## **ORIGINAL ARTICLE**



# Ground gradient affects stride-to-stride fluctuations and gait variability in overground walking

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#### Abstract

**Purpose** This study aims to explore the impact of ground gradient on gait variability.

**Methods** Ten healthy adults  $(39.3 \pm 4.14 \text{ years})$  performed overground walking under three gradient conditions: uphill  $10.1^{\circ}$  (17.81%),  $-10.1^{\circ}$  downhill (-17.81%), and level  $0.54^{\circ}$  (0.95%). Gait kinematics were recorded using inertial measurement units, and stride time intervals were evaluated for variability magnitude and temporal structure via Coefficient of Variation (CV) and Detrended Fluctuation Analysis (DFA- $\alpha$ ). Heart rate was recorded and served as a measure of exertion.

Results Significant differences in both CV and DFA- $\alpha$  emerged among conditions (p<0.001). Downhill walking exhibited the highest CV (4.67  $\pm$  1.65%) and the lowest DFA- $\alpha$  (0.62  $\pm$  0.13). In contrast, uphill walking showed intermediate values (CV: 3.67  $\pm$  0.84%; DFA- $\alpha$ : 0.76  $\pm$  0.09), while level walking displayed the lowest CV (1.98  $\pm$  0.62%) and the highest DFA- $\alpha$  (0.84  $\pm$  0.1), demonstrating a parabolic effect of ground gradient with gait variability for both CV and DFA- $\alpha$ . Downhill walking also elicited faster average velocities (1.57  $\pm$  0.14 m/s) compared to uphill (1.38  $\pm$  0.09 m/s) and level (1.46  $\pm$  0.08 m/s) walking.

Conclusion Interestingly, while uphill walking resulted in the highest heart rate ( $141.9 \pm 13.8$  bpm), DFA- $\alpha$  values of stride time intervals time series did not differ significantly from level walking, suggesting that metabolic effort may not be associated with the temporal structure of gait variability. Overall, it appears that during downhill walking, pronounced neuro-mechanical demands, likely imposed by eccentric effort, affect the amount and temporal structure of variability.

**Keywords** Variability · Gradient walking · Gait · Complexity · Ground inclination

## **Abbreviations**

**OMV**: Optimal Movement Variability **DFA**: Detrended Fluctuation Analysis

CV: Coefficient of Variation

DOMS: Delayed-Onset Muscle Soreness

IMU: Inertial Measurement Unit

GPS: Global Positioning System

**IQR**: Inter-quartile Range

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STI: Stride Time Intervals
ANOVA: Analysis of Variance

 $\eta_n^2$ : Eta-Squared

## Introduction

Human gait is a complex, adaptive process that reflects the intricate interplay between the musculoskeletal and nervous systems. Gait pattern is inherently variable, and it is widely acceptable that this variability does not reflect noise but carries significant information about the underlying motor control strategies and the adaptability of the system to environmental demands. Understanding this variability is crucial for insights into locomotor function, rehabilitation, performance enhancement, and prevention of movement disorders. Gait variability could be evaluated with respect to its amount using linear metrics such as the standard deviation or the coefficient of variation that reveal deviations around a central point, the mean. It can also be evaluated with respect



to its temporal structure, using the so-called nonlinear metrics (Dierick et al. 2021; Wurdeman and Stergiou 2013). The Optimal Movement Variability (OMV) hypothesis (Stergiou and Decker 2011) posits that variability is optimized for healthy motor function, allowing for adaptability in response to changing tasks and environments. Deviations from this optimum level can lead to decreased performance and an increased risk of injury.

The OMV hypothesis proposes that healthy motor systems exhibit fluctuations in movement patterns that strike a balance between excessive rigidity and excessive randomness, reflecting an adaptable, fractal-like structure that enhances resilience (Stergiou and Decker 2011). This behavior is not fixed but varies across the lifespan—developing in childhood as sensorimotor exploration refines into stable yet adaptable patterns (Harbourne and Stergiou 2009) and degrading with age or pathology, often manifesting as either overly rigid (e.g., Parkinsonian gait) or excessively irregular (e.g., ataxic or fall-prone gait) dynamics (Hausdorff 2009). Detrended Fluctuation Analysis (DFA) quantifies this balance through the scaling exponent ( $\alpha$ ), where values near  $\sim 0.8-1.0$  in healthy adults gait indicate persistent, long-range correlations that support adaptive responses to perturbations (Hausdorff 2007; Ravi et al. 2020), yet the exact "optimal" range may shift with task, age, or pathology. Large deviations from this range are clinically meaningful:  $\alpha \le 0.5$  (uncorrelated randomness) may reflect disrupted sensorimotor integration (e.g., cerebellar dysfunction), while  $\alpha > 1.0$  (overly rigid patterns) suggests diminished adaptability as seen in Parkinson's disease (Hausdorff 2009). Critically, such deviations compromise system robustness overly rigid patterns reduce the capacity to adjust to external demands (e.g., uneven terrain), while excessive randomness reflects inefficient capacity for adaptability in the imposing constraints (Buzzi et al. 2003; Lipsitz 2002). The OMV framework thus posits that fractal scaling reflects an active, health-relevant trade-off between stability and flexibility, with implications for diagnosing and rehabilitating motor deficits (Stergiou et al. 2006). Previous research has demonstrated that a range of factors, including environmental conditions, influence gait variability (Kesler et al. 2005; Tamburini et al. 2018). For example, walking on uneven terrain increases the amount of variability (Kent et al. 2019), while walking in varying lighting conditions (near-darkness) increases the amount of variability in middle-aged and older adults (Huang et al. 2017; Naaman et al. 2023), possibly challenging the adaptation capacity of the motor system. Another important environmental factor that is often present is ground gradient, which changes the mechanical and neuromuscular demands of walking and requires different motor control strategies (Diedrich and Warren Jr., 1998). When walking uphill, the timing and weighting of muscle synergies during the touchdown, mid-stance, and early

push-off phases are different from when walking on a flat surface. This is because the central nervous system has to be flexible to meet the different mechanical demands (Janshen et al. 2017; McGowan et al. 2009). Uphill walking is characterized by increased activation of hip, knee, and ankle extensors, which contribute significantly to body support and forward propulsion, with these adaptations becoming more pronounced at faster velocities (Franz and Kram 2012; McGowan et al. 2009). Conversely, downhill walking involves increased muscle activation, particularly in knee extensors, along with an increased amount of variability in terms of intra-limb coordination and intersegmental dynamics (Dewolf et al. 2020; Franz and Kram 2012). On downhill slopes, the contribution of individual joints to total support moments shifts, transitioning from ankle-dominated to knee-dominated strategies (Hong et al. 2014). These neuromuscular adjustments enable the central nervous system to modulate motor control elements, aligning them with the mechanical demands imposed by the gradient. Research has also highlighted the metabolic implications of gradient walking, identifying an optimal economical slope at approximately -10.2%, where the balance of positive and negative external work minimizes energy expenditure (Minetti et al. 1993). Therefore, it appears that the neuromuscular system adapts to different slopes, adjusting coordination and effort to accomplish the task.

However, less is known regarding the effects of gradient walking on the magnitude and the temporal structure of gait variability. Specifically, we found only three studies partially related to this research question. Hunter et al. (2010) examined the trade-off between energetic cost and stability during downhill walking on slopes of 0, 0.05, 0.10, and 0.15 gradients at 1.25 m/s. They found that when subjects adopted a relaxed walking strategy, allowing gravity to take the lead, energetic cost decreased. However, the amount of variability of stride time (a stability indicator, per the authors) increased on the steeper slopes. Yet when they used their normal preferred walking speed, they prioritized stability over minimizing energetic cost, even at the expense of higher energy expenditure (Hunter et al. 2010). A more recent study (Jones et al. 2024) found that during running stride time, DFA- $\alpha$ displayed a moderate decrease during downhill running compared to uphill running. They also found that both the amount and temporal structure of variability were influenced more by the gradient (uphill vs. downhill) rather than by the elapsed exercise duration. So, they claimed that changes in variability between and within runs should be understood in the context of course elevation profiles before any health conclusions are made. Notably, Vieira et al. (2017) evaluated 49 healthy young adults walking on treadmill inclines of  $\pm 6\%$ ,  $\pm 8\%$ , and  $\pm 10\%$ and reported that trunk-acceleration variability increased significantly in medial-lateral, anterior-posterior, and vertical directions during both uphill and downhill conditions; that



local dynamic stability (maximum Lyapunov exponent,  $\lambda_s$ ) was reduced on all inclines—with the greatest  $\lambda_s$  values uphill—and margin of stability (MoS ML) decreased downhill; and that gait regularity (sample entropy) decreased almost linearly from downhill to uphill slopes, indicating a progressive loss of regularity with steeper gradients. Taking everything into account—one study examined running, while the others evaluated the amount of variability, local stability and regularity on a downhill gradient—it appears there is still a knowledge gap that needs to be addressed regarding the effects of gradient walking on the magnitude and the temporal structure of gait variability especially on natural overground conditions.

Addressing this knowledge gap is crucial for multiple reasons. It could provide insights into neuromuscular control and how the body adapts to a specific environmental constraint, aid in the early detection of subtle motor impairments, often amplified by the biomechanical demands of gradients, and offer a more profound understanding of locomotor efficiency and fatigue. It could also be used in rehabilitation to help tailor treatments to each patient based on how they react to gradient walking. It could also be very important in preventing falls by finding patterns of instability, especially in older adults or people who have trouble moving around. Finally, it can inform the design of assistive devices and rehabilitation technologies, enhancing their adaptability to varied terrains (Zignoli et al. 2023). The above rationale highlights the critical importance of addressing this research question.

Therefore, the present study aims to investigate how ground gradient affects gait variability in a natural, uncontrolled outdoor environment. We used DFA- $\alpha$  to examine the temporal structure of variability and the stride time coefficient of variation (CV) to examine the magnitude of variability. This work will help us to further explore the relationship between environmental demands, motor control strategies, and movement adaptability under different walking conditions. Additionally, this study aims to understand how these changes in gait variability align with the OMV hypothesis. Therefore, we hypothesized that downhill and uphill walking will lead to altered gait variability compared to natural ground walking, due to increased mechanical demands that affect neuromuscular control strategies. Speed and heart rate were also evaluated between conditions for physiological comparisons.

#### Methods

#### **Participants**

Ten healthy adults (3 females, 7 males) with the following descriptive characteristics (mean  $\pm$  standard deviation): age  $39.3 \pm 4.14$  years, height  $178.1 \pm 8.19$  cm, and weight

 $82.0\pm10.4$  kg voluntarily participated in the study. Inclusion criterion was an age range of 30 to 45 years as individuals in this group are generally active and less likely to exhibit agerelated declines that could confound the study's outcomes. Exclusion criteria included serious injuries and lower limb or spine surgery, neurological, pulmonary, cardiovascular, and other diseases or drugs affecting balance or gait, pregnancy, alcohol or drug consumption 12 h before measurement, and medium or severe physical activity that could have induced delayed onset muscle soreness (DOMS) 48 h prior to measurement. The research was approved by the Ethics Committee of the University (EC-12/18–5-2020) and conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants included in the study.

## **Experimental protocol**

Participants wore sports clothes and their own comfortable sports shoes. They were instructed to walk an asphalt course of 896 m with an average inclination of 10.1° (17.81%, Fig. 1) at a preferred walking speed, maintaining a stable pace and refraining from stopping during the trial. Inclination data were obtained by performing three measurements on different trajectories along the width of the course (896 m) using a digital inclinometer (DOT, Movella, Enchede, NL) sampling at 75 Hz and the average value of these measurements is reported above. Gait assessment was conducted outdoors on three inclinations: uphill, downhill, and level. For the level walking condition, a different course was selected with a length of 1100 m and an average inclination of  $0.6^{\circ}$  (1.05%). The participants completed the tasks in a randomized order, with at least a 10-min rest period between each condition. It is important to report that all

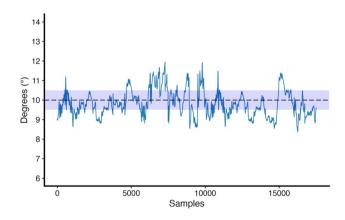


Fig. 1 Inclination profile of the course where uphill and downhill walking tests were performed. Dashed line represents the average value and shaded are  $a\pm 1$  standard deviation. The average value is represented as a line for esthetic reasons although it is a discrete value and lacks time dimension



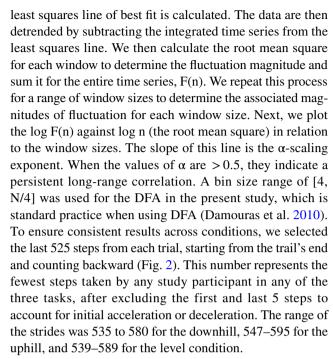
participants consistently initiated their walks (data collection) from the same position on the route and traversed along the same lane for each walk to ensure methodological consistency and reliability. To ensure recovery, we confirmed that the heart rate returned to baseline before initiating the next walk after the uphill task.

## Instrumentation

Lower extremity kinematics were quantified using a wireless Bluetooth, 9-degree-of-freedom inertial measurement units—IMUs (k-Sens [now K-Move]), Kinvent, Biomechanique, Montpellier, France) with a 1000 Hz sampling frequency. This sensor has been successfully used in other studies (Chalitsios et al. 2024). Gait speed was recorded using a GPS unit (Samsung Galaxy no. 205-006142) sampling at 20 Hz, which was strapped to the upper arm of each participant. The IMU sensor was attached to the lower right foot (same for all participants), specifically at the metatarsal region, using a special case that utilized the shoelaces for attachment and further secured with tape wrapped around the shoe to minimize movement. We also used a Polar H10 (Polar Electro Oy, Kempele, Finland) heart rate sensor to continuously monitor heart rate. All three measurement systems (IMUs, GPS, and Polar heart rate sensor) were synchronized using a Bluetooth-based start trigger via a custom control app. Before each trial, devices were paired to the app, and pressing a single 'Start' button sent a simultaneous command to initiate data logging. Each device recorded a timestamped flag upon trigger reception, enabling precise post hoc alignment. Despite differing sampling rates (IMUs: 1000 Hz, GPS: 20 Hz, Polar: 500 Hz), this method reliably synchronized data streams within the measurement window.

## Data pre-processing and analysis

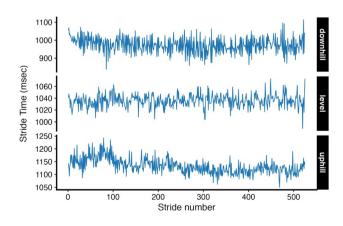
We applied no filtering to the accelerometer to maintain the nonlinear dynamic properties of the recorded time series (Rapp et al. 1993; Theiler and Eubank 1993). We measured the time between two consecutive foot strikes of the same foot using a peak-to-peak algorithm on the largest part of the acceleration signal. We used this method to determine the inter-stride intervals (ISI). Thereafter, each time series was explored for outliers using Tukey's rule, in which any data point that fell  $\pm$  1.5, the inter-quartile range (IQR) is considered one. We found no outliers. To quantify the magnitude of stride-to-stride variability, we used  $CV = \frac{\sigma}{u}$  where  $\sigma$ stands for standard deviation and  $\mu$  for mean. We used Detrended Fluctuation Analysis (DFA) to determine the exponent for the stride time intervals (STI) time series. The DFA algorithm (Peng et al. 1995) integrates a time series, divided into window sizes of length n. In each window, a



To ensure consistency across conditions, we standardized the number of strides analyzed by setting n to 525 strides (counting from the trial end and backwards). This approach allowed for a uniform comparison of DFA- $\alpha$  values across different walking conditions, ensuring that each participant's data was evaluated on an equal footing regardless of the terrain.

## **Statistical analysis**

Descriptive statistics are presented as the mean and standard deviation. One-way repeated measures ANOVA was performed between the three conditions (uphill, downhill, and level) for the dependent variables; CV and DFA-α. In cases where the assumption of sphericity was violated, the Greenhouse–Geisser correction was applied. The analysis was



**Fig. 2** Raw stride time series for a representative subject across the different inclinations (uphill, downhill, or level walking)



performed after verifying that the distribution of the variables did not differ significantly from the normal distribution according to the Shapiro-Wilk test. We quantified effect size using partial eta-squared  $(\eta_n^2)$ , interpreting the thresholds as follows: small effect (0.01), medium effect (0.06), and large effect (> 0.14). Post hoc analysis was performed to examine pairwise differences using a Bonferroni correction. This study was designed to ensure adequate statistical power based on a priori considerations. Specifically, our sample size was selected in accordance with the power analysis literature for DFA in gait variability studies. This literature demonstrates that for within-subject designs, sample sizes in the range of our study are sufficient to detect meaningful effects with a high probability (power > 0.80) (Kuznetsov and Rhea 2017). This sample size was determined to be both scientifically appropriate and practically feasible, reflecting the significant logistical demands of conducting instrumented gait analysis across three distinct conditions in an outdoor environment. To supplement this, a post hoc sensitivity analysis was conducted using G\*Power (version 3.1.9.6). For a large observed effect size  $(\eta_p^2 = 0.7)$  and a significance level of  $\alpha = 0.05$ , this analysis confirmed that the achieved statistical power was 0.97 (97%), underscoring the high sensitivity of our study to the detected effects. All statistical analyses were performed using R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria).

#### Results

The average stride time intervals were for uphill:  $1001.34 \pm 134.72$  ms, level:  $1035.4 \pm 47.97$  ms and downhill:  $914.6 \pm 92.87$  ms. The average speed was significantly different between conditions ( $F_{2.18} = 12.69$ , p < 0.001,  $\eta_p^2 = 12.69$ ).

Fig. 3 Distributions of the speed and heart rate for the different walking conditions. Statistically significant differences between conditions are shown with the black horizontal lines and asterisks

0.58). Post hoc analysis further revealed significant differences between downhill  $(1.58\pm0.14 \text{ m/s})$  and level  $(1.46\pm0.08 \text{ m/s})$  as well as between downhill and uphill  $(1.38\pm0.09 \text{ m/s})$  walking (Fig. 3). There were no differences between level and uphill walking. Recorded heart rate was also significantly different between conditions  $(F_{2,18}=67.93, p<0.001 \text{ and } \eta_p^2=0.88)$ . Post hoc comparisons revealed significant differences between uphill  $(141.9\pm13.8 \text{ bpm})$  and downhill  $(106.4\pm6.6 \text{ bpm})$  and between uphill and level  $(102.9\pm4.9 \text{ bpm})$  walking. There were no differences between level and downhill walking (Fig. 3).

Regarding the magnitude of variability, that was evaluated with the stride time coefficient of variation (CV), we found significant differences between conditions ( $F_{2,18} = 12.43$ , p < 0.001 with a large effect size ( $\eta_p^2$ ) of 0.58). Post hoc analysis (Fig. 4) revealed significant differences for all comparisons. Specifically, we found significant differences between downhill (4.67  $\pm$  1.65%) and level walking (1.98  $\pm$  0.62%), uphill (3.67  $\pm$  0.84%) and level walking, as well as between downhill and uphill walking.

Regarding the temporal structure of variability, that was evaluated with the DFA- $\alpha$ , we found significant differences between conditions ( $F_{2,18}=23.31,\,p<0.001$  with a large effect size ( $\eta_p^2$ ) of 0.72). Post hoc comparisons (Fig. 4) revealed significant differences between downhill (0.62 ± 0.13) and level walking (0.84 ± 0.1), and between downhill and uphill walking (0.76 ± 0.09). No significant differences were found between level and uphill walking.

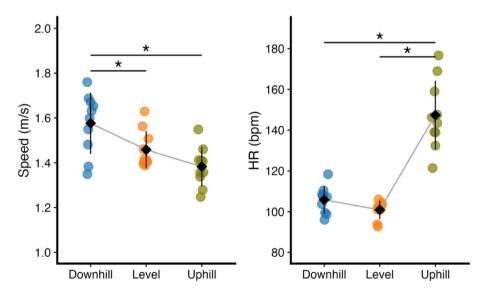
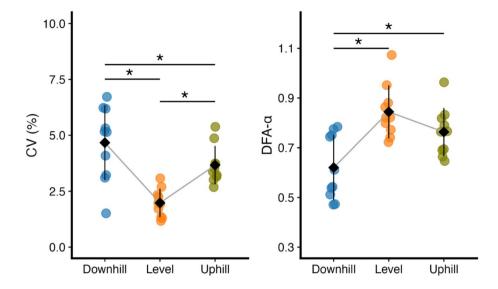




Fig. 4 Distributions of the CV and DFA- $\alpha$  for the different walking conditions. Statistically significant differences between conditions are shown with the black horizontal lines and asterisks



# **Discussion**

The present study investigated how ground gradient affects gait variability in a natural, uncontrolled outdoor environment. We employed DFA-α for assessing the temporal structure of variability and utilized the stride time coefficient of variation (CV) to evaluate the variability's magnitude. Additionally, this study investigated how any possible changes in gait variability align with the OMV hypothesis. Therefore, we hypothesized that downhill and uphill walking will lead to altered gait variability compared to natural ground walking, due to increased mechanical demands that affect neuromuscular control strategies. We also evaluated speed and heart rate between conditions for physiological comparisons. CV and DFA-α showed a U-shaped pattern across conditions, with CV the highest during downhill walking, intermediate during uphill, and the lowest on level walking, with all differences significant. DFA-α was the lowest downhill and the highest on level ground, with no significant difference between uphill and level walking. The heart rate, which indicates exertion, was the highest uphill, while the speed was the greatest downhill.

Our results for level walking regarding the DFA- $\alpha$  were comparable to normative healthy values reported in the literature (threshold of 0.82, CI: 0.72—0.92) (Ravi et al. 2020). These findings are consistent with the Optimal Movement Variability Hypothesis, which suggests that healthy motor systems operate within a specific variability range that supports adaptability and efficiency (Stergiou et al. 2006). While deviations from this range, toward excessive randomness or rigidity, have been associated with impaired function in clinical populations, we emphasize that in our healthy cohort, such deviations, particularly during downhill walking  $(0.62\pm0.13)$ , do not imply dysfunction but rather reflect the increased neuromuscular and mechanical

demands imposed by the task. DFA-α values approaching 0.5 (uncorrelated white noise) during downhill walking suggest a shift toward less-structured variability, indicative of a more challenging control state (Dotov et al. 2017; Hausdorff et al. 1997). This is consistent with prior findings showing that downhill locomotion due to its gravitational and eccentric load characteristics, imposes unique demands on the neuromuscular system (Dewolf et al. 2020; Franz and Kram 2012). While level and uphill walking preserved the fractal structure of gait dynamics, the observed changes in downhill conditions may have important implications for populations with reduced neuromuscular capacity. Nonetheless, we recognize that without accompanying functional or health-related measures, interpretations regarding the optimality or functional implications of these changes should be made cautiously and warrant further investigation.

Importantly, there was also an increase in the selfselected speed during downhill walking. Gottschall and Kram (2006) in their study indicated that the increased braking impulses reduce the magnitude of the upward motion of the center of mass (ascents) while amplifying the downward motion (descents). So, the gravitational pull, coupled with the braking forces, minimizes the need for active propulsion and thus allows the body to take advantage of gravity as a primary driving force, akin to passive walking, which likely results in the observed increase in speed compared to level or uphill walking. At the same time, downhill walking imposes greater mechanical demands on lower limb joints compared to level walking (Hong et al. 2014) and requires distinct muscle recruitment strategies, primarily involving the knee extensors (Franz and Kram 2012). As the downhill grade increases, posterior pelvic tilt and lateral trunk bending toward the stance limb increase, along with peak dorsiflexor and extensor moments at the ankle and knee, respectively (Hong et al. 2014). Notably, downhill walking



elicited the lowest DFA- $\alpha$  despite not having a significant difference in heart rate compared to level walking. Therefore, these findings suggest that the temporal structure of variability may be primarily under neuro-mechanical control, rather than metabolic control, in response to various environmental demands. This is emphasized by the lack of significant differences in the DFA- $\alpha$  between uphill and level walking, while the heart rate increased in uphill walking.

Regarding the magnitude of variability, our results for CV are similar to previous studies that reported stride time CV values below 6% during preferred-speed level overground walking in healthy adults aged 21-47 years (Beauchet et al. 2009; Gabell an Nayak 1984). The significantly increased CV values for downhill and uphill walking in comparison with level walking do not agree with the linear decrease in speed and the linear increase in heart rate with increased grade. Given that metabolic cost is primarily influenced by positive ground gradients with a highly linear increase (Jeffers et al. 2015; Jessup et al. 2023; Minetti et al. 1993), it is unlikely that the increased CV values observed during downhill walking are driven by any difference in metabolic cost. Instead, the heightened mechanical and neuromuscular demands encountered when walking downhill primarily drove this increase. Interestingly, uphill walking affected CV values but not the DFA-α. This shows that the magnitude and temporal structure of variability could be affected diversely from metabolic demands. This further emphasizes the need in future studies of variability to incorporate both aspects, magnitude, and temporal structure.

These findings have practical implications for rehabilitation and training. For example, uphill walking, which preserves fractal organization (1/f noise), could be a safer option for improving lower limb strength and cardiovascular endurance without significantly disrupting stride consistency, making it suitable for populations with limited neuromuscular control. In training, the dissociation between metabolic cost and gait variability offers opportunities for targeted interventions. Downhill walking, despite its lower metabolic cost, imposes significant neuro-mechanical demands, making it valuable for enhancing neuromuscular coordination and eccentric strength. Careful consideration is needed when selecting a downhill slope according to the OMV hypothesis, which posits a 'sweet spot' where sufficient eccentric demand fosters beneficial adaptations—like enhanced neuromuscular coordination—without exceeding thresholds that increase randomness in gait variability. Loss of fractal complexity (e.g., reduced DFA- $\alpha$ ) may diminish performance benefits and elevate injury risk. Thus, moderate slopes can enhance adaptations, but excessive gradients that drive variability into randomness are likely counterproductive for most individuals. Uphill walking, on the other hand, provides a more stable and metabolically demanding option for improving cardiovascular fitness and strength without compromising stride consistency. While our findings highlight altered neuromuscular coordination (Ihlen and Vereijken 2010; Ting et al. 2015) during downhill walking, we refrain from attributing these changes solely to supra-spinal mechanisms without direct neurophysiological measurements. Future work combining DFA- $\alpha$  with EEG/EMG could disentangle these levels of control.

This study is novel for its application of overground walking conditions to investigate variability across different ground gradients. Overground walking more accurately reflects natural locomotor behavior compared to treadmill-based studies, as it includes environmental variations and natural gradients. In addition to studying the effect of ground gradient in a natural environment, an approach that we believe enhances the ecological validity of the findings and provides insights into realworld walking dynamics, a key methodological strength of this study is the inclusion of a sufficient number of strides to satisfy the theoretical assumptions required for reliable DFA-α estimation. By analyzing a standardized minimum of 525 strides per participant across all conditions, this study adhered to established guidelines for ensuring robust and reliable estimation of DFA-α (Kuznetsov & Rhea 2017; Marmelat and Meidinger 2019). This approach addresses a common limitation in gait variability research, where insufficient stride counts may lead to less reliable conclusions. The adherence to these methodological standards ensures the robustness of the results and contributes to the reliability of DFA metrics for future investigations in similar contexts.

Nevertheless, our findings come with certain limitations, one of the most interesting being the potential role of lower limb eccentric strength. Considering that downhill walking substantially increases eccentric demands to counteract gravity, it is plausible that individuals with higher eccentric strength might show minimal changes in gait variability despite walking on a negative gradient. In other words, stronger individuals could be more resistant to the destabilizing forces associated with downhill walking, maintaining a more consistent stride pattern. Future research should investigate how eccentric strength influences gait variability across various gradients, offering clearer insights into inter-individual differences in neuro-mechanical adaptations. Additionally, examining whether manipulating stride fluctuations (i.e., sensory feedback Raffalt et al. 2023; Vaz et al. 2019)) can modify DFA-α in gradient conditions represent an intriguing avenue for further exploration, as it may provide valuable information on how external cues influence the fractal properties of gait.

# **Conclusion**

This study highlights the distinct adaptations to varying ground gradients, showing that downhill walking disrupts stride consistency probably due to increased eccentric



demands, while uphill walking, despite its higher metabolic cost, maintains a more stable temporal structure of gait. The differences between the magnitude and temporal structure of variability regarding uphill walking highlight the need to incorporate both types of metrics in future studies of variability. Practical applications include utilizing downhill walking to enhance neuromuscular coordination and eccentric strength while considering its stability challenges and leveraging uphill walking for strength and endurance training with minimal disruption to gait consistency. Future research should explore individual differences in eccentric and concentric strength and environmental influences on gait variability to further refine our understanding of locomotor adaptations.

Author contributions Conceptualization: Christos Chalitsios, Thomas Nikodelis; Formal analysis and investigation: Christos Chalitsios, Thomas Nikodelis; Writing—original draft preparation: Christos Chalitsios; Writing—review and editing: Christos Chalitsios, Thomas Nikodelis, Nick Stergiou;

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**Data availability** Data produced from the study are available upon reasonable request.

#### **Declarations**

Conflicts of interest The authors have no competing interests to declare that are relevant to the content of this article.

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