in decoupling were not explained by metabolic responses, neuromuscular fatigue indices, mechanical loading during the downhill bout, or anthropometric variables. One possible explanation for this unresolved inter-individual variability is that relevant neuromechanical determinants were not directly assessed in the present study. Although the post-downhill reductions observed in MVC and rapid force production indicate an acute neuromuscular impairment, no direct measures of tendon properties, lower-limb stiffness, or joint-level mechanics were collected, limiting the mechanistic interpretation of why some athletes exhibited greater cardiometabolic decoupling and larger performance decline than others. Recent evidence indicates that higher vertical and leg stiffness are associated with better running economy

(Liu et al., 2022; Van Hooren et al., 2024) and that greater passive Achilles tendon stiffness is related to a lower oxygen cost during running (A. Konrad et al., 2023). In parallel, downhill running has been shown to impair maximal force production and rapid force-generating capacity, although the phase-specific pattern may depend on the experimental model (Varesco et al., 2022). Although these mechanisms were not measured here, they may plausibly contribute to the individual differences in efficiency loss observed under fatigue and should be considered in future studies combining physiological monitoring with direct biomechanical and muscle–tendon assessments. From a practical perspective, these findings highlight the potential relevance of monitoring internal–external load coupling as a simple field-based indicator of durability. HR–speed or VE–speed drift have been proposed as meaningful markers of efficiency loss under fatigue and may help identify athletes capable of maintaining performance despite substantial physiological strain (Rothschild et al., 2025). However, the lack of mechanistic predictors in the present data underscores the need for future research to explore more refined biomechanical, neuromuscular, or perceptual markers to better understand the origins of athlete-specific fatigue resilience.

5.6 Conclusion

This study examined whether increasing stride frequency during prolonged steep downhill running could reduce mechanical stress, attenuate neuromuscular fatigue, or improve subsequent uphill performance. Although the ISF condition effectively reduced impact-related forces, it substantially increased metabolic and ventilatory cost, resulting in greater internal load accumulation. Importantly, these physiological differences did not translate into differences in muscle damage or neuromuscular impairment: both stride-frequency conditions induced comparable reductions in maximal voluntary torque, rate of force development, and EMG-derived indicators of fatigue. As a consequence, the uphill TT was more impaired after ISF, suggesting that the increased metabolic strain accumulated during the descent outweighed any mechanical benefits of higher cadence.

A central contribution of this work concerns the concept of durability. While VO_2max and local knee-extensor endurance strongly predicted uphill performance in a fresh state, none of the traditional physiological or neuromuscular markers explained the inter-individual differences in fatigue-induced performance decline. Instead, cardiometabolic decoupling (HR-, VE-, and Rf-speed ratios) emerged as the most consistent correlate of performance deterioration, supporting the idea that durability represents an independent performance trait not fully captured by standard laboratory metrics.

Limitations of this study include the all-male sample, the use of controlled treadmill downhill running rather than natural terrain, the relatively short uphill time trial, and the focus on quadriceps EMG rather than a broader set of muscle groups involved in downhill running. The modest sample size, although typical for laboratory-based research, also limits the sensitivity of regression analyses exploring predictors of durability.

Future research should examine stride-frequency manipulation in more technical and variable outdoor downhill conditions, include female athletes, and test longer uphill efforts that better reflect race demands. Mechanistic studies incorporating additional neuromuscular or muscle–tendon metrics may help clarify the origins of cardiometabolic decoupling and fatigue resilience. Finally, intervention studies could evaluate whether targeted eccentric training or technical downhill skill development can improve durability and reduce internal–external load drift during extended trail-running efforts.

Chapter 6 – Shoes testing and development

6.1 Collaborative framework and research context

As part of this PhD program, a collaborative agreement was established with Treré Innovation s.r.l., with the aim of supporting the development of trail running footwear through scientific testing and evaluation. The overarching goal of this partnership was to contribute to the design and validation of trail running shoes integrating newer technologies, advanced materials, and performance requirements specific to off-road running.

Throughout the project, several prototype models were provided by the company at different stages of development. These prototypes were tested under various experimental and field conditions, allowing the assessment of their functional properties and their potential impact on running performance and biomechanics. While the degree of product development varied across prototypes, these collaborations offered valuable opportunities to bridge applied research and industrial innovation in trail footwear.

Nevertheless, the industrial development process evolved independently from the academic timeline, which limited the opportunity for a fully integrated co-design phase. As a result, the prototypes were mainly evaluated from a functional and biomechanical standpoint, without detailed information on material composition or midsole architecture. Consequently, the research focused on analysing athletes' responses to the tested models rather than on the mechanical characterization of the footwear itself.

The following sections summarize the experimental activities conducted with the available prototypes, including both laboratory and field-based testing, aimed at exploring how different shoe designs may influence running biomechanics, comfort perception, and overall performance in trail running contexts. Although the studies presented in this chapter were exploratory and primarily aimed at providing applied feedback for product development, they can also be interpreted within the broader framework of this dissertation. In particular, the prototype evaluations offer a translational perspective on how footwear design may interact with some of the biomechanical and neuromuscular demands identified in the previous chapters, including the need for foot stability on uneven terrain and the balance between mechanical protection and metabolic cost during uphill and downhill running. For this reason, the results reported here should not be interpreted as definitive validation of specific shoe technologies, but rather as preliminary observations on how selected design solutions may respond to the functional constraints of trail running.

6.2 Laboratory evaluation of hybrid (gravel/off-road) running shoe prototypes

6.2.1 Introduction

Recent innovations in running footwear have increasingly aimed to optimize both energetic efficiency and mechanical performance by refining midsole geometry, material composition, and stiffness characteristics. While these developments have been widely investigated in road running, their application to off-road or mixed-terrain footwear remains less explored. Hybrid or “gravel” shoes—designed for use across both compact trails and unpaved surfaces—must balance cushioning, traction, and stability while maintaining energetic efficiency comparable to road models.

This preliminary project aimed to provide a laboratory-based evaluation of prototype hybrid running shoes developed by Treré Innovation s.r.l., in comparison with a commercially available reference model (Hoka). The focus was to assess differences in comfort, energetic cost, and biomechanical behaviour under controlled treadmill conditions, in order to generate feedback for further product refinement and scientific understanding of shoe–runner interactions on mixed terrain.

6.2.2 Methods

Three shoe models were tested: two prototypes provided by Treré Innovation (UYN Blue and UYN Red), featuring identical midsole materials and geometries but differing in the inclusion of a polyester plate insert within the midsole, and one commercial reference shoe (Hoka). All tests were conducted on a motorized treadmill under standardized environmental conditions.

Participants and protocol

Athlete performed trials with all three shoe models in a randomized order, running at two fixed speeds (12 km·h^{-1} and 17 km·h^{-1}). Between conditions, sufficient rest was provided to ensure full recovery and stable physiological measurements.

Measurements

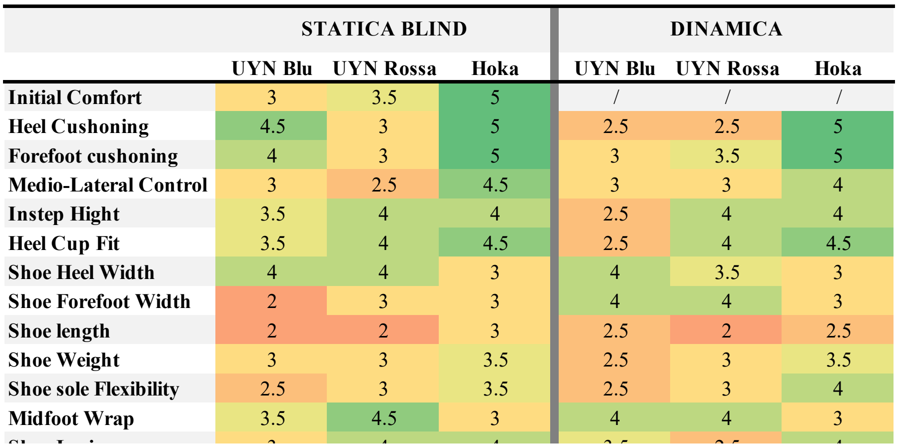
The following parameters were evaluated for each footwear condition:

- Subjective comfort: assessed through a blind static and dynamic questionnaire using a 1–5 Likert scale, covering heel cushioning, stability, flexibility, and overall perception.
- Energetic cost: oxygen consumption ($\dot{V}O_2$) was measured at 12 $km \cdot h^{-1}$ under steady-state conditions, and converted to energy cost ($J \cdot kg^{-1} \cdot m^{-1}$).
- Kinematic analysis: a 3D motion capture system (200 Hz) recorded six reflective markers positioned on the shoe (heel, malleoli, toe, and forefoot regions) to compute stride cycle duration, contact time, duty cycle, and foot rotation angles.
- Plantar pressure: in-shoe pressure insoles (99 sensors) measured total vertical force, force distribution (rearfoot, midfoot, forefoot), and the trajectory of the centre of pressure (COP).

All data were averaged over steady-state running phases, and results were analysed descriptively, given the exploratory nature of the study.

6.2.3 Results

Comfort ratings revealed the commercial Hoka model received the highest overall evaluation (average 4–5/5), while the UYN prototypes showed lower ratings in heel cushioning and overall comfort, particularly the Red version.



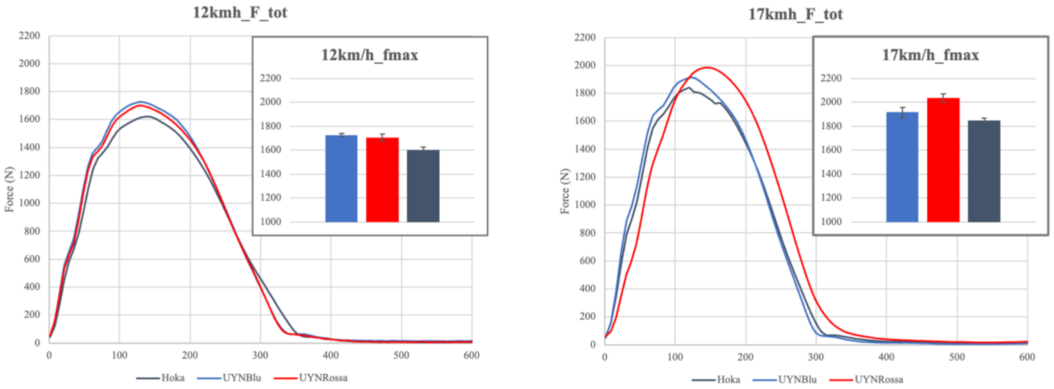
	STATICA BLIND			DINAMICA		
	UYN Blu	**UYN Rossa**	**Hoka**	**UYN Blu**	**UYN Rossa**	**Hoka**
Initial Comfort	3	3.5	5	/	/	/
Heel Cushioning	4.5	3	5	2.5	2.5	5
Forefoot cushioning	4	3	5	3	3.5	5
Medio-Lateral Control	3	2.5	4.5	3	3	4
Instep Hight	3.5	4	4	2.5	4	4
Heel Cup Fit	3.5	4	4.5	2.5	4	4.5
Shoe Heel Width	4	4	3	4	3.5	3
Shoe Forefoot Width	2	3	3	4	4	3
Shoe length	2	2	3	2.5	2	2.5
Shoe Weight	3	3	3.5	2.5	3	3.5
Shoe sole Flexibility	2.5	3	3.5	2.5	3	4
Midfoot Wrap	3.5	4.5	3	4	4	3

Table 12 Static and dynamic comfort ratings for the three tested shoe models (UYN Blue, UYN Red, and Hoka). Mean scores (1–5 scale) are shown for each comfort parameter under static blind and dynamic conditions. Higher scores indicate greater perceived comfort. Colour gradients represent relative evaluations, with green indicating higher comfort and red indicating lower comfort.

Energetic cost differed only slightly between conditions: UYN Blue (3.54 J·kg⁻¹·m⁻¹), UYN Red (3.79 J·kg⁻¹·m⁻¹), and Hoka (3.51 J·kg⁻¹·m⁻¹), indicating slightly higher efficiency for Hoka and UYN Blue compared to UYN Red.

Stride parameters (cycle duration, contact time, and duty cycle) were similar across models, though the UYN Blue showed marginally shorter contact times and a lower duty cycle, potentially reflecting improved transition dynamics.

Plantar pressure and force distribution analyses showed consistent total vertical forces across footwear, with minor shifts in regional loading: UYN Red exhibited greater midfoot and forefoot loading, while Hoka displayed a more balanced COP trajectory.



	UYN Blu	UYN Rossa	Hoka
Force (N)	1726.8	1703.6	1598.9
SD (N)	13.5	26.6	24.9

	UYN Blu	UYN Rossa	Hoka
Force (N)	1915.8	2037.2	1848.5
SD (N)	41.1	33.5	18.6

Figure 25 Total vertical ground reaction force during running at 12 km·h⁻¹ (left) and 17 km·h⁻¹ (right) for the three shoe models (UYN Blue, UYN Red, and Hoka). Mean force–time curves are shown for each shoe, with corresponding mean peak force values reported in the insets and summary tables below.

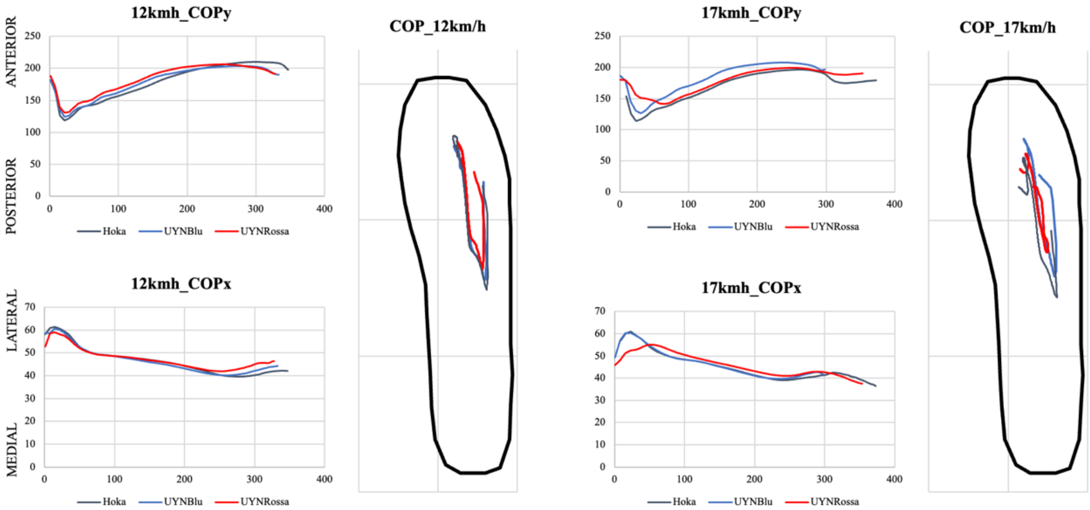


Figure 26 Centre of pressure (COP) trajectories during running at 12 $km \cdot h^{-1}$ and 17 $km \cdot h^{-1}$ for the three shoe models (UYN Blue, UYN Red, and Hoka). Mean anterior–posterior (COPy, top) and medial–lateral (COPx, bottom) displacements are shown, with the corresponding COP paths (right panels) plotted within the footprint outline. Higher values on the y-axis indicate forward progression, while higher x-axis values represent a more lateral COP position. Data indicate comparable COP trajectories among shoes, with minimal differences in both directions across speeds.

Kinematic analysis revealed comparable foot roll and inclination angles among shoes, with Hoka demonstrating slightly reduced torsional movement during stance. Stride frequency and length were not significantly influenced by footwear type.

6.2.4 Discussion

Overall, this laboratory evaluation identified only small differences among the tested models. The commercial Hoka shoe demonstrated slightly superior comfort and marginally lower energetic cost, while the UYN Blue prototype showed comparable biomechanical performance and potential for refinement.

These findings suggest that the tested prototypes provided adequate mechanical behaviour and running efficiency for hybrid or off-road use, though improvements in cushioning distribution and weight balance could further enhance comfort and perceived smoothness.

From a biomechanical perspective, these observations may also be interpreted in light of the findings reported in Chapter 2, where running on uneven terrain was associated with greater activation of the tibialis anterior and peroneus longus, suggesting an increased demand for foot stabilization and control of inversion–eversion movements. In this context, footwear solutions designed to improve rearfoot guidance and lateral support may be functionally relevant in off-road running. In the present prototypes, the insert extended from the plantar region towards the lateral heel, and this configuration may have contributed to the perception of stability and to the maintenance of comparable kinematic behaviour across conditions. However, because the mechanical properties of the insert and midsole were not directly characterized, this interpretation should be considered only as a functional hypothesis rather than as evidence of a specific stabilizing mechanism.

This project served as an initial stage of applied validation within the broader collaboration between academia and industry, offering valuable insights for future development of trail and gravel running footwear. The results underline the relevance of combining biomechanical and perceptual data in prototype testing, particularly when designing shoes for mixed-terrain conditions where stability, cushioning, and energy return must be carefully balanced.

6.3 Laboratory and field evaluation of trail running shoe prototypes

6.3.1 Introduction

This project aimed to evaluate prototype trail running shoes provided by Treré Innovation (models XCC and X02, with and without carbon plate respectively) compared to two commercial references (Nnormal Tomir and La Sportiva Bushido II). The investigation focused on the energetic, neuromuscular, and biomechanical responses during uphill and downhill running, both in controlled laboratory conditions and in real outdoor environments. The goal was to assess the functional performance and comfort of the prototypes, contributing to the validation of their design for trail running use.

6.3.2 Methods

Four trained male trail runners participated in two testing sessions:

- ➢ Indoor testing: uphill treadmill running (15% incline) for 5 min at a self-selected submaximal speed.
    - Measured variables: ventilatory and metabolic parameters (Cosmed CPET), surface EMG activity of six lower-limb muscles (Peroneus Longus, Tibialis

  Anterior, Rectus Femoris, Vastus Lateralis, Biceps Femoris, Gluteus Medius), and plantar pressure (Novel Pedar insoles).
  - After each condition, participants rated shoe comfort using a standardized 1–5 Likert scale.

- ➢ Outdoor testing: performed on a 100 m mountain trail (≈ 15% gradient), alternating uphill and downhill runs under two intensities: self-selected “race pace” and all-out effort.
  - Collected data: EMG of the same six muscles, in-shoe pressure data, and comfort ratings.
  - Each participant completed six runs per shoe (three uphill + three downhill) in randomized order.

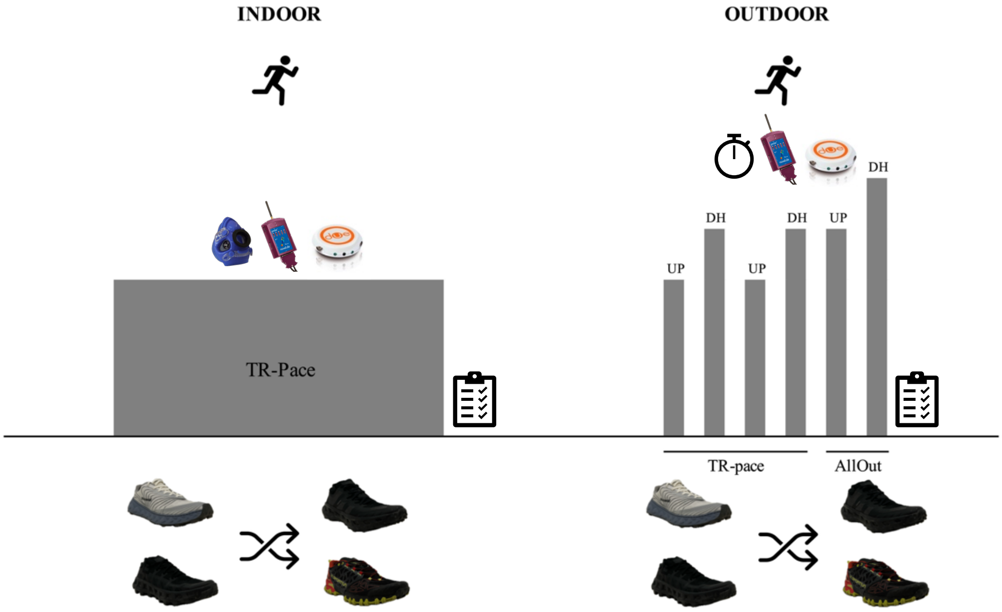


Figure 27 Schematic overview of the experimental protocol. The study included indoor treadmill test at individual self selected TR pace and outdoor trials on natural terrain combining uphill (UP) and downhill (DH) segments at self selected TR pace and all-out intensity.

6.3.3

Indoor testing

No significant differences were found among the four shoe models in oxygen consumption, ventilation, or heart rate.

Soggetto	**Scarpa**	**Speed**	**Slope**	**Rf**	**VE**	**VO2**	**VCO2**	**RQ**	**HR**
---	**---**	**km/h**	**%**	**1/min**	**L/min**	**mL/min**	**mL/min**	**---**	**bpm**
S1	Baseline	---	---	11.80	7.93	288.15	233.43	0.80	59
	Nnormal	7.4	15	31.78	95.13	4170.13	3755.40	0.90	150
	Bushido	7.4	15	32.46	95.87	4159.67	3721.94	0.89	155
	XOOC	7.4	15	31.03	94.40	4162.66	3727.89	0.90	157
	X02	7.4	15	32.42	93.37	4148.82	3590.78	0.87	156
S2	Baseline	---	---	19.08	20.12	543.67	476.74	0.88	66
	Nnormal	5.5	15	36.91	89.37	2913.59	2617.82	0.90	138
	Bushido	5.5	15	41.69	99.42	2965.58	2727.53	0.92	141
	XOOC	5.5	15	37.32	93.79	3114.21	2802.51	0.90	144
	X02	5.5	15	41.69	99.42	2965.58	2727.53	0.92	141
S3	Baseline	---	---	10.04	13.17	334.05	341.91	1.02	90
	Nnormal	5.5	15	33.55	88.23	3011.02	3032.03	1.01	173
	Bushido	5.5	15	29.96	82.33	3005.50	3011.26	1.00	171
	XOOC	5.5	15	32.88	85.60	2936.04	3018.46	1.03	169
	X02	5.5	15	31.08	87.24	3060.62	3272.66	1.07	162
S4	Baseline	---	---	13.76	12.70	382.17	347.60	0.91	79
	Nnormal	7	0.15	25.32	78.50	3530.96	3370.12	0.95	152
	Bushido	7	0.15	25.84	80.99	3402.15	3321.76	0.98	144
	XOOC	7	0.15	26.89	85.45	3587.57	3397.38	0.95	156
	X02	7	0.15	28.99	87.77	3542.58	3395.35	0.96	154

Table 13 Individual physiological responses during uphill treadmill running across footwear conditions. Respiratory frequency (Rf), minute ventilation (VE), oxygen uptake (VO_2), carbon dioxide output (VCO_2), respiratory quotient (RQ), and heart rate (HR) are reported for each subject (S1–S4) at the tested speed and slope under different footwear conditions (Baseline, Normal, Bushido, XOOC, X02).

EMG analysis revealed only minor variations in muscle activation, with slightly higher activity of Vastus Lateralis and Rectus Femoris in the X02 and Bushido II models, but without statistical relevance.

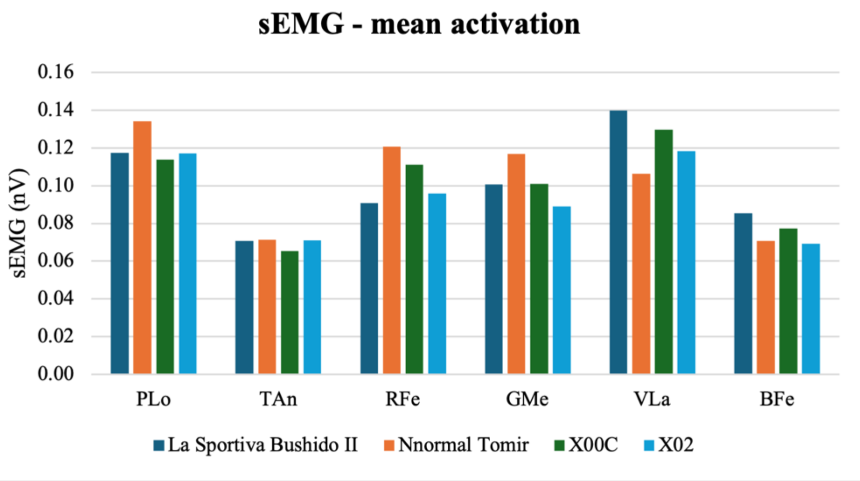


Figure 28 Mean muscle activation across footwear conditions during uphill running. Values represent the average activation across subjects for six lower-limb muscles: PLo, TAn, RFe, GMe, VLa, and BFe, tested with four footwear models: La Sportiva Bushido II, Nnormal Tomir, X00C, and X02.

Plantar pressure and force distribution showed comparable contact times and total forces across footwear.

Subjective comfort ratings identified the X02 model as the most appreciated, particularly for heel fit, forefoot cushioning, and flexibility.

Outdoor testing

Both uphill and downhill running showed similar neuromuscular activation patterns across shoes. During all-out efforts, activation increased slightly in Gluteus Medius and Vastus Lateralis, yet no systematic differences between footwear were detected.

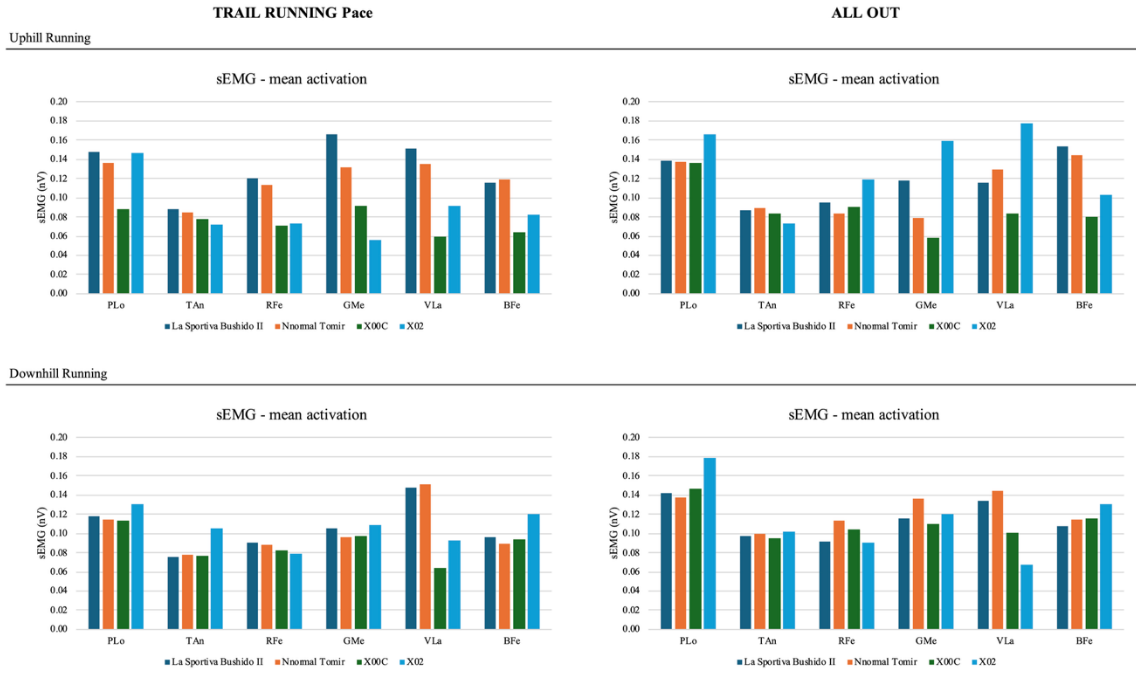


Figure 29 Mean muscle activation across footwear conditions during trail running at different paces and slopes. Average activation (mean sEMG) across subjects for six lower-limb muscles (PLo, TAn, RFe, GMe, VLa, BFe) during uphill and downhill running, performed at trail running pace (left) and all-out pace (right). Data are reported for four footwear models: La Sportiva Bushido II, Nnormal Tomir, X00C, and X02.

Plantar pressure analysis confirmed consistent force distribution among models and terrain conditions.

Comfort evaluation again favoured the X02, which obtained the highest overall rating (4.6 / 5), while Bushido II was rated lowest in cushioning and fit.

6.3.4 Discussion

Overall, the laboratory and field data revealed minimal performance and biomechanical differences between prototypes and commercial references. Although energetic and neuromuscular parameters were comparable, subjective assessments consistently indicated greater comfort and better perceived stability for the X02 model.

These findings suggest that the prototypes achieved a functional level similar to established trail shoes, supporting their suitability for mixed and technical terrains. Within the broader framework of this dissertation, these applied findings are also relevant because they suggest that footwear design may influence trail-running performance not only through comfort, but also through its interaction with the mechanical and neuromuscular demands of the task. In previous chapters, downhill and uneven running were shown to increase external loading, destabilization demands, and fatigue-related performance deterioration. In this context, shoe features intended to improve cushioning, rearfoot control, or longitudinal stiffness may be relevant because they could influence how mechanical load is distributed and perceived during uphill and downhill running. The inclusion of composite or plated elements in some of the tested prototypes reflects this broader design rationale. However, since no direct mechanical characterization of the footwear was available and only minimal between-shoe differences were observed in the present exploratory testing, no conclusion can be drawn regarding their specific effect on fatigue accumulation or durability. Rather, these results support the feasibility of integrating footwear testing into a broader performance model in which mechanical support, comfort, and energetic cost should be considered together. Nonetheless, the absence of detailed information on midsole structure and materials limits a deeper interpretation of the observed differences.

In summary, this study provided a combined laboratory–field assessment of prototype trail shoes, demonstrating that functional testing in both controlled and ecological environments can effectively guide iterative product refinement. The X02 model showed promising comfort and performance characteristics, offering a solid base for further development in collaboration between research and industry. Taken together, these exploratory projects suggest that prototype footwear evaluation can complement biomechanical research in trail running by providing preliminary, practice-oriented observations on how cushioning, stability-oriented structures, and stiffness-related design choices may relate to the specific neuromuscular and mechanical constraints of off-road locomotion.

Chapter 7 – Additional project conducted during an international research stay: performance variability across elite trail running competitions

7.1 Additional project conducted during an international research stay: performance variability across elite trail running competitions

During my international research stay at LUNEX University, I was involved in several research and academic activities. Among these, I developed an independent project that is aligned with the core themes of this thesis but methodologically separate from the main experimental studies. In collaboration with the host research group, I performed an exploratory analysis of a large performance database including multiple elite trail running competitions, with the aim of quantifying race-to-race performance variability and assessing how results in one event can predict outcomes in subsequent races. This project is still ongoing, and the analyses presented here are preliminary and mainly hypothesis-generating, intended to provide additional context for the interpretation of the main findings of the thesis.

7.2 Introduction and aim

Trail running is characterized by a wide variability in course profiles, terrain characteristics and environmental conditions, ranging from short technical races with steep ascents and descents to long-distance ultra events (ITRA; Scheer et al., 2020). Unlike track and road running, where distances and course layouts are highly standardized, trail running competitions take place on heterogeneous routes with differing elevation gain, surface properties and technical difficulty. This lack of standardization, combined with variable weather, temperature and altitude, makes it challenging to monitor performance over time and to compare results across races or seasons in a consistent way.

Several studies have attempted to describe pacing strategies and performance profiles in trail running by analysing intermediate split times and race dynamics (Corbí-Santamaría et al., 2023; De Waal et al., 2025; Genitrini et al., 2022; Gutiérrez et al., 2025; Jaén-Carrillo et al., 2025; Markovic et al., 2025; Suter et al., 2020). However, these investigations have generally reported substantial variability in both pacing patterns and performance outcomes, and are often limited to single events, specific distances or relatively small samples. At the same time, in elite settings, distinguishing a meaningful improvement from random variation is essential. A small but real improvement is often defined as 0.3 times the standard deviation of an athlete's variability (Malcata & Hopkins, 2014; Skattebo & Losnegard, 2018), but applying this criterion requires accurate, sport-specific estimates of within-athlete performance variability, which are currently lacking in trail running.

Within this context, the present project was designed to address this gap. This study aimed to quantify race-to-race performance variability and assess predictability in elite trail running competitions of various distances over a single season.

7.3 Materials and methods

7.3.1 Data source and athlete selection

Race results were retrieved from publicly available databases of international trail and mountain running competitions from the 2024 season. To focus on high-level performers, only male elite athletes were included, defined as those with an ITRA Performance Index ≥ 825. The ITRA Performance Index provides an estimate of an athlete's performance level on a 0–1000 scale, calculated as a weighted average of the runner's five best results over the previous 36 months, with recent and top performances contributing more to the score. Based on this criterion, a total of 941 athletes were included in the database.

7.3.2 Race selection and categorisation

A total of 216 races from the 2024 season were selected from major international and national circuits, including the Golden Trail World and National Series, Skyrunning World and National Series, ISF Vertical Kilometer (VK) Open Championship, World and European Championships, all WMRA (World Mountain Running Association) events, and major ultratrail races. Races were categorised according to the official ITRA classification into uphill-only (UP) and distance-based categories (XXS, XS, S, M–L, XL–XXL). Separate datasets were created for each category to allow distance-specific analyses of performance variability.

7.3.3 Data processing and statistical analysis

Athlete–race matching and data processing were performed using a custom MATLAB script, which identified repeated participations of the same athlete across races within each category. Athletes who did not compete in a given category and races with fewer than five eligible elite athletes were excluded from the corresponding analyses. For each athlete and race category, within-athlete variability was quantified as the coefficient of variation (CV) and standard deviation (SD) of race times across competitions. Between-athlete variability was expressed as the standard deviation of individual CVs within each category. Performance predictability across races was assessed using intraclass correlation coefficients (ICC), calculated with Hopkins' spreadsheet (Hopkins, 2015).

7.4 Results

Overall, within-athlete race-to-race variability ranged from ~1 to 8% across race categories, with shorter races showing lower variability and higher predictability, and intermediate–long distances presenting slightly greater variability. Intraclass correlation coefficients indicated moderate-to-very-high performance predictability for most categories, with some intermediate distances showing only moderate reliability. Detailed values for each race distance are reported in Table 14 and illustrated in Figure 30.

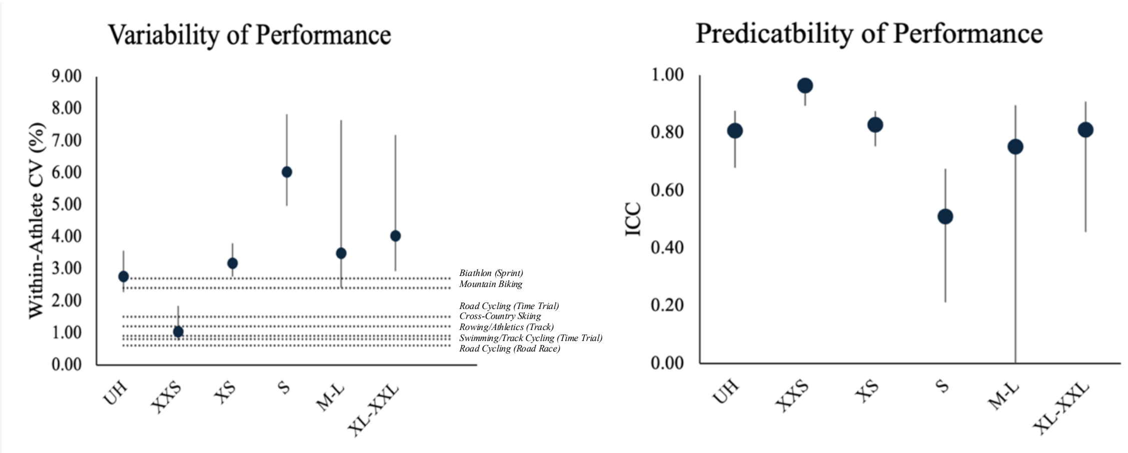


Figure 30 Within-athlete race-to-race variability and performance predictability across elite trail running race categories. The left panel shows mean within-athlete coefficient of variation (CV) in finishing time for each race category, with vertical bars indicating between-athlete spread. The right panel shows intraclass correlation coefficients (ICC) with 95% confidence intervals, expressing the predictability of performance across races in each category. Dashed horizontal lines in the left panel represent reference values for within-athlete variability reported in other endurance sports (Skattebo & Losnegard, 2018; Malcata & Hopkins, 2014).

Distance	Sex	Year	n	Avg Time (min)	Between Subject SD % (Variability)	Within Subject SD % (Variability)	95% CI		ICC (predictability)	95% CI		Descriptor ICC
UH	M	2024	149	50.35	6.38	2.77	2.27	3.57	0.81	0.68	0.88	High
XXS	M	2024	139	51.42	5.58	1.05	0.77	1.85	0.96	0.89	0.98	Very high
XS	M	2024	291	116.15	7.81	3.19	2.75	3.79	0.83	0.75	0.88	High
S	M	2024	274	208.22	8.71	6.03	4.96	7.83	0.51	0.21	0.67	Moderate
M-L	M	2024	143	352.88	7.10	3.50	2.42	7.63	0.75	0.00	0.89	Moderate
XL-XXL	M	2024	137	945.29	9.43	4.04	2.93	7.17	0.81	0.46	0.91	High

Table 14 Race-to-race variability and performance predictability across elite male trail running race categories. For each race category, the table reports sample size (N), mean finishing time, between-subject variability (SD%), within-subject race-to-race variability (SD%), and intraclass correlation coefficients (ICC) with 95% confidence intervals, together with qualitative descriptors of ICC magnitude.

7.5 Conclusions and limitations

In summary, this exploratory project provides initial benchmarks of race-to-race performance variability and predictability in elite trail running. Across a wide range of race categories, within-athlete variability in finishing times remained relatively small, with shorter uphill and short-distance events exhibiting lower variability and higher predictability than intermediate and longer races. These findings suggest that, despite the inherent heterogeneity of trail running courses, elite athletes tend to display reasonably stable performance profiles within a given race category over a single season. Although preliminary, these estimates may offer a starting point for defining meaningful performance changes and for interpreting longitudinal trends in elite trail running results.

This project has several important limitations that must be acknowledged when interpreting the findings. First, the analyses are based on a single competitive season and include only male elite athletes above a given ITRA threshold; therefore, the results cannot be generalised to other performance levels, to female athletes or to multi-year trends. A more robust characterisation of performance variability would require extending the analysis across multiple seasons and explicitly incorporating the women’s dataset, which has already been collected using the same criteria (with the elite threshold set at

700 ITRA points). More critically, the main conceptual limitation concerns the use of finishing time as the sole outcome variable. The original approach was inspired by work in more standardised sports, where events are held over comparable distances and conditions, making race times broadly comparable across competitions. In trail running, however, even nominally similar races within the same distance category can differ substantially in elevation gain, altitude, technical difficulty and environmental conditions, and the same event may change from year to year due to weather or course modifications. All these factors make it difficult to separate actual performance changes from variability driven by course and environmental differences, and they weaken the assumptions underlying the calculation of coefficients of variation and intraclass correlations based solely on race times. Several normalisation strategies (e.g., adjusting for distance and elevation, or using flat-kilometre coefficients) were considered and tested, but they did not fully resolve these issues and often produced unstable or imprecise estimates. For these reasons, the present analysis should be viewed as exploratory and hypothesis-generating rather than as a definitive quantification of performance variability in trail running. Future work may need to adopt alternative designs, such as focusing on a single major event (e.g., Sierre–Zinal or UTMB) and following returning athletes across multiple editions, and/or integrating detailed course descriptors and environmental data into more complex statistical models.

Chapter 8 – General discussion

8.1 Recap

Trail running is an endurance discipline in which performance emerges from the interaction of energetic, biomechanical, and neuromuscular demands under highly variable environmental and terrain conditions. Unlike road running, where speed is typically maintained on relatively uniform surfaces and moderate gradients, trail running is characterised by frequent transitions between steep ascents and descents, irregular and unstable ground, and prolonged race durations. These features alter the distribution of mechanical work between joints and muscle groups, shift the balance between concentric and eccentric contractions, and impose substantial demands on balance, proprioception, and technical foot placement.

From a physiological and mechanical standpoint, uphill and downhill running present markedly different constraints. Uphill sections are dominated by high metabolic and cardiorespiratory load, with increased positive work and a strong dependence on aerobic capacity and muscular endurance. Downhill sections, conversely, are mechanically demanding despite having a lower metabolic cost, owing to high eccentric braking forces, rapid stretch–shortening cycles, and the need to stabilize the body over irregular contact points. Superimposed on this, uneven terrain introduces continuous small perturbations to foot strike and joint loading, requiring fine neuromuscular control and potentially increasing both energetic cost and local muscle fatigue. Consequently, trail running performance cannot be attributed to a single limiting factor, but instead reflects how athletes coordinate these subsystems across changing slopes and surfaces, and how they tolerate and manage accumulating fatigue over time.

These characteristics pose specific challenges for scientific investigation. Classical laboratory tests, usually performed on even surfaces and at steady speeds, only partially capture the demands of off-road running and may underestimate the contribution of neuromuscular control and eccentric loading. Field studies, on the other hand, offer ecological validity but often lack detailed control over speed, stride mechanics, or surface properties, which makes it difficult to isolate causal relationships. Moreover, most existing models of endurance performance are based on relatively homogeneous conditions and do not explicitly account for slope-dependent mechanics, terrain irregularity, or the concept of “durability”.

Within this context, the overarching aim of the present doctoral project was to advance the understanding of trail running performance by integrating laboratory-based and field-based approaches. Specifically, the thesis sought to clarify: (i) how running on uneven terrain affects energetic cost and neuromuscular activation when biomechanical variables are tightly controlled; (ii) how uphill and downhill segments contribute to overall race performance and how athletes of different performance levels modulate their kinematics in competition; (iii) how sex differences in physiology and biomechanics manifest in real trail running, when male and female athletes are matched for performance; and (iv) how downhill running strategy, particularly stride frequency, influences neuromuscular fatigue, subsequent uphill performance, and durability.

To address these questions, four complementary studies were conducted. The first (Chapter 2) used a controlled laboratory design to isolate the effect of surface unevenness on metabolic cost and lower-limb muscle activation, keeping speed and spatiotemporal parameters constant. The second (Chapter 3) analysed in-race split times and kinematics in uphill and downhill sections, relating segment performance and stride mechanics to overall race outcome in well-trained and elite athletes. The third (Chapter 4) extended this approach to examine sex differences in trail running, focusing on how male and female athletes with comparable performance adopt distinct mechanical strategies and respond to fatigue over successive race sectors. Finally, the fourth study (Chapter 5) implemented an ecologically oriented protocol combining uphill and downhill efforts to test whether manipulating stride frequency during downhill running modifies neuromuscular fatigue, affects subsequent uphill time-trial performance, and reveals physiological and neuromuscular correlates of greater durability.

Taken together, these studies were designed not as isolated experiments but as complementary perspectives on the same problem: how trail runners manage the combined energetic, biomechanical, and neuromuscular demands imposed by uneven terrain, steep gradients, and cumulative fatigue. By bridging standardised laboratory models with high-resolution in-race analyses and a targeted fatigue intervention, the thesis aims to contribute to a more comprehensive framework for understanding performance in trail running and to provide a basis for refining future performance models in this discipline.

8.2 Summary of mail findings

The results of each study (discussed in detail in the corresponding chapters) can be summarised as follows.

Study I – Running on uneven ground: energetic cost and muscular involvement

This study showed that, when running mechanics are rigorously standardised, surface irregularity per se imposes a clear additional physiological and neuromuscular cost. When participants ran on uneven terrain while maintaining identical speed, step frequency and step placement as on an even surface, activation of ankle stabiliser muscles was systematically higher on the uneven surface. This increased neuromuscular demand was accompanied by higher cost of running, elevated cardiometabolic responses, and greater perceived exertion, indicating that the “price” of stabilising the ankle joint and coping with local perturbations is paid both metabolically and neuromuscularly. Contrary to the initial hypothesis, the variability of muscle activation did not increase when the running pattern was constrained to be similar between conditions, suggesting that the primary effect of uneven ground under controlled mechanics is a higher tonic level of activation rather than a more variable activation pattern. Overall, this study demonstrates that terrain irregularity, even when global kinematics are kept constant, meaningfully increases the physiological and neuromuscular load of running, reinforcing the importance of considering surface characteristics when modelling off-road running demands and designing training or injury-prevention strategies.

Study II – Kinematics and performance on uphill and downhill trail running in elite and well-trained athletes

In a highly competitive Skyrunning race, this study examined how uphill and downhill sections contribute to overall performance and how kinematic patterns differ across performance levels. At the global level, the uphill segment accounted for the largest proportion of variance in overall race time, underscoring its central role in determining final classification. However, as performance level decreased, athletes lost a greater relative amount of time in the downhill compared with the uphill section. This pattern indicates that, beyond a certain level, downhill ability becomes a key discriminator between top and lower-placing athletes and represents a larger margin for improvement in sub-elite competitors. Kinematic analyses showed that differences between performance groups were predominantly mediated by running speed, with associated changes in stride parameters. In uphill segments, faster ascent speeds were mainly achieved through higher stride frequency, whereas in downhill segments higher speed was primarily associated with increased stride length. When kinematic variables were normalised for speed, most between-group differences disappeared, suggesting that metabolic, neuromuscular, and technical factors, rather than distinct kinematic "styles", are likely to underpin performance differences at a given speed. Over the course of the race, uphill kinematics remained relatively stable, while downhill kinematics shifted towards longer strides and lower step frequencies in the later segment, a pattern that may reflect accumulating fatigue, adaptation to technical demands, or deliberate pacing strategies. Taken together, these findings highlight the decisive impact of downhill performance on race outcomes in elite Skyrunning and point to downhill-specific technical and neuromuscular training as a key performance lever.

Study III – How sex differences shape performance and running kinematics in competitive trail running

This study investigated how sex differences in trail running manifest when male and female athletes are carefully matched either for relative performance or for absolute performance. When matched for relative performance, men completed the race faster than women and were superior in both uphill and downhill sections, while maintaining a similar distribution of time between segments. In contrast, when matched for absolute performance, women devoted a larger fraction of total race time to the uphill section (being slower there), but partly compensated through comparatively faster downhill running. Kinematic analyses revealed that, in both uphill and downhill running, men in the relative-performance-matched group achieved higher speeds through the expected combination of longer stride length, shorter contact time, and lower duty factor. However, once running speed was included as a covariate, most sex-related differences in kinematics were largely attenuated or disappeared, indicating that speed is the main determinant of the observed mechanical differences. Across segments, both sexes exhibited similar patterns of kinematic adaptation. In uphill sections, a shift towards shorter strides and higher stride frequency was observed in later segments. In downhill sections, an increase in speed and stride length was accompanied by a reduction in stride frequency, with comparatively small changes. When men and women were matched for absolute performance, they exhibited broadly similar kinematic profiles and segment-by-segment adaptations, suggesting that, at comparable speeds, mechanical strategies are largely shared across sexes. Overall, these results indicate that male athletes have a clear performance advantage, particularly in uphill running, but also suggest that female athletes may adopt efficient mechanical solutions, such as slightly lower duty factors at comparable speeds, and that downhill skill and exposure may be especially relevant to their competitive success.

Study IV – Effects of downhill stride frequency on neuromuscular fatigue, uphill performance, and durability in trail runners

This study evaluated whether increasing stride frequency during prolonged steep downhill running could reduce mechanical load, attenuate neuromuscular fatigue, and improve subsequent uphill performance, within a protocol that mimicked typical trail-running terrain transitions. Contrary to the initial hypothesis, a 10% increase in stride frequency during the downhill bout induced clear acute changes in mechanical and metabolic load, but did not reduced muscle damage or preserve neuromuscular function compared with preferred stride frequency. The increased-cadence condition was characterised by higher metabolic and ventilatory demands and greater internal load accumulation, while the preferred-cadence condition exhibited higher impact forces. Despite these differences, both conditions resulted in similar decrements in maximal voluntary torque, rapid force production, and EMG-based indices of fatigue, and comparable elevations in blood markers of muscle damage. Importantly, uphill time-trial performance was impaired after both downhill conditions, with a larger performance deterioration following the high-cadence descent, indicating that the added metabolic strain outweighed any mechanical benefit of reduced impact forces. Under fresh conditions, uphill performance was strongly predicted by VO_2max and local knee-extensor endurance, and to a lesser extent by rapid force production, confirming their relevance as determinants of steep uphill running capacity. Under fatigued conditions, however, none of these traditional physiological or neuromuscular markers explained inter-individual differences in performance decline. Instead, cardiometabolic decoupling, quantified as the disproportionate rise in heart rate and ventilatory responses relative to speed, emerged as the most consistent correlate of the loss in performance. These findings support the notion that “durability” constitutes a distinct performance trait, more closely linked to the ability to limit internal–external load decoupling during prolonged stress than to classical laboratory measures or to specific downhill stride-frequency strategies.

Collectively, these studies indicate that trail running performance emerges from the interaction between terrain-specific energetic and neuromuscular costs, speed-driven kinematic strategies (particularly in uphill and downhill sections), and an independent dimension of durability characterised by cardiometabolic decoupling, rather than by isolated biomechanical patterns or traditional laboratory markers alone.

To visually synthesize the main findings of the present thesis within a broader framework, Figure 31 proposes an integrative conceptual model of trail running performance, in which classical endurance determinants are combined with terrain-specific cost, slope-dependent mechanical demands, and fatigue- and durability-related processes.

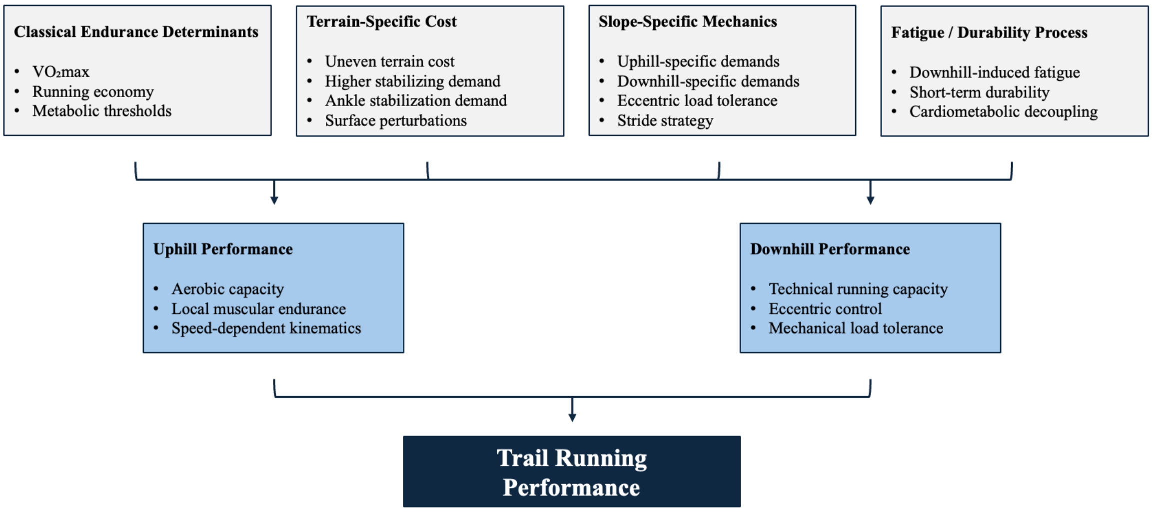


Figure 31 Proposed conceptual model of trail running performance. The figure summarizes the main findings of the present thesis by integrating classical endurance determinants with terrain-specific cost, slope-specific mechanical demands, and fatigue- and durability-related processes. The boxes represent broader conceptual domains, while the listed items reflect the specific mechanisms or constructs most clearly supported by the present work. This model is intended as a conceptual synthesis rather than as a quantitative predictive model.

8.3 Limits and future perspectives

The studies presented in this thesis have some methodological and contextual limitations. First, they necessarily balance experimental control and ecological validity in different ways. Laboratory protocols (uneven terrain and downhill stride-frequency manipulation) simplified real trail conditions by standardising speed, step frequency and surface characteristics, and by using treadmill-based downhill running and relatively short uphill efforts. Field studies, in turn, were based on a single race, short filmed sections and video-derived spatiotemporal parameters, without concurrent physiological or neuromuscular testing. These choices were essential to ensure feasibility, especially with high-level athletes, but they limit both the generalisability and the mechanistic detail of the findings.

A second set of limitations concerns the variables measured and the populations studied. Plantar forces, joint-level kinematics, core and upper-limb muscle activity, and broader neuromuscular or tendon characteristics were not assessed, and only a limited range of speeds and slopes was examined in controlled conditions. Moreover, the work focused mainly on well-trained and elite athletes, and in some protocols exclusively on men. This strengthens the performance relevance of the results but makes between-athlete differences more subtle and reduces their applicability to recreational runners and more heterogeneous groups.

These limitations provide clear directions for future research. Continuous monitoring of biomechanics, physiology and neuromuscular function over entire races, and across different courses and distances, would allow a more complete description of how athletes manage uphill, downhill and uneven terrain over time. In parallel, extending laboratory protocols to a wider range of slopes, speeds and surfaces, and using more flexible analytical approaches, could better capture non-linear interactions between stride mechanics, terrain and fatigue. Finally, future studies should include larger and more diverse samples—particularly female and sub-elite or recreational runners—and test targeted interventions (e.g., downhill technical training, eccentric strength programmes, durability-oriented conditioning) to determine whether the determinants of performance and durability identified in this thesis can be effectively modified in practice.

More specifically, several methodological developments may help strengthen future research in this area. First, longer-duration experimental protocols, including more prolonged downhill and subsequent uphill efforts, would allow a more complete investigation of how acute fatigue responses evolve toward more extended manifestations of durability. Second, more ecologically valid outdoor interventions, particularly on technical downhill terrain, are needed to verify whether the biomechanical and physiological responses observed in controlled laboratory models are maintained under real trail-running conditions. Third, the systematic inclusion of female athletes should be prioritized to better capture sex-specific responses to terrain, fatigue, and performance decline. Finally, the integration of wearable technologies, such as inertial sensors, in-shoe pressure systems, portable metabolic devices, and continuous heart-rate monitoring, may offer a promising opportunity to quantify internal–external load coupling, locomotor adjustments, and fatigue-related changes in real-world trail settings, while preserving the ecological complexity that characterizes this discipline.

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