ARTICLE

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Does physical fitness level affect body balance and physiological responses after a 4 km load carriage task?

¿El nivel de aptitud física afecta el equilibrio corporal y las respuestas fisiológicas después de realizar un ejercicio en el cual se transporta una carga durante 4 km?

Abstract: It is not known whether higher physical fitness level (PFL) is really an advantage for military personnel in operational tasks. Objective: To investigate the effects of PFL on body balance and physiological responses in a 4 km load carriage task. Body balance was analyzed (n=22) using personal equipment (22 kg) before and after the 4km load carriage (treadmill). Heart rate (HR) was assessed throughout the task and PFL considered the result of the most recent Brazilian Army physical fitness test. Two-way mixed ANOVA and independent t-tests were applied ($p<0.05$). Load carriage significantly increased sway area (72.21±30.94 to 102.68±48.57 mm^2) and other balance variables, without effects of PFL. The group with excellent PFL had lower mean HR values (104.55±9.37 bpm) compared with the group with good or very good PFL (115.07±10.14 bpm). Carrying 22 kg for 4 km worsened body balance and the military personnel with better PFL performed this task with less cardiac effort.

Keywords: postural control; cardiovascular system; weight-bearing; military sciences; military personnel.

Resumen: No está claro si un mejor nivel de aptitud física (PFL) es realmente una ventaja para el personal militar en funciones operativas. Propósito: investigar los efectos del PFL en el equilibrio corporal y las respuestas fisiológicas, en un ejercicio en el cual se transporta una carga durante 4 km. Se registró el equilibrio corporal (n=22) utilizando el equipo personal (22 kg), antes y después del transporte de la carga durante 4 km (cinta ergométrica). La frecuencia cardíaca (FC) se evaluó a lo largo del ejercicio, y con el resultado más reciente de la prueba de aptitud física del ejército se consideró el PFL. Se aplicaron pruebas del tipo ANOVA de diseño mixto bidireccional y t independientes ($p<0.05$). El transporte de carga aumentó significativamente el área de oscilación (72,21±30,94 a 102,68±48,57 mm2) y otras variables de equilibrio, sin efecto del PFL. Se observaron valores medios de FC más bajos para el grupo con el PFL excelente (104,55±9,37 lpm), en comparación con el grupo con el PFL buena/muy buena (115,07±10,14 lpm). Transportar 22 kg durante 4 km empeoró el equilibrio corporal y los militares con mejor PFL realizaron este ejercicio con menos esfuerzo cardíaco.

Palabras clave: control postural; sistema cardiovascular; soporte de peso; ciencias militares; militares.

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1 INTRODUCTION

Military personnel are required to perform many combat qualification and training activities. These physical and operational tasks involve complex challenges of varying degrees of difficulty, such as physical activities and strenuous tactical and technical maneuvers. For example, military troops walk long distances carrying heavy loads of supplies, ammunition, and equipment, besides facing psychological and physical obstacles, often resulting in what the literature calls combat fatigue (MALA et al., 2015).

The load carried by soldiers is usually stored in backpacks or packages that tend to require a mechanical body response and postural adjustments to maintain standing balance (KNAPIK; REYNOLDS; HARMAN, 2004). Postural balance can be defined as the processes for maintaining the body within a base of support by the congruence between the inputs of the visual, proprioceptive, and vestibular systems and the appropriate outputs of the neuromuscular system (KLEINER; SCHLITTLER; SÁNCHEZ-ARIAS, 2011; SHUMWAY-COOK; WOOLLACOTT, 2016). Usually, this physical capacity is objectively measured by a force plate (DUARTE; FREITAS, 2010) to evaluate the displacement of the center of pressure (COP). This parameter refers to the location of the vector resulting from the ground reaction forces applied on the base of support, which constitutes of the base of the feet (WINTER, 1995). Many variables are estimated from the COP displacements, such as area, velocity, amplitude, standard deviation, and frequency, which help understand the individual's body sway pattern (DUARTE; FREITAS, 2010).

Previous studies showed that supporting a load (without carrying it) significantly increases the COP sway area, mean velocity, and sway path length (SPL) (GOLRIZ et al., 2015; HELLER; CHALLIS; SHARKEY, 2009; RUGELJ; SEVŠEK, 2011; ZULTOWSKI; ARUIN, 2008). However, the behavior of these COP-based variables after load carriage tasks, which are very common in the routine of military troops, who need to carry equipment and armaments by themselves, is still unknown. Although Dahl et al. (2016) evaluated the effect of this activity on postural alignment (but not postural balance), resulting in greater forward head posture after a six-minute walk with a two-strap backpack, their studied time (only six minutes) does not represent real occupational tasks.

Moreover, load carriage can clearly become very strenuous depending on the specific performance conditions, and measuring heart rate (HR) during the activity is a way to quantify the level of effort made by the troops. The scientific literature describes this parameter as a physiological marker of fatigue and effort used to quantify the stress resulting from physical training load (FERREIRA et al., 2017; FREITAS; MIRANDA; BARA FILHO, 2009). HR recording is a simple, non-invasive, and frequently applied method, which has been widely used to prescribe training loads due to its ease of use in monitoring the intensity of physical activities (LOPES; OSIECKI; RAMA, 2012).

Besides HR, rating of perceived exertion (RPE) is another physiological marker of physical activity intensity. This scale was developed by Gunnar Borg (1982) as a tool to quantify the perception of effort in a given task and is widely used for training prescription due to its low cost and easy application (ESTON, 2012). In general, studies on the physiological demands of individuals who carry loads over long distances showed an increase in HR and other variables such as oxygen consumption and RPE (GILES et al., 2019; GRENIER et al., 2012; PIHLAINEN et al., 2014). The joint assessment of postural balance, HR, and RPE during a load carriage task shows biomechanical, neurological, and physiological responses. This integrated view would help evaluate the physical performance of soldiers in this operational task.

Brazilian operational military bases (which include long-distance load carriage tasks) require military units to have a high physical fitness level (PFL), and this level is classified as good, very good, or excellent in Brazilian Army (BRASIL, 2022). However, although military personnel are recognized as a well-conditioned group, it is not known whether higher PFL is really an advantage for operational tasks such as load carriage. Moreover, no study has assessed whether the aforementioned variables are associated with each other and could really promote an integrated view, as previously proposed. Individuals with better cardiopulmonary fitness are expected to have lower mean absolute HR during physical activities, according to a previous study (DU et al., 2005). However, to our knowledge, no study has evaluated the association between physiological demand and PFL for this specific military task: load carriage. We hypothesize that well-conditioned military personnel would perform load carriage tasks with lower body balance and physiological responses, suggesting a possible delay in the development of fatigue.

Studying the effects of load carriage on postural balance and physiological variables may show the physical repercussions of this task performed by many occupational groups, such as military personnel, both during training and in real-life scenarios. With this knowledge, military physical instructors will be able to develop specific strategies to minimize the effects and ensure the readiness of the troops even after a long-distance load carriage. Therefore, this study aimed to investigate the effects of PFL on body balance and physiological responses in a 4 km load carriage task and the relationship between balance and physiological changes caused by the task.

2 METHODS

2.1 Sample and study design

This cross-sectional observational study included a sample of 22 men from the Physical Education College of the Brazilian Army(EsEFEx), a military organization located in Rio de Janeiro, Brazil (convenience sample). Being men aged 18 to 30 years with a minimum score of “good” on the latest Army physical fitness test (APFT – *Teste de Aptidão Física*), which is applied every four months, were the inclusion criteria. These criteria represent the military personnel who usually perform load carriage tasks in the Brazilian Army: soldiers serving in operational bases, which require a minimum score of “good.” No individual had a history of musculoskeletal or neurological disease. All participants completed and signed the informed consent form. This study was approved by the local Research Ethics Committee (Protocol No. 83493618.1.00000.5235).

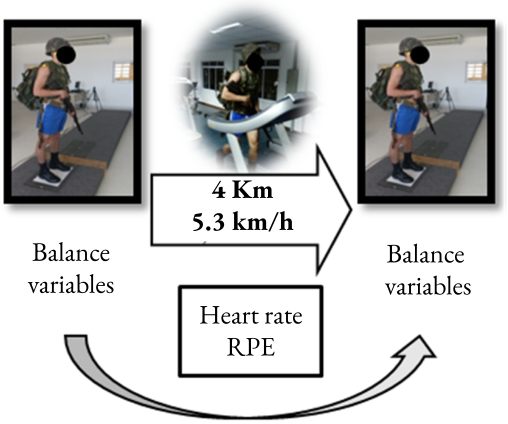
A sample size of 20 was estimated by G*Power (version 3.1.9.7, Germany) for the following parameters: power (0.80), coefficient α (0.05), and effect size (0.35). This study also used a F-tests and ANOVA (repeated measures, within-between interaction). Since no previous study had done this assessment (before and after load carriage with body balance variables), the effect size value was chosen to classify this effect as a "not high" effect size. Usually, 0.40 or 0.50 is adopted as the limit for high effects (for eta analysis), thus, a value just below it (0.35) was used in G*Power. In total, 22 soldiers were selected to prevent that possible dropouts could reduce the study analysis power.

2.2 Simulated 4 km road march

All participants walked the distance of 4 km in 45 minutes at a constant speed of 5.3 km/h on a treadmill (Technogym Excite Run 900, Italy) at the same time of the day, in a temperature and humidity controlled environment at the EsEFEx Biosciences Laboratory. This protocol was based on the characteristics of road marches established by the guidelines of the Brazilian Army (BRASIL, 2019). Participants wore their personal equipment (mean: 21.8±0.77 kg), which included a rifle, a helmet, a medium-sized backpack weighing 15 kg, suspenders, and a tactical belt with a one-liter water canteen attached on the left side, during the whole experimental protocol.

The experimental procedures were performed in the following order: (i) assessment of postural balance when carrying personal equipment; (ii) HR assessment (Polar FT1, Finland) and RPE when carrying load during a simulated 4 km road march on the treadmill; and (iii) assessment of postural balance with personal equipment after road march (Figure 1). All data for each participant were collected in about 80 minutes.

Figure 1 – Schematic representation of the experimental protocol for postural balance and physiological (heart rate and rating of perceived exertion – RPE) assessments



Δ carriage = variation (Post – Pre)

Source: prepared by the authors, 2023

All personal equipment was weighed on a Filizola® scale, model PL 2007 (Brazil), which was also used to measure the participants' total body mass. Their height was measured with a Sanny® stadiometer (Brazil), according to a standard protocol.

2.3 Assessment of postural balance (stabilometry)

A force plate (Bertec, USA) was used to evaluate the displacement of the center of pressure (COP). Three trials of postural balance assessment were performed to ensure greater data reliability (RUHE et al., 2010), and their mean was used for analysis. Each stabilometric trial lasted 80 seconds, with the initial 20 seconds discarded to avoid possible transient disturbances (CARROLL; FREEDMAN, 1993; LIN et al., 2008). The interval between each measurement was 45 seconds, during which participants remained seated, but without removing the personal equipment. Stabilometric data were collected at a sampling rate of 1 kHz and filtered using a 2nd order bidirectional Butterworth low-pass filter with a cutoff frequency of 5 Hz (DUARTE; FREITAS, 2010).

Participants were instructed to remain in a comfortable position, with their feet approximately shoulder width apart. The position of the participant's feet was marked on a sheet of paper on the force platform to avoid changes in the area of the base of support during the tests. All participants were instructed to avoid movements during the measurements and fixed their eyes on a point 3 m in front of them.

The following dependent variables were estimated from the COP displacement: sway area (mm^2), sway path length (SPL; mm), and, separately, velocity, standard deviation, and amplitude in the anterior-posterior (AP) and medial-lateral (ML) directions (PRIETO et al., 1996). These COP variables were estimated using specific routines in the MATLAB 2020 software (MathWorks, USA).

2.4 Assessment of physiological variables

During the simulated road march, HR was monitored and recorded every five minutes by a portable heart rate monitor (FT1, Polar, Finland). RPE was also recorded every five minutes, using the modified Borg scale, which ranged from 0 (no exertion) to 10 (maximal exertion) (BORG, 2000). Before the task, participants were introduced to the scale and the researchers highlighted the meaning of each number, stating, for example, that the number 5 did not refer to "moderate exertion," as many might think (since it is the midpoint between 0 and 10), but to "severe." Mean HR and mean RPE were estimated considering the nine measurements obtained during the 45-minute walk. Maximal heart rate (max HR) and RPE (max RPE), which represented the highest value measured in the last 15 minutes of load carriage, were also analyzed.

2.5 Physical fitness level (PFL)

The participants' PFL was obtained using their score on the latest Brazilian Army physical fitness test (APFT), which was recorded in individual institutional files. The APFT consists of a 12-minute run, floor push-ups, bar pull-ups, and sit-ups. Military personnel are classified into fitness scores according to their test results: I (insufficient), R (regular), G (good), VG (very good), or E (excellent) (BRASIL, 2022). The final PFL, which is recorded in the individual institutional file, is the worst score among the four tests.

To avoid selection bias and bias from other sources, some standard conducts were established: (i) eligibility data were assessed in the anamnesis form by specific questions; (ii) the researcher who helped the participant to complete this form did not evaluated him in the simulated 4 km road march; and (iii) the APFT scores were the last data included in the spreadsheet used for the analysis.

2.6 Data analysis

Initially, participants were divided into two groups according to their PFL: one group included participants with an excellent APFT score (EX; n = 11) and the other participants with a good or very good APFT score (GVG; n = 11). Data distribution was assessed using the Shapiro-Wilk normality test. In order to identify the effects of load carriage, considering PFL, two-way mixed ANOVA (for postural balance variables) and t-tests (for independent samples - cardiac behavior during the load carriage task: mean HR and max HR) were performed. For RPE, the PFL of groups was analyzed using Fisher's exact test, which compared the frequencies of RPE scores for both groups (EX vs. GVG).

Finally, Pearson's correlation tests between stabilometric and HR variables were used to assess the relationship between changes in postural balance caused by load carriage (Δ carriage, for variables with significant changes from the previous analysis) and the cardiac demand of the task (mean HR and max HR).

Data were presented as mean ± standard deviation and frequency. All analyses were performed using IBM SPSS Statistics version 27. Statistically significant differences were considered for p-values < 0.05. The correlation coefficient (r) was considered very strong when $r \geq 0.9$, strong when r ranged from 0.6 (inclusive) to 0.9, regular when r ranged from 0.3 (inclusive) to 0.6, and weak when r ranged from 0 to 0.3 (CALLEGARI-JACQUES, 2003).

3 RESULTS

We evaluated 22 soldiers (27.09 ± 2.07 years of age, 75.70 ± 9.14 kg body weight, and 1.77 ± 0.07 m tall). Regarding the latest APFT score, 31.80% of participants had good (G) PFL, 18.20% had very good (VG) PFL, and 50.00% had excellent (EX) PFL. The anthropometric characteristics of the EX group (n = 11) were 27.09 ± 1.97 years of age, 70.26 ± 7.73 kg body weight, and 1.75 ± 0.07 m tall. Participants in the GVG group (n = 11) were 27.09 ± 2.26 years old, 1.79 ± 0.07 m tall, and weighed 81.13 ± 7.13 kg.

Two-way mixed ANOVA showed changes due to the main effect of load carriage, with significantly increases in sway area (F = 13.174; p = 0.002), medial-lateral (ML) standard deviation (F = 16.836; p = 0.001), and ML amplitude (F = 26.648; p < 0.001) (Table 1). We observed no difference for the main effect of PFL or interaction.

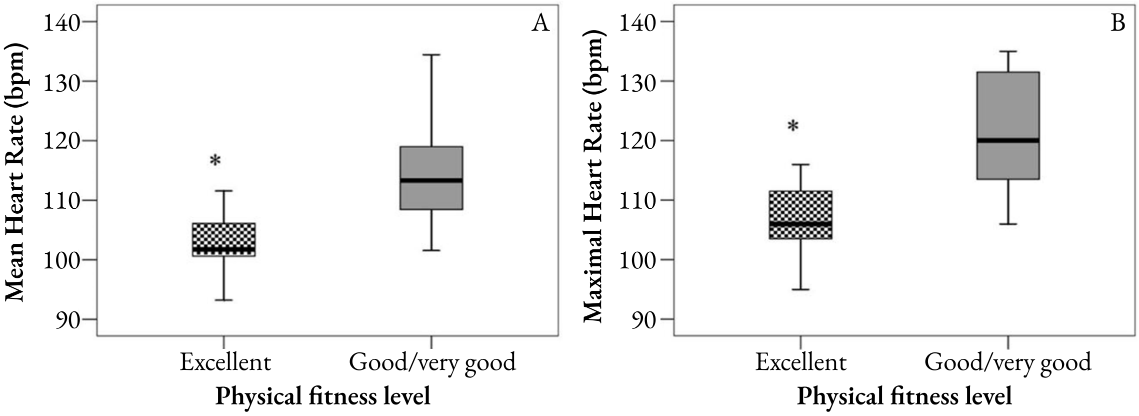
Table 1 – Body balance variables before and after the 4 km load carriage for the total sample (n = 22) and for the groups with excellent (n = 11) and good or very good (n = 11) physical fitness level

Variables	Before load carriage	After load carriage
Sway area (mm2)		
EX	69.03 ± 38.59	94.04 ± 57.18
GVG	75.39 ± 22.33	111.32 ± 38.98
Total	72.21 ± 30.94	102.68 ± 48.57*
Mean ML velocity (mm/s)		
EX	2.61 ± 0.75	2.53 ± 0.73
GVG	2.56 ± 0.50	2.71 ± 0.56
Total	2.59 ± 0.62	2.62 ± 0.64
Mean AP velocity (mm/s)		
EX	4.51 ± 0.76	4.37 ± 0.93
GVG	4.68 ± 1.15	4.86 ± 0.97
Total	4.59 ± 0.95	4.61 ± 0.96
ML standard deviation (mm)		
EX	1.88 ± 0.71	2.46 ± 0.97
GVG	1.80 ± 0.41	2.56 ± 0.55
Total	1.84 ± 0.57	2.51 ± 0.77*
AP standard deviation (mm)		
EX	3.28 ± 1.01	3.64 ± 1.55
GVG	3.72 ± 0.83	3.79 ± 1.01
Total	3.50 ± 0.93	3.71 ± 1.28
ML amplitude (mm)		
EX	9.88 ± 3.44	12.15 ± 4.30
GVG	9.37 ± 1.95	13.47 ± 2.40
Total	9.62 ± 2.74	12.81 ± 3.46*
AP amplitude (mm)		
EX	17.24 ± 5.07	17.67 ± 5.66
GVG	19.10 ± 3.81	19.91 ± 5.18
Total	18.17 ± 4.48	18.79 ± 5.41
SPL (mm)		
EX	340.11 ± 60.55	328.33 ± 71.38
GVG	347.05 ± 65.98	362.36 ± 62.94
Total	343.58 ± 61.90	345.34 ± 67.94

Data are presented as mean ± standard deviation. AP: anterior-posterior direction.
EX: group with excellent PFL (n = 11). GVG: group with good or very good PFL (n = 11).
ML: medial-lateral direction. SPL: sway path length. * p< 0.05
Source: prepared by the authors

Regarding cardiac behavior, according to PFL, the difference was statistically significant between the groups. Participants with excellent PFL showed less cardiac effort than participants with good or very good PFL, with lower mean and maximal HR values (Figure 2).

Figure 2 – Boxplots (minimum, 1st quartile, median, 3rd quartile, and maximal values) for mean heart rate (A) and maximal heart rate (B). *Statistical difference between the groups (t-test for independent samples)



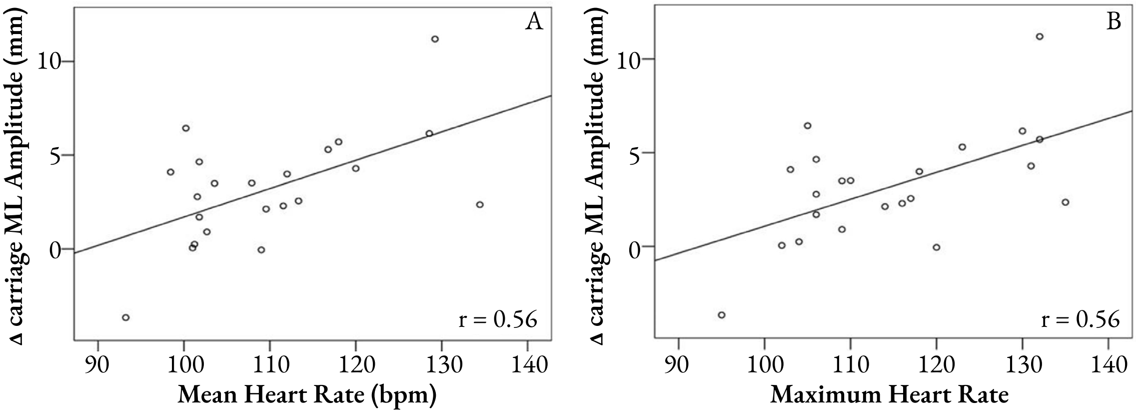
Maximal Heart Rate = Maximum heart rate

Source: prepared by the authors

Despite the difference found for heart rate, RPE showed no difference between the EX and GVG groups for both mean and max RPE. The analysis of mean RPE values for the EX group showed that seven participants reported score 2 and four reported score 3. In the GVG group, four participants reported 2 and seven reported 3. Fisher's exact test showed no statistical difference for mean RPE ($p = 0.40$). Regarding max RPE, six participants in the EX group reported 2, four reported 3, and one reported 4. In the GVG group, two participants reported 2, eight reported 3, and one reported 4. Similarly to mean RPE, max RPE showed no statistical difference in Fisher's exact test ($p = 0.19$).

Finally, cardiac variables showed a significant, positive, and regular correlation with Δ carriage ML standard deviation (mean HR: $r = 0.50$, $p = 0.019$; max HR: $r = 0.46$, $p = 0.033$) and Δ carriage ML amplitude (mean HR: $r = 0.56$, $p = 0.007$; max HR: $r = 0.56$, $p = 0.007$) (Figure 3). Another significant correlation was found between mean HR and Δ carriage sway Area ($r = 0.45$, $p = 0.038$). The correlation between max HR and Δ carriage sway Area showed no significant results ($r = 0.38$, $p = 0.083$).

Figure 3 – Scatter plots for Δ carriage ML amplitude vs. mean heart rate (A) and maximum heart rate (B) r: Pearson correlation coefficient



Source: prepared by the authors

4 DISCUSSION

This study aimed to identify the effects of 4 km load carriage (with about 22 kg) on postural balance and cardiac responses, comparing individuals with different physical fitness levels. The results showed that: (i) carrying the personal equipment for 4 km changed sway area, ML amplitude, and ML standard deviation; (ii) PFL was associated with HR during treadmill walking, but not with changes in postural control; and (iii) postural and physiological changes resulting from load carriage have a positive correlation.

4.1 Postural balance and load carriage

The changes observed in postural balance after load carriage may be due to a possible fatigue caused by the task. In prolonged military exercises, carrying personal equipment seems to lead to significant lower limb muscle fatigue, which affects body balance, increasing the challenge and changing proprioception (ARLIANI et al., 2013; MARCHETTI; ORSELLI; DUARTE, 2013). Maintaining postural balance after minor disturbances is difficult due to changes in position sense (ALLEN; PROSKE, 2006) and ankle joint stability (YAGGIE; MCGREGOR, 2002), and the impaired synergy of hip and knee muscles to react to these disturbances (GRIBBLE; HERTEL, 2004). Moreover, fatigue affects neuromuscular function itself, worsening the ability to contract muscles quickly and appropriately for postural adjustments.

Many studies point to fatigue as a factor that impairs postural balance (ALLEN; LEUNG; PROSKE, 2010; NARDONE et al., 1997; RAHNAMA et al., 2003). For example, Baroni et al. (2011) confirmed the direct dependency relationship between fatigue and postural balance (in which increased fatigue leads to greater imbalance), but their study used a cycle ergometer fatigue protocol, unlike our study. Other authors found similar results using isokinetic dynamometry of isolated muscles (GRIBBLE; HERTEL, 2004; YAGGIE; MCGREGOR, 2002). Our study shows that postural balance is modulated by a previous load carriage task and the positive correlations observed between postural and physiological changes enhance this discussion, highlighting that the higher the cardiac effort during the task, the worse the body balance. Since this is an observational cross-sectional study, this correlation does not guarantee a cause and effect relationship, but considering all these results, the integrated view (biomechanical, neurological, and physiological behavior) mentioned in the introduction section seems to make sense.

Our results showed significant changes in ML postural adjustments after load carriage, which could be due to a possible load asymmetry in the limbs (ZULTOWSKI; ARUIN, 2008). During the 4 km walk, participants were allowed to carry the rifle in the position they considered most comfortable, but they alternated the weapon between the two hands, which may have caused the differences observed mainly in the ML axis. The human body has postural strategies to maintain balance in relation to the axes of movement. Studies observe more ankle and hip strategies for the AP axis (HORAK; NASHNER, 1986) and the load-unload strategy for the ML axis (WINTER, 1995), which shows a certain postural asymmetry. Thus, unsurprisingly, the ML axis had the greatest effect on stabilometric parameters after physical demand in this study.

To our knowledge, this study was the first to assess postural balance after a long-distance walk with load carriage. The only study that addressed static postures after load carriage assessed postural alignment after a six-minute walk. Dahl et al. (2016) found an increase in neck hyperextension after the walk, but did not analyze postural balance.

4.2 Physical fitness level relationship with heart rate and stabilometric changes

Our results showed that the physical fitness level was associated with cardiac behavior in the load carriage task. Participants with better APFT scores performed the 4 km walking with lesser cardiac effort, reflected in lower mean and maximal HR values. In addition, the increase in post-walking stabilometric parameters was not the same for all individuals in the sample. Those who presented more changes in postural balance after the 4 km walk are those who also showed greater cardiovascular effort. Participants who showed better physical fitness were probably more adapted to the task, which may have contributed to less HR adjustments during low physical effort.

Although the association between cardiac behavior in a long-distance load carriage task and fitness levels has never been investigated, the results corroborated the traditional findings of exercise physiology studies. Individuals with better fitness levels would present better efficiency/

economy of movement, demanding less of their cardiovascular system in a given physical task (LITLESKARE et al., 2020). Although physical fitness level was found to have influence on the HR behavior, no impact on body balance was observed. The high level of experience of the participants and the low difficulty level of the balance assessment (60 s keeping upright position with personal equipment) may explain this result.

4.3 Limitations and highlights

No tests were made to specifically assess muscle peripheral fatigue associated with the 4 km walking, which is one of the limitations of the present study. Another important limitation was the use of a treadmill to simulate a road march in a controlled environment, when actual load carriage tasks are usually carried out by the troops on uneven terrain, with steep slopes, and under the most varied weather conditions. However, as this was the first study examining the effects of long-distance load carriage on postural balance, we opted to work in a controlled environment to allow a comprehensive and individualized follow-up.

Using the RPE scale with military service members is not an easy task. Since these individuals are usually physically fit (due to their military training), they commonly underestimate the perceived effort in most tasks. Thus, being familiar with the meaning of each of the scale's values is essential, which was employed in the present study. Finally, we note that among the studies issuing load carriage and support, a sample of 22 individuals is larger than many of them, further increasing the relevance of our findings.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

Carrying 22 kg for 4 km worsened the body balance with modifications in the sway area, medial-lateral standard deviation and amplitude. Participants with better physical fitness level performed this task with less cardiac effort but no influence was observed on body sway. Finally, participants performing the 4 km load carriage with less cardiac effort also presented less postural balance variations, in function of load carriage.

Further research on this topic could evaluate heart rate behavior after the performance of the load carriage task in order to identify for how long military personnel are able to maintain conditions close to the pre-effort, which would improve the ability to accomplish assigned missions. Another suggestion is assessing the effects on postural balance of symmetrical load carriage and verifying whether these effects disappear, as usually occurs in load support tasks. Furthermore, verifying the modifications after load carriage under field conditions and for longer distances would also be valuable.

AUTHORSHIP AND CONTRIBUTIONS

Miriam Raquel Meira Mainenti, Ricardo Alexandre Falcão, Luis Aureliano Imbiriba – study conception and design; data collection, analysis, and interpretation; critical revision of the article for important intellectual content; final approval of the version submitted to *Coleção Meira Mattos*.

Jonathan Vieira da Silva, Victor Vinícius Ribeiro Lima – data collecttion, analysis, and interpretation; writing of the article; final approval of the version submitted to *Coleção Meira Mattos*.

Fabio Alves Machado – data interpretation; critical revision of the article for important intellectual content; final approval of the version submitted to the *Coleção Meira Mattos*.

Adriane Mara de Souza Muniz – data analysis and interpretation; critical revision of the article for important intellectual content; final approval of the version submitted to *Coleção Meira Mattos*.

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