

Nonlinear analysis reveals duration and gradient-dependent disruption of load carriage gait variability during an outdoor 6.72 km time trial in military personnel

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ABSTRACT

This investigation assessed the effect of gradient and duration on the gait variability exponent, DFA- α , in military personnel affixed with dual inertial measurement units performing a load carriage time-trial. Gait data ($N = 14$) were partitioned into 256 stride time segments by gradient (uphill, downhill) using a gait event algorithm. Detrended fluctuation analysis calculated DFA- α per segment, which was averaged across one-third durations (phases 1–3) per gradient. Two-way repeated measures ANOVA examined effects of gradient, duration, and interaction on DFA- α , with Bonferroni-adjusted post-hoc comparisons. There was a significant main effect of duration (phase 1: 0.593 ± 0.021 ; phase 2: 0.563 ± 0.031 ; phase 3: 0.493 ± 0.021 ; $F = 3.833$, $p = 0.035$, $\eta_p^2 = 0.228$), but not gradient (uphill: 0.486 ± 0.031 ; downhill: 0.614 ± 0.035 ; $F = 4.252$, $p = 0.060$, $\eta_p^2 = 0.246$), or interaction ($F = 0.019$, $p = 0.981$, $\eta_p^2 = 0.001$). Pairwise comparisons revealed significantly lower DFA- α during phase 3 than phase 1 ($p = 0.016$). Elapsed duration and uphill gradient, despite a large, but non-significant effect, may represent factors altering gait variability for injury risk.

1. Introduction

Load carriage is a demanding military occupational task performed in the daily physical training and operations of modern Warfighters (Vaara et al., 2022; Knapik et al., 2004). Load carriage performance comprises a multi-dimensional interaction within the locomotor system to produce coordinated muscle contractions and limb movements that generate a targeted gait optimal to achieve task goals (Fox B et al., 2020; Fallowfield et al., 2012; Shi et al., 2019). Locomotion behaviors encountered during load carriage, such as ruck marching (i.e., natural ambulation), forced-marching (i.e., walking at a velocity beyond gait transition velocity where one would naturally jog), and running (i.e., natural or forced) are common military-relevant perturbations that alter individual gait patterns to accomplish unit-wide outcomes (Krajewski

et al., 2023). However, such perturbations have been shown to contribute to load carriage-related musculoskeletal injury (MSKI) prevalence rates of 21–31 % during military training courses (Jensen et al., 2019; Lovalekar et al., 2020). Although gait patterns constitute a continuous, cyclical pattern deemed repeatable during otherwise unperturbed, steady-state conditions (Ahamed et al., 2021), such behaviors demonstrate a subtle degree of stride-to-stride fluctuation, known as gait variability (Bellenger et al., 2019), which may serve as an important biomarker reflecting the health of the locomotor system (Stergiou and Decker, 2011), an indicator of fatigue (Bellenger et al., 2019; Fuller et al., 2024a, 2024b), and risk factor for MSKI (Nesterovica-Petrikova et al., 2023; Fonseca et al., 2020; Springer et al., 2016).

Gait variability is the temporal inter-stride fluctuation in gait parameters that occurs during normal bipedal ambulation (Hausdorff

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et al., 1999). Gait variability has traditionally been assessed by leveraging linear statistical measurements, such as standard deviation or coefficient of variation, of stride parameters (Bartlett et al., 2007). Although such measures provide insight into the magnitude and breadth of variation of parameters, application of linear methods fails to capture the temporal and dynamic nature of gait by considering gait parameters independent of intra-individual variation (Hunter et al., 2023). Nonlinear methods, however, may provide a foundation to overcome these limitations by leveraging the theory of dynamical systems to measure the magnitude of and temporal changes in inter-stride fluctuations of gait time series data captured during load carriage tasks (Brink et al., 2024). Nonlinear methods may provide an advantage in evaluating the sequential changes of gait due to its self-similar, fractal properties that may otherwise be left uncaptured using linear techniques (Hausdorff et al., 1996).

Detrended fluctuation analysis (DFA) is a nonlinear analytic technique that quantifies fractal properties or statistical self-similarity of time-series data by scaling long-term autocorrelations of non-stationary signals from the fractal scaling exponent, alpha (Warlop et al., 2018). DFA-alpha (DFA- α) quantifies the statistical self-similarity of gait parameters within an inter-stride interval, such as stride time (time elapsed from heel strike to ipsilateral heel strike (Brink et al., 2024)). DFA- α values ($0 \leq \alpha \leq 1.0$) classify gait variability patterns based on the likelihood of a parameter value following a value of similar direction, and the extent to which such values are correlated with previous ones (Wilson et al., 2023). Statistical persistence ($0.5 > \alpha < 1.0$) indicates that parameter values are positively correlated, such that values in one direction are likely to be followed by values in the same direction (i.e., long stride intervals followed by longer stride intervals). In contrast, anti-persistence ($\alpha < 0.5$) indicates that successive parameter values are negatively correlated, such that values in one direction are likely to be followed by values in the opposite direction. (Fuller et al., 2024a; Wilson et al., 2023; Jones et al., 2024). At the extremes, randomness ($\alpha = 0.5$) indicates that values are not correlated with any previous value, and over-persistence ($\alpha > 1.0$) indicates that values show a strong positive correlation to prior values (Ahamed et al., 2021; Krajewski et al., 2020a). Categorizing DFA- α values provides an overview of the locomotor system behavior (Krajewski et al., 2023). Gait variability patterns of 'persistence' reflect a theoretically ideal gait owing to its stable yet adaptive structure to create appropriate motor solutions, known as corrections in gait, to achieve task goals (Ahamed et al., 2021). However, 'anti-persistence' or 'randomness' reflect inconsistent gait patterns due to fatigue or reduced gait coordination under heightened locomotor task difficulty that requires greater motor solutions to achieve task goals (Bellenger et al., 2019; Fuller et al., 2024a; Brink et al., 2024). This may reduce one's motor function capacity, described as the ability to generate motor solutions against perturbations, which may bring the system to actional boundaries toward failure (i.e., fall) or MSKI (Krajewski et al., 2020b).

Military personnel performing in-training load carriage tasks creates an opportunity for monitoring DFA- α for altered gait patterns indicative of reduced gait coordination and fatigue, such as randomness and anti-persistence, as risk factors for load-carriage-related MSKI (Bellenger et al., 2019; Fuller et al., 2024a; Brink et al., 2024; Edwards, 2018). Previous investigations observed that personnel performing load carriage under operationally-relevant loads (>30 % body mass) exhibit altered gait patterns during treadmill-based ambulatory tasks performed at fixed speeds (Krajewski et al., 2023; Brink et al., 2024) or in controlled environments on flat gradients (Ahamed et al., 2021). However, when monitoring occurs in the outdoor military training environment, the additional environmental and contextual factors that may contribute to altered gait patterns during load carriage tasks must be captured. Gradient is a well-established environmental factor that negatively affects load carriage gait dynamics (Jones et al., 2024; Padulo et al., 2023; Kimel-Naor et al., 2017); however, its influence on DFA- α during outdoor load carriage remains uninvestigated (Brink et al., 2024;

Walsh and Harrison, 2022). Elapsed duration is an important contextual factor that is negatively associated with DFA- α and serves as an indicator of fatigue during prolonged load carriage tasks (Bellenger et al., 2019; Brink et al., 2024). Given that gradient and duration of task are not encountered independently in in-field settings, investigating their interaction during load carriage tasks may elucidate their influence on gait variability (Brink et al., 2024). Understanding the influence of gradient and duration on DFA- α during in-training load carriage time trial assessments may help identify ecological factors associated with altered gait dynamics and determine risk factors for load-carriage-related MSKI. This could also support the development of gait monitoring interventions to help detect aberrant gait patterns during training that may inform in-training modification strategies for reducing MSKI risk and optimizing load carriage performance.

The purpose of this investigation was to assess the influence of gradient and duration and their interaction on DFA- α measured during an outdoor 6.72 km load carriage time trial performed by a group of military personnel undergoing a United States Marine Corps (USMC) Mountain Leadership Course (MLC). First, it was hypothesized that personnel would show different DFA- α values between uphill and downhill gradients of the event, with lower DFA- α values observed during the uphill gradient than the downhill gradient owing to heightened locomotor task difficulty level. Second, it was hypothesized that DFA- α would reduce towards randomness and anti-persistence with increased duration in both uphill and downhill gradients as a potential indicator of fatigue. Third, it was hypothesized that there would be an interaction effect of gradient and duration on DFA- α , such that DFA- α would show differential changes over the duration of the MET depending on whether participants were on the uphill or downhill gradient.

2. Materials and Methods

2.1. Participants and procedures

The USMC MLC is an arduous, 6-week military training course designed to evaluate and improve academic, physical, and tactical readiness in mountainous environments at moderate altitudes (2438–3048 m) among US Marines during the winter and summer seasons. The current investigation represents a secondary analysis from a previously conducted parent study designed to identify predictors of MSKI and MLC completion. Data were collected during one iteration in the summer of 2024. This study was approved by the University of Pittsburgh Institutional Review Board (STUDY22020119), the USMC Institutional Review Board, and the Office of Naval Research (ONRN00014-23-1-2795 & ONRN62909-21-1-2015) Human Research Protection Office and conducted following the Declaration of Helsinki.

Data for the current investigation were drawn from participating personnel with IMU data captured during a 6.72 km load carriage time trial performed at the start of a 6-week training course. Participants included in this investigation were healthy males ≥ 18 years who were free from MSKI and conditions that would prevent participation in the parent study, and provided written informed consent before parent study participation. Participants' height (cm) and mass (kg) were measured using a stadiometer instrumented with a digital scale (Healthometer Professional 500 KI, McCook, IL) with percent body fat (%) assessed using bioelectrical impedance analysis (InBody 770, InBody USA, Cerritos, CA) before the time trial event.

2.2. Mountain Endurance Test - 6.72 km load carriage time trial

The MLC Mountain Endurance Test (MET) is a single load carriage time trial that occurred in the morning (0630–0800, 7.22 °C, 0 cm precipitation, 53 % humidity) on an outdoor closed-course loop of firmly packed dirt and gravel mixture (~ 10 m wide) that comprised a 3.37 km ascent for a total elevation gain of 237 m (+6.5 % gradient) and 2.78 km

descent for a total elevation loss of 214 m (-7.7% gradient) and 0.21 km flat portion to the finish line (minimum elevation: 2072 m; maximum elevation: 2309 m). Participants began the event *en masse* and performed the event to achieve an individual best time (hh:mm:ss). Participants were allowed to utilize any self-selected locomotion behavior, including walking, forced-marching, and running, necessary to achieve their best time. Participants wore combat utilities (no helmet) and boots with 25 kg rucksacks. Researchers stopped IMU recordings and removed the devices upon MET completion.

2.3. Wearable data collection

2.3.1. Inertial measurement unit

Two Vicon IMeasureU Blue Trident™ (IMeasureU™, Auckland, NZ) tri-axial dual-g IMU sensors (Low-g: ± 16 g, 1125 Hz; High-g: ± 200 g; 1600 Hz) collected high-resolution acceleration, gyroscope, and magnetometry data to quantify each participant's spatiotemporal gait patterns throughout the MET. Before device attachment, de-identified accounts were created using the IMeasureU Step™ application (iOS 2.4.447) on tablets (Apple Inc., Cupertino, CA) for in-field data collection. Each device was assigned a de-identified code for the left and right limb by device serial number. All IMUs were placed in IMeasureU™ straps and attached to participants' distal medial tibia located 5 cm above the superior border of the medial malleolus on the exterior of participants' boots with the head of the device directed towards the toes to ensure the z-axis aligned perpendicular to the ground. Placement of the device occurred while participants were seated on the ground in dorsiflexion ($>90^\circ$) to allow a natural range of motion at the ankle joint during locomotion. Additionally, researchers applied a pre-wrap (3M Coban™, Thermo Fisher Scientific Inc., Waltham, MA) around the device and boot followed by synthetic kinesiology tape (SB SOX Pro™, SB SOX LLC, Irving, TX) (Wilson et al., 2023) to ensure security of the device and mitigate IMU migration throughout the event based on previous in-field investigations (Burland et al., 2021). This IMU device has shown good absolute and relative agreement measured by a two-way agreement intraclass correlation (ICC = 0.80) and Pearson correlation coefficient ($r = 0.81$), respectively, for calculating nonlinear time series features, including DFA- α , when compared to the instrumented treadmill criterion during load carriage under 23 kg body-borne load (Slattery et al., 2024).

2.3.2. Wrist-worn device

The Garmin Instinct Solar (Garmin Ltd., Olathe, KS), a commercial-grade wristwatch device, was worn by participants to continuously monitor elevation (m) data during the MET. This device contains a multi-sensor set embedded within a fiber-reinforced polymer case, including a triaxial accelerometer and gyroscope, to collect elevation data at an epoch-by-epoch (12 Hz) frequency. Devices were distributed at the start of the MLC for continuous physiological monitoring during the course. Participants' raw elevation data captured during the MET were offloaded via Garmin Connect™ in.FIT file format and transformed using an open-source Flexible and Interoperable Data Transfer Software Development Kit to configure data into a suitable format (.CSV) for subsequent analysis (FIT Protocol | FIT SDK). Previous studies assessing the reliability and validity of elevation data recorded by Garmin wrist-worn devices report a minimal relative between-device variability (1.5 %) during outdoor mountain climb activities, with an accuracy difference as much as 3 % compared to global navigation satellite system receiver devices (Menaspá et al., 2014).

2.4. Data processing

Raw data captured by IMUs were uploaded to IMeasureU Step™ software and trimmed to match the start and end of the time trial for each participant and exported for processing using custom MATLAB™ 2023a (Mathworks, Inc., Natick, MA, USA) scripts. The initial 60 s of

trimmed raw data were excluded via MATLAB™ due to potential start-up effects on gait (i.e., speed fluctuations), and the final 60 s of trimmed data due to potential drastic increases in speed to the finish line (Fuller et al., 2024a). Trimmed IMU data of the entire event were filtered with a fourth-order Butterworth low-pass filter with a cut-off frequency of 10 Hz for analysis (Brink et al., 2024). Filtered data were partitioned into two main sections (uphill and downhill) based on changes in elevation by leveraging continuous monitoring data collected by the wrist-worn device during the MET. Course sections were generated by aligning elevation data with resultant acceleration data conjoined using discrete universal timestamps (ms) between devices based on an identical reference point. An uphill section of 3.37 km in length was generated and utilized for analysis that started at the minimum elevation point of the course (2072 m; 365 m from the start line) to the maximum elevation point of the course (2309 m; 3.73 km from the start line) for a total elevation gain of 237 m. A downhill section of 2.78 km in length was generated and utilized for analysis that started at the maximum elevation point of the course to the bottom of the course (2095 m; 6.51 km from the start line) for a total elevation loss of 214 m. A flat section of 0.210 km in length starting from the bottom of the course (2094.89 m; 6.51 km from the start line) to the finish line (total elevation gain of 9.00 m) was not considered for analysis owing to its shortened distance that prohibited the accrual of sufficient stride numbers for analysis. Hence, only the uphill and downhill sections were included. A graphical portrayal of the course by section with elevation and resultant acceleration data from a representative participant is shown in Fig. 1.

Universal timestamps at the start and end of each gradient section were extracted for partitioning of the filtered IMU data by section. An unsupervised gait event identification algorithm from Purcell et al. (2006) was applied to the filtered data by section that utilized accelerometer data from the right shank to identify initial contact (IC) and terminal contact (TC) gait events and calculate stride times (S_T ; ms) into individual segments of 256 strides as recommended by Kiernan et al. (2023). This algorithm allowed the input of constraints to remove any abnormal prolonged pauses in the gait cycle based on human load carriage literature by imputing the following parameters: maximum step frequency: $4.75 \text{ steps} \cdot \text{s}^{-1}$; minimum stance time: 95 ms; maximum stance time: 540 ms; minimum swing time: 200 ms; maximum swing time: 1200 ms (Kasović et al., 2024). S_T was calculated as the time elapsed from heel strike to ipsilateral heel strike (Brink et al., 2024). This calculation was performed by extracting peaks of the acceleration data from the right foot in the anterior-posterior direction, which corresponded to heel strikes (Brink et al., 2024). S_T was the chosen gait parameter due to its common representation in biomechanical studies wherein nonlinear methods have been applied (Krajewski et al., 2023; Brink et al., 2024; Ahamed et al., 2018).

DFA exponents (α) were calculated on discrete S_T time series data for each 256-stride segment by condition after applying an outlier removal filter on the S_T time series data (Mean $S_T \pm 3$ SD) utilizing open source code from the University of Nebraska, Omaha, Biomechanics Core repository (v2.2.0.0) (NONANLibrary/LICENSE). Mean and standard deviations (SD) of DFA- α were calculated for each segment by gradient (uphill and downhill). To standardize the number of segments per gradient, all segments within each gradient were partitioned into one-third duration phases (Phase 1, 2, 3) with each containing the necessary number of segments needed to split each gradient section into thirds. Phase 1 represented 0–33 %, Phase 2 represented 34–66 %, and Phase 3 represented 67–100 % of the number of segments within each gradient section. Hence, this approach enabled the assessment of the temporal changes in gait parameters across each gradient. Time to complete each gradient section was calculated as the difference in timestamps between the start and end of each section after converting timestamps to seconds (s). Velocity ($\text{m} \cdot \text{s}^{-1}$) per gradient section was calculated as the time to complete each gradient section divided by section distance.

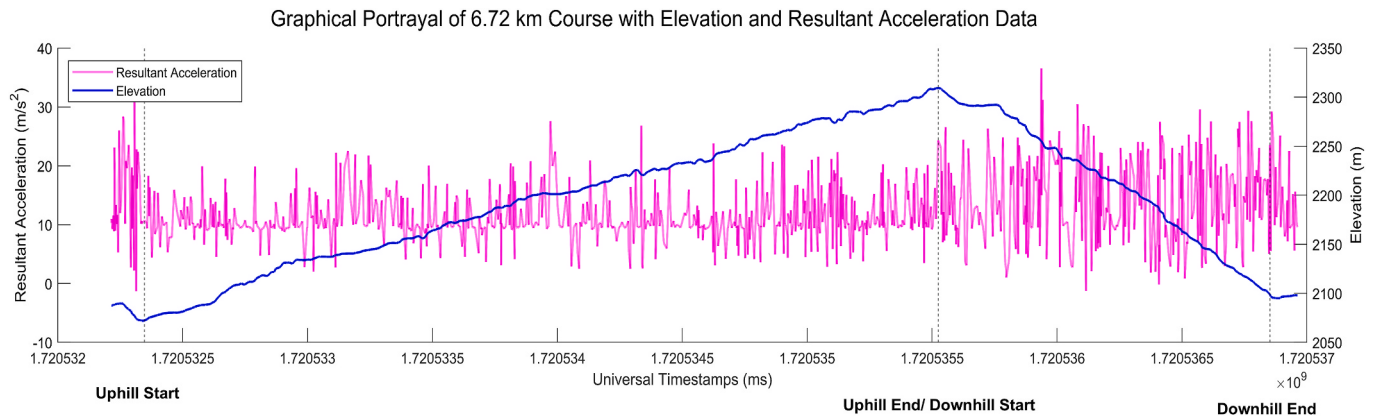


Fig. 1. Graphical portrayal of the 6.72 km time trial with elevation data from sea level (m) on the right y-axis and resultant acceleration data on the left y-axis. An uphill section ('Uphill Start') began at the minimum elevation of the course (2072 m above sea level; 365 m from the start line) to the maximum elevation point (2309 m above sea level) for a total elevation gain of 237 m. A downhill section ('Uphill End/Downhill Start') began at the maximum elevation of the course (2309 m above sea level) to the bottom of the course ('Downhill End'; 2095 m above sea level) for a total elevation loss of 214 m.

2.5. Detrended fluctuation analysis

Gait variability was analyzed utilizing the fractal methods of DFA (Ahamed et al., 2021; Hausdorff et al., 1996; Peng et al., 1993) on S_T time series data for each 256-stride segment within each gradient. The DFA creates a one-dimensional signal $x(i)$, $i = 1, \dots, L$, wherein x is the initial signal of size L , and an integrated signal (x) is calculated based upon Equation (1):

$$Y(k) = \sum_{i=1}^k (B(i) - B_{avg})$$

where B_{avg} is the mean value of the signal (B = signal value at a specific time point). The unified time series Y is then divided into segments of length l , and the linear approximation Y_l is obtained through a least-squares fit of each segment separately to reveal the trend of each section. The mean fluctuation (root mean square) of the incorporated and detrended time series was computed using Equation (2):

$$F(l) = \sqrt{\frac{1}{L} \sum_{k=1}^L (Y(k) - Y_l(k))^2}$$

The calculations mentioned above were repeated for a range of l . This analysis aimed to identify the relationship between $F(l)$ and the size of segment l due to this relationship serving as an indicator of a scaling phenomenon. In general, $F(l)$ increases with increases in the range of segment l . Double plot logarithmic graph ($\log(F(l))$ vs $\log(l)$) was generated, and this graph was used to acquire the scaling exponent (α). A linear dependency implies the existence of self-fluctuations, and $F(l)$, which is the slope of the line outlining the scaling α exponent, increases with l based on a power law, as illustrated in Equation (3):

$$F(l) - l^\alpha = > \log((F(l)) - \alpha * \log(l))$$

DFA yields the α scaling exponent in which represents the correlational structure of the signal. Anti-persistent stride-to-stride fluctuations were represented as $\alpha < 0.5$. Random stride-to-stride fluctuations (i.e., white noise; uncorrelated or stochastic) were represented as $\alpha = 0.5$. Persistent stride-to-stride fluctuations (i.e. pink noise; positive long-range correlations) were represented as $0.5 < \alpha \leq 1.0$. Over-persistent stride-to-stride fluctuations (i.e., brown noise; long-range correlations) were represented as $\alpha \geq 1.0$ (Peng et al., 1995).

Preliminary analysis identified a subset of participants who had both wrist-worn device data and IMU data during the MET ($N = 7$). Therefore, elevation sections were partitioned among participants without wrist-worn device data into uphill and downhill sections utilizing a

nearest-pairs technique such that participants who had finished the time trial with both IMU and wrist-worn device data were paired with a participant of closest finishing time without wrist-worn device data, and the resultant acceleration data of both participants were overlaid with paired elevation data to cross-reference the start and end of each gradient section of the participant without elevation data. This was conducted by observing substantial increases in resultant acceleration during the transition from the uphill to downhill section of the event in participants without wrist-worn device data based on previous investigations (Waite et al., 2021).

2.6. Statistical analysis

Descriptive statistics (mean $\pm$ SD) were calculated for all variables. Two-way repeated measures analysis of variance (ANOVA) was conducted to examine the effects of gradient (uphill, downhill), elapsed duration (phase 1, phase 2, phase 3), and interaction between gradient and duration on DFA- α . Significant interactions were followed by analyzing the simple main effect of duration at each level of a gradient. Non-significant interactions were followed by an assessment of the main effect of gradient and the main effect of duration wherein significant main effects were followed by post hoc pairwise comparisons using Bonferroni adjustment. Estimated marginal means $\pm$ standard error (SE) were expressed for the results of the two-way repeated measures ANOVA. Effect sizes (η_p^2) were used to inform the magnitude of the effect of gradient and duration on DFA- α , with values 0.01, 0.06, and 0.14 identified as lower thresholds for small, medium, and large effect sizes (Lakens, 2013). Power analysis using G*Power (v.3.1.9.4) was conducted to determine the required sample size for a two-way repeated-measures ANOVA. Based on expected differences in DFA- α reported in similar studies (large effect sizes: $\eta_p^2 \geq 0.215$, $f = 0.538$) (Chalitsios et al., 2025), and assuming a significance level of $\alpha = 0.05$ and power of 0.80, the power analysis indicated that a minimum of 13 participants would be required to detect a statistically significant effect. Statistical significance was set at $\alpha = 0.05$, two-tailed.

3. Results

3.1. Participants & course segmentation

Fourteen men (29.0 ± 6.1 y, 177.5 ± 10.7 cm, 83.1 ± 10.4 kg, 14.0 ± 3.6 %) completed the MET with available IMU data for analysis. Mean $\pm$ SD of time to complete the MET was 01:07:45 $\pm$ 00:06:59 s with time to complete the uphill and downhill gradients of the course as 00:41:48 $\pm$ 00:07:31 s and 00:21:00 $\pm$ 00:04:27 s, respectively. Mean $\pm$ SD of

velocity during the uphill and downhill gradients were $1.54 \pm 0.29 \text{ m s}^{-1}$ and $2.32 \pm 0.59 \text{ m s}^{-1}$, respectively. The mean $\pm$ SD of the number of segments in the uphill gradient was 7.94 ± 4.66 (range: 9–20), and within the downhill gradient was 3.66 ± 1.90 (range: 4–8). Participants' segments were partitioned into three phases with increasing elapsed duration. Mean $\pm$ SD number of segments per phase in the uphill gradient was 4.71 ± 1.20 , and the downhill gradient was 2.02 ± 0.75 . Participants' total counts of 256-stride intervals by gradient and counts by phase are shown in Fig. 2.

3.2. Comparison of DFA- α between gradients and durations

Two-way mixed measures ANOVA revealed a significant main effect of duration with a large effect size, (Phase 1: 0.593 ± 0.021 vs. Phase 2: 0.563 ± 0.031 vs. Phase 3: 0.493 ± 0.021 , $F = 3.833$, $p = 0.035$, $\eta_p^2 = 0.228$), suggesting that DFA- α values were significantly different between phases, irrespective of the gradient. There was not a significant effect of gradient, but the effect size was large (Uphill: 0.486 ± 0.031 vs. Downhill: 0.614 ± 0.035 , $F = 4.252$, $p = 0.060$, $\eta_p^2 = 0.246$), indicating that DFA- α values may differ across uphill and downhill portions of the course, but the effect remains uncertain. The duration $\times$ gradient interaction was also not significant with a small effect size ($F = 0.019$, $p = 0.981$, $\eta_p^2 = 0.001$), indicating that the effect of duration on DFA- α did not significantly differ between gradients.

Post hoc pairwise comparisons assessed the significant main effects of DFA- α between phases. The analysis revealed that DFA- α was significantly lower during the third phase compared to the first phase owing to a 17 % reduction in estimated marginal mean value (Phase 1: 0.593 ± 0.021 vs. Phase 3: 0.493 ± 0.021 , 95 % CI = [0.018, 0.182], $p = 0.016$)

with DFA- α moving from a gait variability pattern of persistence towards random/anti-persistence, irrespective of gradient (Fig. 3). There was not a significant difference in DFA- α between the first and second phases owing to a 5 % reduction in estimated marginal mean value (Phase 1: 0.593 ± 0.021 vs. Phase 2: 0.563 ± 0.031 , 95 % CI = [−0.93, 0.154], $p = 1.000$) or second and third phases from a 12.4 % reduction in estimated marginal mean value (Phase 2: 0.563 ± 0.031 vs. Phase 3: 0.493 ± 0.021 , 95 % CI = [−0.025, 0.165], $p = 0.196$), irrespective of gradient (Fig. 3).

4. Discussion

This investigation aimed to assess the effect of gradient and duration on the gait variability metric, DFA- α , among military personnel who performed an outdoor 6.72 km load carriage time trial during USMC MLC. Our first hypothesis that personnel would show significantly different DFA- α values between gradients, with lower values in the uphill gradient was only partially supported. Although the results did not reach statistical significance ($p = 0.060$), a large effect size ($\eta_p^2 = 0.246$) and contextualizing the estimated marginal means into their gait variability classifications indicated a distinct contrast between gradients as the uphill portion showed a gait variability pattern of slight anti-persistence (0.486 ± 0.031), whereas the downhill portion showed persistence (0.614 ± 0.035). Hence, these results may suggest that DFA- α may be altered during uphill load carriage ambulation compared to downhill ambulation, irrespective of duration. Our second hypothesis that DFA- α would show a reduction during each gradient towards randomness and anti-persistence with increased duration was met as there was a significant difference between the first and final phase,

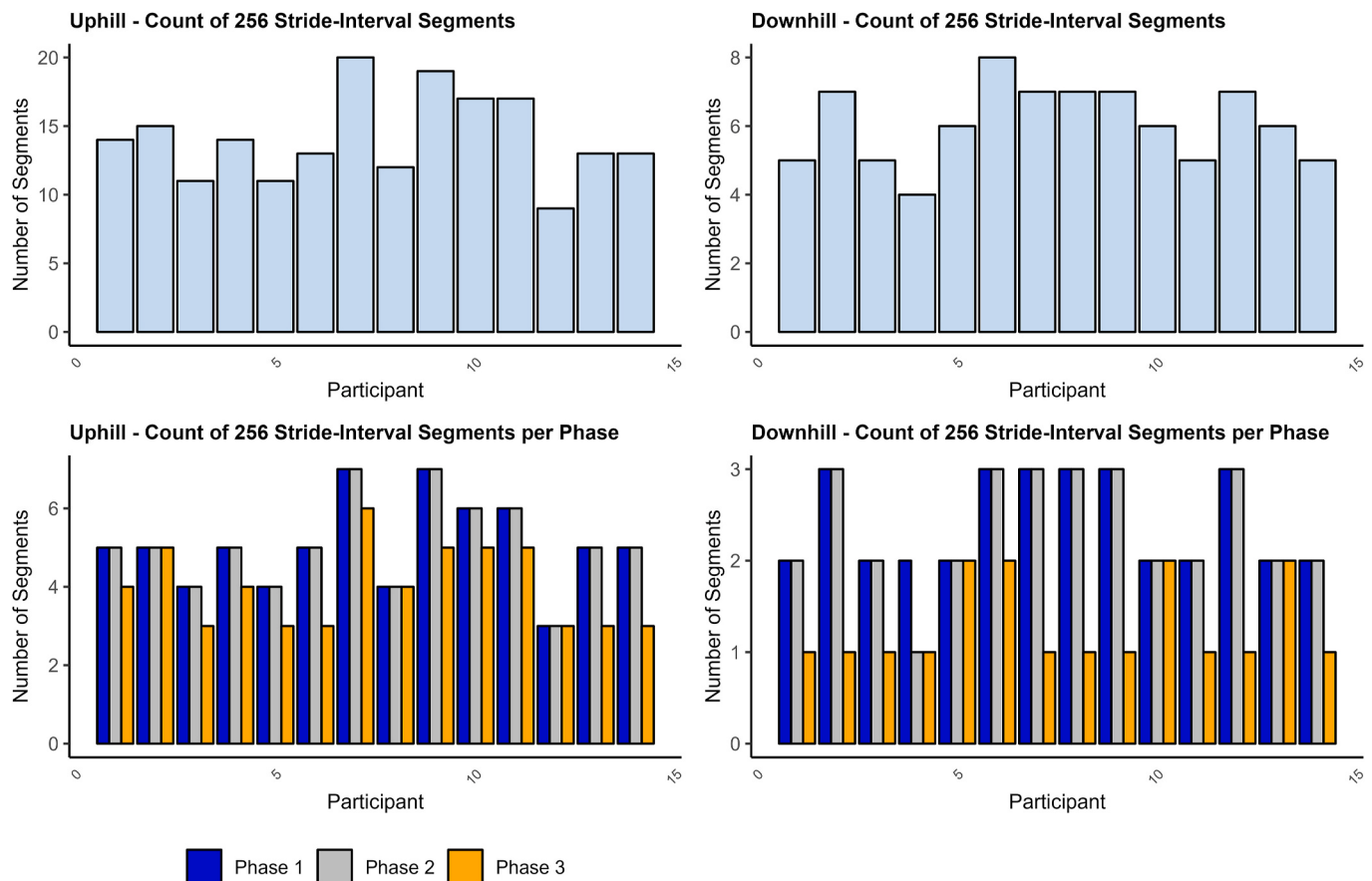


Fig. 2. Count of the number of 256-stride segments obtained during each uphill (top left facet) and downhill (top right facet) gradients with their elapsed duration phases (bottom facets) for each participant. All participants obtained three phases (Phase 1- blue; Phase 2 – gray; Phase 3 – orange) for analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

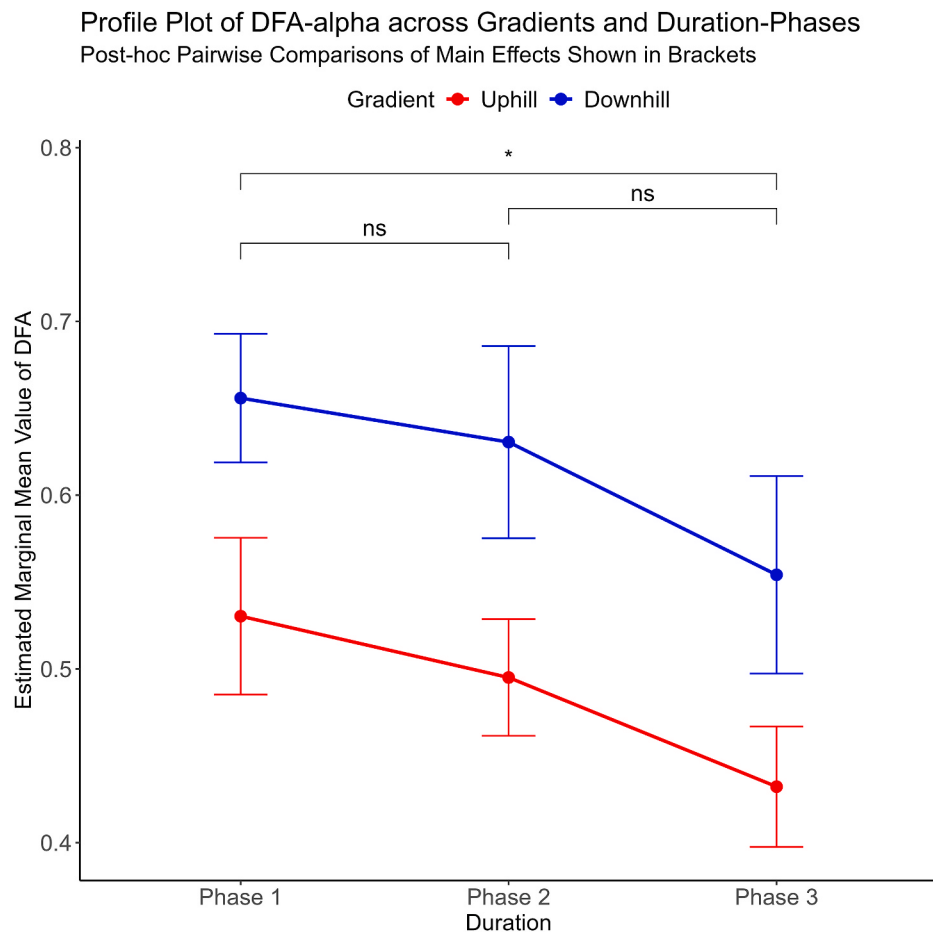


Fig. 3. Values are shown as estimated marginal means with standard error bars. Mean profile plot evaluated the interaction effect of duration and gradient on DFA- α . There was no significant interaction of gradient and duration on DFA- α , but there was a significant main effect of duration, where DFA- α significantly reduced from the first to the third phase. Asterisk (*) indicated statistical significance ($p < 0.05$).

irrespective of gradient, owing to a 17 % reduction in DFA- α with a large effect size ($\eta_p^2 = 0.228$) during the final phase compared to the first phase. Indeed, this reduction in DFA- α resulted in an estimated marginal mean value reflecting the transition of a gait variability pattern from persistence to slight anti-persistence (Fig. 3). Finally, our third hypothesis that there would be an interaction effect between gradient and duration on DFA- α was not met as the gradient changes were found to influence DFA- α similarly across each phase rather than differently over time (Fig. 3). Together, these results may suggest that military personnel performing a load carriage time trial may experience a reduction in gait coordination during an uphill portion of the event and potential fatigue-related changes in gait during its later stages with no evidence of a combined influence of gradient and phase on gait coordination. Hence, these findings may suggest that both environmental and contextual factors may have detrimental effects on DFA- α independently, thus driving gait patterns associated with reduced motor function capacity and MSKI risk.

Wearable monitoring of gait dynamics in the outdoor military training environment requires an understanding of the environmental factors that lead to aberrant gait patterns during in-training tasks, such as time-to-complete assessments. Gradient is a well-established factor that alters load carriage gait dynamics (Jones et al., 2024; Padulo et al., 2023; Kimel-Naor et al., 2017). Our results demonstrated a notable reduction in DFA- α in the uphill portion of the event compared to the downhill portion (Fig. 3), which remained lower than the downhill portion throughout the event. Although this difference was statistically non-significant, the observation of the large effect size presents opportunity for *post-hoc* power analysis to assure sufficient power and

contextualize this result. Hence, the following parameters were imputed: $N = 14$, $\alpha = 0.05$, number of measurements = 2, $\varepsilon = 1$, effect size $f = 0.571$ (converted from observed partial $\eta_p^2 = 0.246$), and the observed average within-subject correlation $r = 0.088$. From these inputs, the achieved *post-hoc* power was 0.83, which was above the conventional 0.80 threshold. Sensitivity analyses ($1 - \beta = 0.80$) returned a minimum detectable effect size of $f = 0.547$ ($\eta_p^2 = 0.231$) under these parameters. Additional sensitivity analyses under alternative correlation assumptions ($r = 0$ and $r = 0.5$) yielded minimum detectable effects of $f = 0.572$ ($\eta_p^2 = 0.247$) and $f = 0.405$ ($\eta_p^2 = 0.141$), respectively. Pairwise contrasts revealed a notable difference in gait variability between gradients (0.128 ± 0.062 , 95 % CI: 0.006, 0.261); however, the wide confidence interval indicates variation in the estimate. Hence, the large effect size, sufficient *post-hoc* power analysis, and contrasts with wide confidence intervals may suggest that the non-significant *p*-value may reflect wide variation in DFA- α rather than the absence of a true effect. As this task was conducted with a self-selected locomotor strategy, individuals may have switched between strategies within gradients to contribute to these results. However, Brink et al. (2024) also observed lower DFA- α values during an uphill portion of a fixed-speed load carriage ambulation at 3.0 % gradient than a downhill slope of -3.0 % gradient in US Army soldiers to support our results (Brink et al., 2024). Further examination of results from Brink et al. (2024) revealed that participants exhibited a mean DFA- α value at that inclination of 0.718 (95 % CI: 0.610, 0.826) (Brink et al., 2024). This value was 68 % higher than the marginal mean value during the uphill section in our participants. However, the aforementioned investigation was conducted on a motorized treadmill, which can

elevate DFA- α values compared to outdoor surfaces owing to greater motor control (Lindsay et al., 2014), and performed on half the gradient magnitude than the current investigation (3.0 % vs. 6.5 %). Greater gradient percentages have been shown to alter gait variability patterns owing to increased locomotor task difficulty level, decreased stability, and increased magnitude of neuromuscular muscle signal outputs that can impair the neuromuscular control system's ability to accommodate greater perturbations to the system (Walsh and Harrison, 2022). Together, these results may extend previous research to suggest that an uphill gradient during an outdoor load carriage assessment may serve as an ecological environmental factor that alters gait dynamics to a greater extent than a downhill gradient.

Utilizing DFA- α for detecting altered locomotor patterns, such as randomness or anti-persistence, under contextual factors has been proposed as useful for forecasting MSKI risk and risk factors, such as fatigue (Bellenger et al., 2019; Fuller et al., 2024a; Brink et al., 2024). Our analysis observed a significant main effect of duration on DFA- α independent of gradient with a large effect size (Fig. 3), such that the final phase of each gradient observed a significantly lower DFA- α value than the first phase (mean difference: 0.100) to align with our second hypothesis. For the uphill condition, participants performed a gait variability pattern of slight persistence in the first phase (0.530 ± 0.045 , 95 % CI = [0.433, 0.628]), toward randomness in the second phase (0.495 ± 0.034 , 95 % CI = [0.423, 0.568]) to anti-persistence in the final phase (0.432 ± 0.035 , 95 % CI [0.357, 0.507]). Similarly, the downhill condition began with a gait variability pattern of persistence (0.656 ± 0.037 , 95 % CI [0.576, 0.736]) and then shifted toward a pattern of randomness in the final phase (Phase 2: 0.631 ± 0.055 , 95 % CI [0.511, 0.750]; Phase 3: 0.554 ± 0.057 , 95 % CI [0.431, 0.677]) (Fig. 3). Reduction in DFA- α during prolonged load carriage tasks has been suggested as an indicator of fatigue that may be detrimental to the integrity of the soldier's ambulatory stability and operational performance (Brink et al., 2024). Although similar observations were made during treadmill-based ambulatory tasks at fixed speeds (Krajewski et al., 2023; Brink et al., 2024) or on flat gradients (Ahamed et al., 2021), the current results may extend these observations by suggesting that the duration of the task, irrespective of the gradient, may serve as an ecological contextual factor that may indicate the onset of fatigue. A 17 % reduction in DFA- α may also be interpreted based on Dingwell and Cusumano (2010) as participants utilizing a larger set of motor solutions (i.e., increased control effort) to achieve the desired task goal during the final phase (Krajewski et al., 2020b). This alternative explanation may reflect that participants were exerting greater stride-to-stride control over their stride times owing to greater caution during the event for a potential reduction in motor function capacity. Unfortunately, one cannot conclude from the results whether the observed gait dynamic changes occurred due to degraded physiological control or adaptation in response to physical and/or physiological limitations. However, Black et al. (2007) suggested that altered gait variability during prolonged tasks may suggest a locomotor system operating closer to the actional boundaries that would be more likely to fail (fall, MKSI) with additional perturbations (Black et al., 2007). This reduction in DFA- α during each gradient may serve as an important contextual factor for load-carriage-related MSKI risk (Nesterovica-Petrikova et al., 2023; Brink et al., 2024).

Since gradient and task duration are not encountered independently in field settings, investigating their interaction during load carriage may clarify their combined influence on gait variability. Our analysis revealed no significant interaction between gradient and duration on DFA- α , indicating that gradient affected DFA- α similarly across phases rather than changing over time as hypothesized. As shown in the profile plot (Fig. 3), DFA- α progressed from slight persistence in the first phase to anti-persistence in the third phase of the uphill gradient. Upon reaching the ascent, participants appeared to 'reset' their gait coordination by returning to persistence in the first phase before following a similar temporal decline toward randomness. This pattern may reflect

increased locomotor difficulty and/or fatigue during the ascent followed by a regained gait coordination early in the downhill and potential fatigue in its later stages, which may have reduced the likelihood of an interaction. Several additional factors may also explain the null result. Military personnel are trained to sustain performance under load (Feigel et al., 2025), which may buffer against interactive effects. In addition, the use of three phases may have been too coarse to capture subtle nonlinear dynamics. Individual differences in fatigue tolerance, gait mechanics, and recovery capacity could also have obscured group-level effects (Bellenger et al., 2019; Fuller et al., 2024b). Moreover, real-world terrain, pace regulation, and external factors (i.e., load carriage stability) may have exerted stronger influences on DFA- α than the interaction itself. Finally, linear interaction test may overlook complex nonlinear dependencies (Kristensen and Hansen, 2004). Future work should consider nonlinear approaches, such as polynomial terms, spline-based models, or generalized additive mixed models with random slopes, to capture time-varying effects that simple phase-based analysis may miss (Mundo et al., 2022). Together, although these results suggest that gradient and duration independently shaped DFA- α , adaptive strategies, task constraints, and reset effects during terrain transitions may have mitigated any observable interaction.

Reductions in DFA- α during load carriage represent variable stride-interval patterns that may provide meaningful analytics to detect task difficulty and fatigue-related changes in gait during prolonged load carriage tasks in active operations and military training courses. Employment of real-time monitoring of DFA- α trends towards randomness and/or anti-persistence may allow leadership to take a proactive approach in field training exercises, such as adjusting load carriage pacing strategies or redistributing external load to assist in managing personnel fatigue, maintaining optimal performance, and reducing load-carriage-related MSKI risk. By providing objective measures of gait strategies used during tasks, military practitioners or leadership can streamline decision-making processes to enhance task completion for operational readiness. Interventions allowing the evaluation of DFA- α in real-time during load carriage may help detect when (duration) and under which circumstances (uphill gradient) reduced DFA- α occurs.

Although this investigation revealed novel results, it is not without limitations. First, this investigation comprised a subset of participants who wore both devices, thus requiring the estimation of the start of the downhill gradient to be based on a nearest-pairs technique. However, previous investigations report the use of acceleration data as a suitable method to determine slope changes (Waite et al., 2021). Second, the number of segments was not controlled, thus requiring gradients to be partitioned into phases. This led to an unequal number of segments (Fig. 2). However, participants contained an equal segment number for the first two phases to mitigate this limitation. Third, this investigation did not include formal measures of fatigue in which may have provided contextual supportive data to justify the changes in DFA- α over the three phases per gradient. Future studies should include direct physiological markers of fatigue to validate this relationship. Nevertheless, this investigation found a notable effect of duration and gradient on DFA- α to extend previous research to signify that both elapsed duration and uphill gradients may negatively affect gait dynamics to serve as ecological determinants of load-carriage-related MSKI risk (Brink et al., 2024).

5. Conclusion

DFA- α during an outdoor 6.72 km load carriage time trial is significantly reduced with increased elapsed duration of the task, independent of gradient, with a notable reduction in DFA- α during a 6.5 % uphill gradient, independent of duration, suggesting that elapsed duration and uphill gradients may serve as contextual and environmental factors associated with gait alterations indicative of reduced gait coordination and potential fatigue for increased load-carriage-related MSKI risk. Reduced DFA- α over time may indicate fatigue and greater motor solution utilization that may inhibit one's ability to overcome larger

perturbations. Our results provide the opportunity and practical support for implementing gait monitoring interventions to evaluate DFA- α in real-time during load carriage tasks. This may help detect when (duration) and under which circumstances (uphill gradient) reductions in DFA- α occur. Detected reductions in DFA- α may serve as prompts to signal the adjustment of pacing strategies or load distribution modifications across a team to manage fatigue-related impacts on gait patterns.

CRedit authorship contribution statement

Evan D. Feigel: Writing – original draft, Visualization, Software, Formal analysis, Data curation, Conceptualization. **Ayden McCarthy:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Joel T. Fuller:** Writing – review & editing, Methodology, Conceptualization. **Lily Rosenblum:** Writing – review & editing, Software, Methodology, Data curation. **Mita Lovalekar:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Tommi Ojanen:** Writing – review & editing, Data curation. **Kai Pihlainen:** Writing – review & editing, Data curation. **Brian J. Martin:** Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Kristen J. Koltun:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Tim L.A. Doyle:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Bradley C. Nindl:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of interest

None.

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