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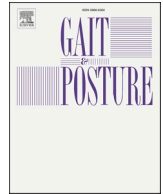
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The effect of external loads and biological sex on coupling variability during load carriage

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ABSTRACT

Background: Load carriage is a fundamental requirement for military personnel that commonly results in lower-limb injuries. Coupling variability represents a potential injury mechanism for such repetitive tasks and its unknown whether external loads and biological sex affect coupling variability during load carriage.

Research question: Is there a sex-by-load interaction during load carriage at self-selected walking speeds?

Methods: Twenty-six participants (13 males, 13 females) completed three 10-minute treadmill-based trials wearing body-borne external load (0 %BM, 20 %BM, and 40 %BM) at load-specific self-selected walking speeds. A Vicon motion capture system tracked markers with a lower-body direct-kinematic model calculating sagittal-plane segment kinematics of the thigh, shank, and foot across 19 strides. Continuous relative phase standard deviation (CRPv) provided a measure of coupling variability for each coupling angle (Thigh-Shank and Shank-Foot). The CRPv for each load and sex was compared using statistical parametric mapping repeated measures ANOVA and paired *t* tests.

Results: Significant sex-by-load interactions were reported for the Thigh-Shank coupling. Males demonstrated no significant load differences in CRPv, however, females displayed significantly higher CRPv in the 40 %BM than the 0 %BM condition. A significant main effect of load was observed in the Shank-Foot coupling, with the 40 % BM having significantly greater CRPv than the other load conditions.

Significance: Both biological sex and external loads significantly affected CRPv during load carriage at self-selected walking speeds. Females demonstrated greater CRPv at the heavier loads, suggesting that the perturbation from the heavier mass increases coupling variability, which may also be amplified by a greater total passive load due to their relatively higher adipose tissue compared to males. The consistent CRPv in males suggests that higher relative loads may be required to change coupling variability. Collectively, these results suggest that external load affects the coupling variability of males and females differently, providing potential for injury screening and monitoring programs.

1. Introduction

The loads carried by military personnel significantly increases their injury risk, particularly of the lower-limbs [1,2]. Orr et al. [1] reported that 34 % of soldiers will sustain at least one load-carriage related injury across their military career. Importantly, female soldiers appear more likely to sustain either a serious time-loss injury (~2.4 fold increase), or any injury (~2 fold increase) than male soldiers [3]. The frequency of

load-carriage related lower-limb injuries is problematic for military organisations as it directly impacts the physical availability and capability of personnel.

Associations between the variation in the magnitude and relative timing of motion between segments (coupling variability) and various injury and pathological states has been demonstrated [4–6]. The dynamical systems theory suggests that coupling variability represents the flexibility and adaptability of the motor system to achieve a task [4,

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6]. Compared with healthy people, lower coupling variability has been reported in individuals with injury or disease such as low-back pain [6], patellofemoral pain (PFP) [4], and Parkinson's disease [7]. James et al. [8] proposed the overuse injury-variability hypothesis on the principle that greater variability reduces the risk of injury due to the redistribution of the magnitude, direction, rate, or frequency of the stress within the same tissue [8]. However, abnormally high variability can be indicative of unstable and less adaptable coupling patterns that are directly linked to lack health [9] and, researchers have suggested than an optimal bandwidth of variability in may exist that reduces injury risk [5,9]. The high rates of injury during load-carriage activities of suggests that the redistribution of forces, due to the amount of variability in the lower extremities, may play role in lowering injury risk.

The effect of load carriage on coupling variability has been reported in relation to the effect of handling position [10] and assistive devices [11] across several tasks, including treadmill and overground walking [12,13], and running [13,14]. Despite methodological differences, these studies collectively demonstrate that greater external loads are associated with increased coupling variability. Additionally, limited equivocal research has explored the impact of biological sex on coupling variability. Research has demonstrated females display significant decreases in relative variability and ankle joint work with the addition of load [15,16], and increases in total knee joint moments at heel strike [17] during forced marching. Similarly, males and females have displayed significantly different fractality of their gait patterns during load carriage tasks [18,19]. Current research on coupling variability has demonstrated both higher [20] and lower [21] variability in females when compared with males during different tasks. Despite both studies reporting biological sex differences in coupling variability, the tasks completed (45° cutting manoeuvre [21] and running overground [20]) differ in complexity and degrees of freedom used. Little research has focused on lower-limb coupling variability during pervasive military tasks such as loaded marching. As such, generalising extant coupling variability data to the task of marching is not feasible. Recent research that has reported on lower-limb coupling variability during loaded military marching activities was limited to only female participants [13]. As such, no research has investigated the interaction between biological sex and external load on coupling variability during military-relevant load carriage activities.

Therefore, the aim of this study was to investigate the effect of biological sex and external loads on lower-limb coupling variability during walking at self-selected walking speeds. It was hypothesised that lower-limb coupling variability would increase with greater external loads and there would be significant biological sex differences.

2. Methods

All procedures were approved by La Trobe University's Science, Health and Engineering College Human Ethics Sub-Committee (Ethics number: HEC18146) with written informed consent provided before participating. This study was an independent secondary analysis of previously published data [22].

2.1. Participants

Twenty-six healthy young adults (13 females, age: 25.8 ± 6.3 y, stature: 1.67 ± 0.06 m, body mass: 61.9 ± 7.2 kg; 13 men, 21.9 ± 2.0 y, 1.79 ± 0.01 m, 72.4 ± 5.7 kg) volunteered to participate. The sample size utilised for this secondary analysis was deemed appropriate as it was larger than previous studies that have found statistically significant differences using SPM, therefore reducing the risk of type II error [23–25]. To be representative of recruits, participants were not military personnel and had limited external load carriage experience. All were injury free for at least six months immediately prior to participation. To be eligible, participants were required to pass the Australian Army physical fitness entry standards in line with previous research, which

consisted of 8 (female) or 15 (male) push-ups, 45 sit-ups and an estimated VO_2max of $> 38.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [22,26].

2.2. Data collection

Each participant completed three 10-minute walking trials of incremental, torso-borne external load (0 % of body mass [BM], 20 %BM and 40 %BM) applied via a weighted vest (Little Bloke Fitness, Reservoir, VIC, Australia). The load was added symmetrically antero-posteriorly and medio-laterally using 1-kg metal blocks to keep load close to the centre of mass and best reflect the double backpack used in military contexts [27]. A self-selected walking speed was determined for each torso-borne load (0 %BM, 20 %BM, 40 %BM) using the average speed during three minutes of walking around a 'figure-eight' overground lap (length 80 m), which also allowed for habituation of each load [22]. Load specific self-selected walking speeds were used to remove speed as a potential confounding variable [28]. All data were collected on a Trackmaster motorised treadmill (TMx58, Newton, Kansas). Data was collected for 19 consecutive strides over the 6th minute for each load condition from each participant. Trials were conducted in the order 0 % BM, 20 %BM and 40 %BM, allowing for linear increases in muscle stiffness to ensure safe task completion [29]. Relative loads were chosen to remove differences in absolute load as a confounding variable as per previous research [12,22,28]. Each participant had a minimum of 10-minutes passive rest between trials with heart rate returning to within 10 % of resting values prior to starting the next trial.

Thirty-six retroreflective markers were attached bilaterally to each participant on their anterior and posterior superior iliac spines, iliac crests, medial and lateral femoral epicondyles, medial and lateral malleoli, calcanei, and first and fifth metatarsal heads. To measure segment motion, additional markers ($n = 4$ per plate) were affixed to custom moulded thermoplastic plates that were attached laterally on each thigh and shank. Marker trajectories were captured using a 10-camera Vicon V16 opto-reflective motion capture system (Vicon Motion Systems Ltd, Oxford, UK; 100 Hz). Data were processed and labelled using Vicon Nexus (version 2.10.2).

2.3. Data analysis

After raw trajectory data were filtered using a dual-pass second-order low-pass Butterworth filter with a 6 Hz cut-off frequency (determined by a residual analysis and visual inspection), a seven-segment lower-limb and pelvis direct kinematic model was used to calculate required joint centres [22,30]. Knee and ankle joint centres were calculated as the midpoint between the femoral epicondyles and ankle malleoli, respectively, whereas hip joint centres were calculated using a regression equation [31]. Segment-embedded anatomical coordinate systems were defined in accordance with the International Society of Biomechanics recommendations. For each segment, the origin was defined as the distal joint centre and the x-, y-, z-axes were defined in the anteroposterior, longitudinal and mediolateral directions, respectively [32,33]. Segment angles were calculated as the rotation of the segment-embedded coordinate systems relative to the global (laboratory) coordinate system using a z-x-y rotation sequence.

Coupling between segments was assessed using continuous relative phase (CRP). Segment angles were normalised to centre around zero [34] to which the Hilbert Transformation was then applied. The CRP of the Thigh-Shank and Shank-Foot couplings in the sagittal plane were calculated using the following equation:

$$\text{CRP}(ti) = \arctan(H1(ti)x2(ti) - H2(ti)x1(ti) / x1(ti)x2(ti) + H1(ti)H2(ti))$$

where, H_1 and H_2 are the imaginary components of the Hilbert Transform of segment 1 (proximal) and segment 2 (distal), respectively, and x_1 and x_2 are the original signal of segment 1 and segment 2, respectively [34].

CRP values were then segmented to 19 strides (gait cycle; heel contact to heel contact) and time normalised to 101 data points using

cubic-spline interpolation for each participant per condition. Coupling variability was quantified as the standard deviation of the CRP (CRPv) across the 19 strides at each point of the gait cycle and was calculated using circular statistics [35].

2.4. Statistical analysis

To determine the effect of external load and biological sex on walking speed, a repeated measures analysis of variance (ANOVA) was performed in SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) with α set at 0.05. Effect sizes (partial eta squared [η_p^2]) were classified as follows: large effect > 0.14 , moderate effect > 0.06 , and small effect > 0.01 [36]. Where significant interactions or main effects were present, post hoc pairwise comparisons were performed with a Sidak correction for multiple comparisons. Data are presented as mean \pm SD unless stated otherwise.

Statistical analyses of CRPv time series were compared between loads (0 %BM, 20 %BM and 40 %BM) and biological sexes using statistical parametric mapping, repeated measures ANOVA and paired t tests ($\alpha = 0.05$) [37] implemented through the open-source spm1d package (v. 0.4, www.spm1d.org, Pataky, 2012 [38]) in MATLAB (v9.10.0.1649659 (R2021a), The Mathworks Inc., Natick, MA). Standardised mean difference effect sizes for within-subject design (Cohen's d_z [36]) were calculated for each percent of the gait cycle and calculated as per Rosenthal [39].

3. Results

There was no sex-by-load interaction ($p = 0.097$, $\eta_p^2 = 0.092$) nor main effect of biological sex ($p = 0.578$, $\eta_p^2 = 0.013$) for walking speed. However, there was a significant main effect of external load ($p = 0.002$, $\eta_p^2 = 0.231$), with the 40 %BM condition demonstrating a significantly slower walking speed than both the 0 %BM (mean difference \pm standard error: 0.13 ± 0.05 km·h⁻¹, $p = 0.034$, $d_z = 0.84$) and 20 %BM conditions (0.16 ± 0.04 km·h⁻¹, $p = 0.008$, $d_z = 0.66$).

A significant sex-by-load interaction was observed for the Thigh-Shank coupling (percent of gait cycle: 13–15 %, $F[2,48] > 6.311$, $p = 0.047$) (Fig. 1. A). While there were no significant differences in CRPv with changes in external load for the males (Fig. 2. A), the female participants displayed significantly higher CRPv in the 40 %BM condition compared with the 0 %BM condition ($t[1,12] > 3.826$, $p < 0.001$) during the stance phase of gait (Fig. 2. B). There were no significant biological sex differences, however, the female cohort demonstrated

generally lower CRPv than the males, with effect sizes between biological sexes ranging from -0.63 to -0.18 (0 %BM), -0.37 to -0.02 (20 %BM), and -0.06 to -0.12 (40 %BM).

For the Shank-Foot coupling there were no significant sex-by-load interactions, nor a main effect of biological sex on CRPv (Fig. 1. B). However, there was a significant main effect of external load throughout the gait cycle (3–10 %, 21–26 %, 38–56 %, and 64–2 %, $F[2,48] > 6.589$, $p \leq 0.047$) on CRPv. The 40 %BM condition had significantly higher CRPv than both the 0 %BM ($t[1,25] > 3.362$, $p \leq 0.045$) and 20 %BM ($t[1,25] > 3.395$, $p \leq 0.047$) conditions throughout swing and some of stance (Fig. 3).

4. Discussion

This study aimed to quantify the effect of external load and biological sex on coupling variability during walking at self-selected speed with incrementally increased relative external loads. Whilst studies have investigated the effects of sex and load on coupling variability independently, to the author's knowledge this is the first study to explore the interaction between biological sex and load. A significant sex-by-load interaction was observed in the Thigh-Shank coupling, with female participants displaying greater variability in the 40 %BM condition compared with the 0 %BM condition, unlike male participants where there was no change across loads. There was a main effect of load for the Shank-Foot coupling, with CRPv being significantly greater in the 40 %BM condition than both the 0 %BM and 20 %BM conditions.

Only the Thigh-Shank coupling demonstrated a significant sex-by-load interaction, whereby the female participants displayed greater variability during the 40 %BM condition when compared with the 0 %BM condition, with no significant changes in the males. When these data were previously analysed [22] in their zero-dimensional form (discrete spatiotemporal and kinematic measures), there were no significant sex-by-load interactions. Research that has explored the effect of biological sex on coupling variability is equivocal and task-dependent [20, 21]. In the men, CRPv did not change with increasing external loads, which suggests that the loads did not provide enough perturbation to affect coupling variability. Previously, Scott & Ramabhai [40] reported that females worked at a higher physiological intensity than males when carrying equal relative external loads (37 %BM). They suggested that this reflected the greater body fat mass of the females (17 kg) compared with the males (9 kg), which resulted in a greater total passive load for females (41 kg) than the males (36 kg). In support, the previous primary analysis of this data demonstrated a main effect of sex whereby the

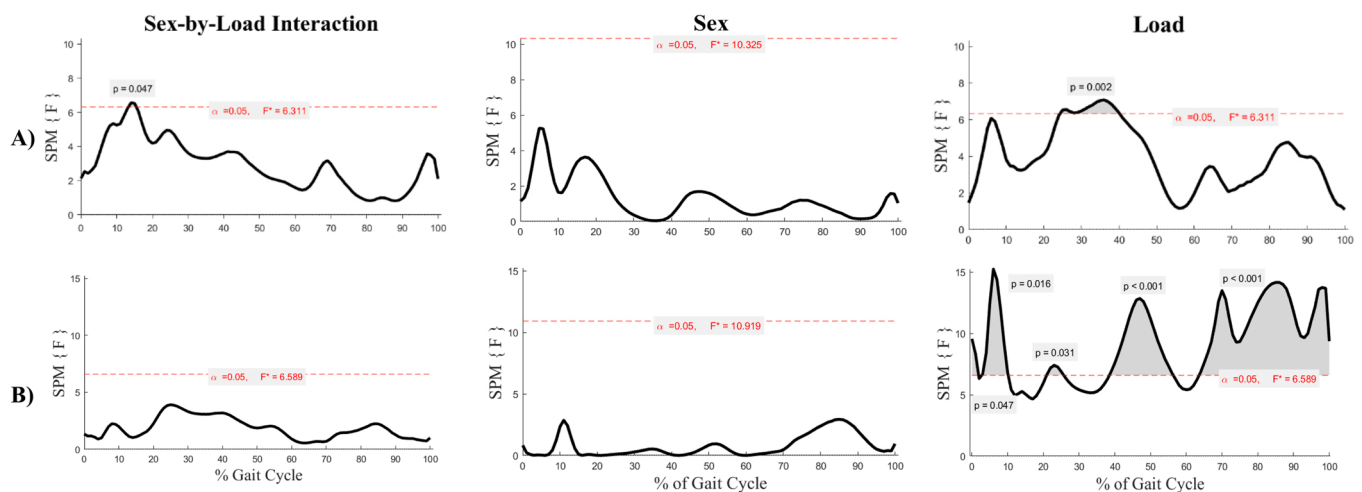


Fig. 1. SPM ANOVA F -statistic (black line) for sex-by-load interaction, the main effect of biological sex, and the main effect of external load for the entire gait cycle. Critical F -threshold is displayed on the red dashed line. Grey shaded region represents the parts of the gait cycle where there is a statistically significant interaction or main effect. (A) Thigh-Shank coupling. (B) Shank-Foot coupling.

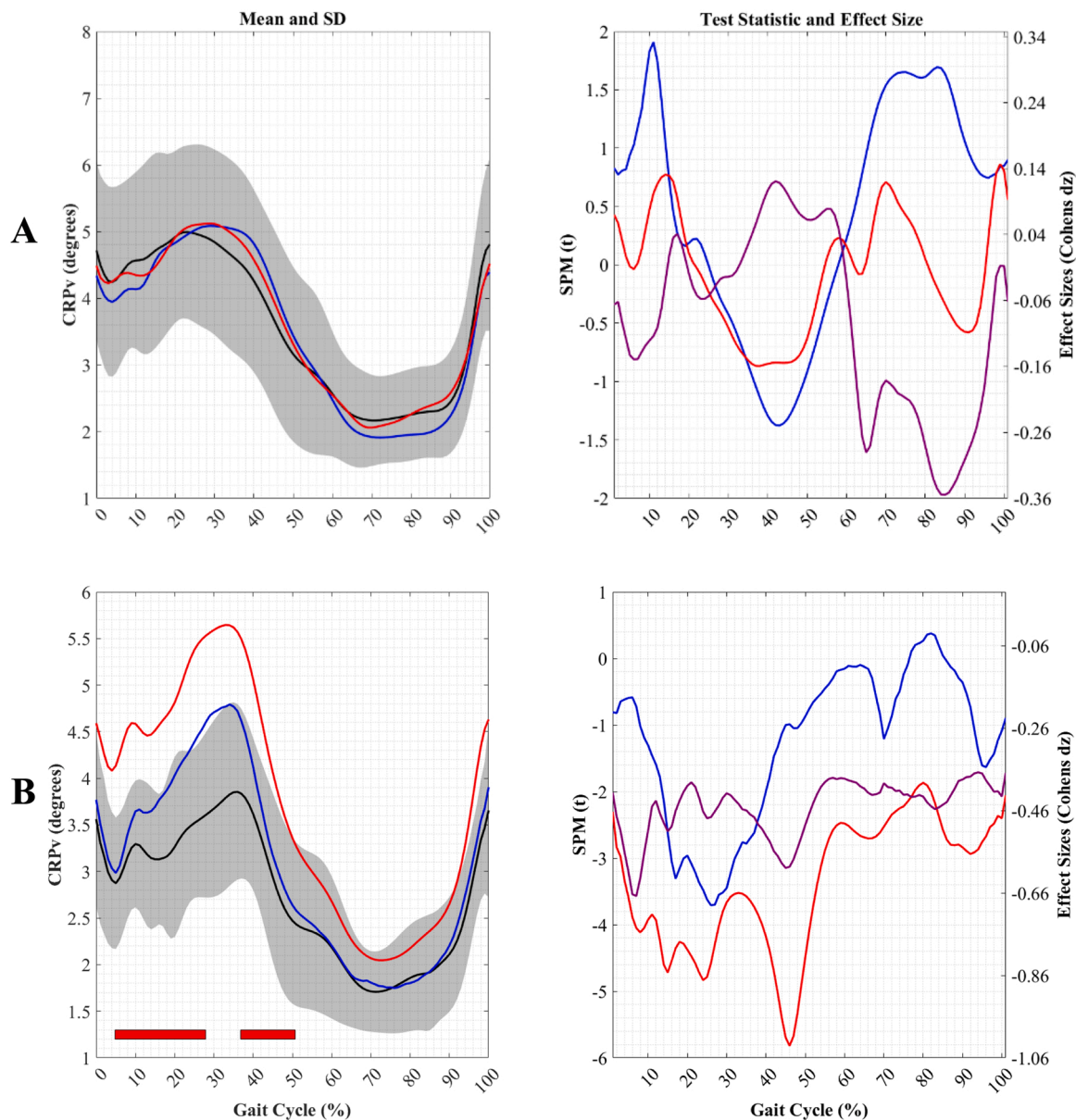


Fig. 2. The mean coupling variability (CRPv), t statistic, and effect sizes of the (A) male and (B) female participants for the Thigh-Shank coupling from 0 % to 100 % (heel strike to heel strike) of the gait cycle. The solid lines (left) represent the mean CRPv of the 0 %BM (black), 20 %BM (blue) and the 40 %BM (red) condition. The shaded grey region represents the standard deviation of the 0 %BM condition. The coloured bars (left) show when the SPM $\{t\}$ critical threshold was exceeded between the 0 %BM and 20 %BM conditions (blue), the 0 %BM and 40 %BM conditions (red), and the 20 %BM and 40 %BM conditions (purple). The solid lines (right) represent the t statistic and effect sizes of the paired t test, comparing the 0 %BM and 20 %BM conditions (blue), the 0 %BM and 40 %BM conditions (red), and the 20 %BM and 40 %BM conditions (purple).

females were observed to elicit a greater physiology intensity (5 ± 2 % VO_2max averaged across all loads) than the males across the same protocol [22]. Further, a main effect of load was also observed indicating that physiological intensity significantly increased between all external load conditions.

The higher physiological intensity of the female participants might help to explain the greater relative increases in CRPv observed, which is consistent with previous research that's reported increased coupling variability during tasks with heavier loads [10–12,14]. Given the lower CRPv observed in the 0 %BM and 20 %BM conditions for the female participants, the introduction of a perturbation (i.e. 40 %BM load), particularly at a time of high knee-joint loading [41], may have increased coupling variability as the motor system attempts to find a suitable movement solution in response to the constraints imposed by the load. However, it is also possible this increased coupling variability

in the 40 %BM condition may result in instability during early stance and increase the risk of acute-type injuries.

For the Shank-Foot coupling, CRPv significantly increased with load for most of the swing phase and during loading response in the early stance phase. The constraints imposed by the heavier loads appear to have perturbed the movement system and forced participants to search for suitable movement solutions in the lead up to, and during initial contact and loading response, when internal loads and risk of injury are high [42,43]. In line with previous research, it can be speculated that the increase in CRPv at higher loads is potentially functional in several ways.

First, it might help individuals to avoid task failure (e.g., falling) under the more challenging constraints, and adapt in response to additional perturbations such as an external impact force or an unexpected change in the walking surface [44]. Ippersiel et al. [44] recently

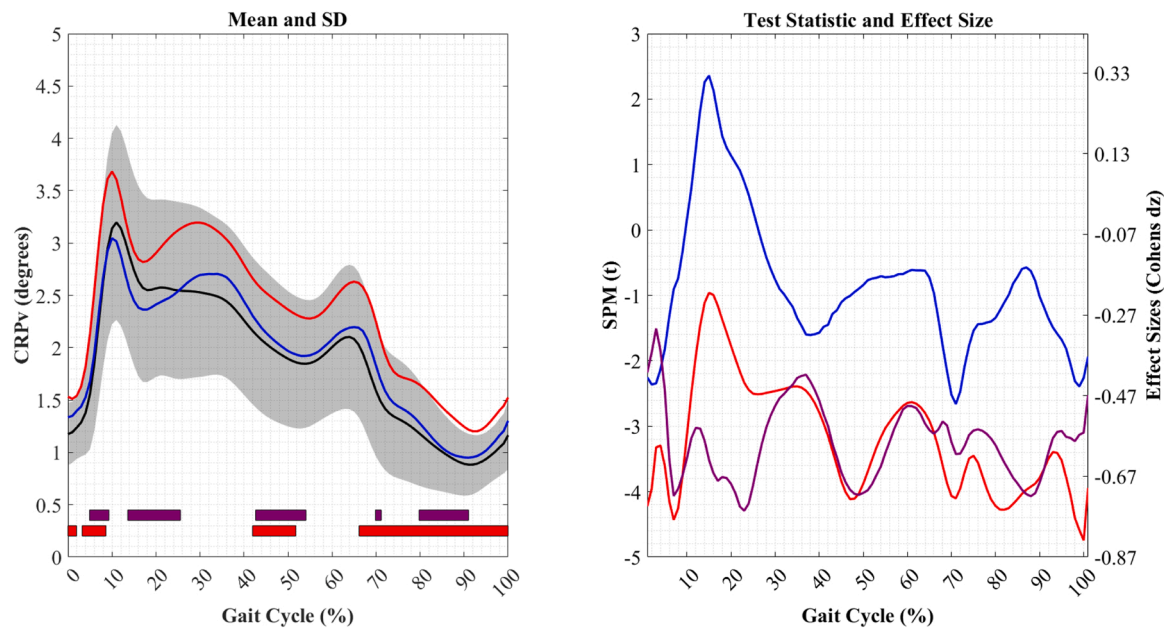


Fig. 3. The mean coupling variability (CRPv), t statistics and effect sizes of all participants for the Shank-Foot coupling from 0 % to 100 % (heel strike to heel strike) of the gait cycle. The solid black line represents the mean CRPv, with the shaded grey region representing the standard deviation of the mean CRPv for the 0 %BM condition. The solid lines (left) represent the mean CRPv of the 20 %BM (blue) and the 40 %BM (red) condition. The solid lines (right) represent the t statistic and effect sizes of the paired t test, comparing the 0 % and 20 %BM conditions (blue), the 0 % and 40 %BM conditions (red) and the 20 % and 40 %BM conditions (purple). The coloured bars (left) show when the SPM (t) critical threshold was exceeded between the 0 % and 20 %BM conditions (blue), the 0 % and 40 %BM conditions (red) and the 20 % and 40 %BM conditions (purple).

reported that coupling variability increased in the ankle-knee and knee-hip couplings (with average increases of 8.28° and 1.48° , respectively) during walking on an uneven surface. They suggested that this is a likely functional response and reflective of adaptations to the more demanding task of walking on an irregular surface. In the current study, placing additional demands on the neuromuscular system by perturbing it with a torso-borne load provoked a similar response in terms of lower extremity coupling variability to walking on an uneven surface. However, further research using specific adaptability experimental protocols is required to determine whether the increased coupling variability under more challenging conditions is functional in this way, and reflective of the adaptability of the neuromuscular system.

Second, the greater variability during, and in the lead up to, the initial loading response might serve to redistribute loads amongst lower extremity tissues, reducing the risk of cumulative overuse injury [8]. However, it is also possible that the variability seen at higher loads could be too large, falling outside of the bandwidth of functional variability [5, 9]; reflecting unstable movement patterns and exposing individuals to increased risk of acute injuries. Rates of acute ankle injuries in military personnel are particularly high during load carriage and the increases in coupling variability seen at high loads could be a contributing factor [2]. More work, including carefully designed prospective experiments, is required to ascertain the relationship between coupling variability and the occurrences of overuse and acute injuries during load carriage.

Third, the greater coupling variability at higher loads might help minimise centre of mass displacement during the gait cycle [14]. This is important because of the relationship between centre of mass displacement and energetic cost [45]. Techniques such as Uncontrolled Manifold (UCM) analysis can be used to decompose variability into ‘good’ and ‘bad’ variability, dependent on the impact on the outcome of a movement. ‘Good’ variability is that which has no effect on the outcome variable (e.g., centre of mass position), indicating the variability in the movement system is helping to maintain a consistent outcome. A conceptually similar technique, Goal Equivalent Manifold analysis, has been recently employed to examine the effects of load carriage on the regulation of stride length and speed [17]. Furthermore,

studies have previously employed UCM analysis to explore the regulation of centre of mass movement during gait [46,47], but not during gait with load carriage. Using techniques such as UCM to identify the extent that the increased coupling variability observed at higher loads contributes to minimizing centre of mass displacement should be a focus of future research. Such research would help to further identify the relationship between both coupling and coordination variability and energetic cost.

4.1. Limitations and future research

This study has potential limitations and has identified areas of future research. One potential limitation of the current study is the use of treadmill-based locomotion, with lower-limb coupling variability being significantly lower during treadmill walking than overground walking [48]. However, as 10–15 strides are necessary to ensure accurate estimates of coupling variability [49], a treadmill is a more practical method as it allows for many consecutive strides to be collected. While self-selected walking speed and relative loads do not reflect standard military practice, they removed fixed walking speed and absolute load as potential confounding variables. Following this initial investigation, future research should replicate this study using fixed walking speeds and absolute loads, which are more representative of military practice. Another incremental progression for future research would be to account for total passive load (fat mass and external mass) when exploring the effects of relative and absolute loads on coupling variability during walking.

Previous research has suggested that the greater coupling variability observed during loaded conditions is a result of a lack of experience in heavy load carriage [12,14]. Further, the available research supports that greater experience in load carriage tasks decreases coupling variability among participants [12]. Future research should therefore aim to understand the effect that load carriage experience has on lower-limb coupling variability in military personnel. Moreover, the length of the current study does not reflect the typically extended length of load carriage tasks undertaken by military personnel post recruit training.

The results may therefore not be representative of movement strategies adopted for extended tasks and future research should explore changes in coupling variability in prolonged load carriage tasks.

Finally, due to the cross-sectional nature of the current study, no relationship can be drawn between changes in lower-limb coupling variability and injury risk. To improve understanding of the underlying causes or effects of coupling variability on injury, prospective studies exploring the relationship between coupling variability and injury occurrence are required to be undertaken.

5. Conclusion

This study examined the effect of biological sex and external load on coupling variability during load carriage at self-selected walking speeds. There was a significant sex-by-load interaction for the Thigh-Shank coupling and main effects of external load for both Thigh-Shank and Shank-Foot. The increase in Thigh-Shank coupling variability at the heaviest relative load was only observed in the female participants, suggesting that the higher total passive load presented a sufficient perturbation that triggered an increase in coupling variability. The increase in Shank-Foot coupling variability at the heaviest relative load support that the loads carried by military personnel may impose a system perturbation that facilitates an adaptive response.

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Declaration of Competing Interest

The authors have no conflicts of interest to report.

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