ARTICLE

Indirect markers of muscle damage in military physical training session with neuromuscular and aerobic exercises in circuit (Cross operational): acute effects and recoverability

Marcadores indiretos de dano muscular em sessão de treinamento físico militar com exercícios neuromusculares e aeróbios em circuito (Cross operacional): efeitos agudos e recuperação

ABSTRACT

The Brazilian army recently developed the military physical training "Cross Operational" (CO), composed of four different difficulty levels. The moderate/high intensity character of CO can damage the muscle fibers, characterized by alterations at the cellular level, which can be evaluated indirectly by intramuscular proteins showing up in the bloodstream. In this context, the analysis of muscle damage biomarkers can be used as part of an initial injury prevention tool and in the evaluation of training progression and post-exercise recovery. This study aimed to observe the acute effect of CO on indirect markers of muscle damage in military personnel of the Brazilian army. Twenty-four male recruits aged between 18 and 26 years participated in the study. The four sessions corresponding to the CO levels were performed in cross-design, with a seven-day washout period, and blood samples were collected at rest, immediately after, 24 and 48 hours after each training session. The measured markers were creatine kinase (CK), myoglobin (Mb) and lactate dehydrogenase (LDH). In all CO levels, CK had a significant increase 24 hours after CO, whereas Mb and LDH increased immediately after CO. Regarding recovery time, LDH returned to baseline values within 48 hours in all levels, whereas CK and Mb, after 48 hours of recovery, reduced but did not return to baseline in level 4. The serum elevations of the biomarkers studied, like those found in studies with other consolidated physical training methods, suggest that CO can contribute to the improvement of the physical training of military personnel.

Keywords: Biomarkers. Muscle damage. Cross promenade. Functional fitness. Tactical athlete.

RESUMO

O Exército Brasileiro (EB) desenvolveu recentemente o treinamento físico militar "Cross Operacional" (CO), composto por quatro níveis de dificuldade. O caráter de intensidade moderada/alta do CO pode causar danos às fibras musculares, caracterizado por alterações a nível celular, podendo ser avaliado indiretamente pelos níveis séricos de proteínas de origem musculoesquelética. Assim, a análise de biomarcadores de dano muscular é utilizada como parte de uma ferramenta inicial de prevenção de lesões e de avaliação da progressão do treinamento e recuperação. Este estudo teve como objetivo observar o efeito agudo do CO sobre marcadores indiretos de dano muscular em militares do EB. Participaram do estudo 24 soldados do sexo masculino com idades entre 18 e 26 anos. As quatro sessões correspondentes aos níveis de CO foram realizadas em *crossover*, com *washout* de sete dias. Amostras de sangue foram coletadas em repouso, imediatamente após, 24 e 48 horas após cada sessão de treinamento. Os biomarcadores foram creatinoquinase (CK), mioglobina (Mb) e lactato desidrogenase (LDH). Em todos os níveis do CO, a CK aumentou significativamente 24 horas após o CO, enquanto Mb e LDH aumentaram imediatamente após o CO. Em relação ao tempo de recuperação, a LDH retornou aos valores basais 48 horas após todos os níveis do CO, enquanto CK e Mb reduziu, mas não retornou aos valores basais no nível 4. As elevações séricas dos biomarcadores estudados, assim como em estudos com outros métodos de treinamento consolidados, sugerem que o CO pode contribuir para a melhoria do treinamento físico de militares.

Palavras-chave: Biomarcadores. Dano muscular. Cross Promenade. Aptidão funcional. Atleta tático.

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1 Introduction

Military personnel need a good level of physical conditioning to perform their functional tasks. Military Physical Training (MPT) aims to improve the physical conditioning of military personnel, aiming to increase professional efficiency, in addition to being a health-promoting instrument (BRASIL, 2015). Knowledge of the impact of MPT on the body is relevant for planning and preventing injuries, therefore studies on exercise physiology in military personnel are also relevant.

Armies around the world have been developing new functional physical training methods, related to skills that allow them to simulate gestures that will be performed in combat. In this sense, the Brazilian Army developed the Cross Operational (CO), a physical training method characterized by combined aerobic and neuromuscular activities (Brasil, 2017). CO was designed based on concepts from current moderate/high intensity training methods, such as CrossFit™, and classic methods, such as Cross Promenade. This model of physical training aims to be attractive and operational, expanding the range of exercises that can be performed in the military's physical preparation, in addition to encouraging practice and satisfaction in the available exercise programs.

Physical training must be planned to have an adequate intensity, so as not to exacerbate the inflammatory response and increase the risk of muscle damage. Assessing the physical and biochemical stress on muscle fibers from a military physical training with moderate/high intensity characteristics, and detecting if the recruit is recovered before starting a new training session is of paramount importance to avoid injuries and overtraining situations. Currently, scientific research on high intensity interval training (HIIT) focuses mostly on aerobic modalities (De Oliveira Ottone and colab., 2019; Kaspar and colab., 2016), whereas studies evaluating muscle damage after HIIT sessions involving resistance or functional exercises are scarce. In this sense, this study aimed to investigate the behavior of indirect markers of muscle damage after different levels of difficulty of CO sessions. The main hypothesis is that there will be significant differences between the different levels of this military physical training method.

2 Theoretical Reference

Recent studies support the use of training models based on low-volume, high-intensity functional exercises in athletes and military personnel, showing important improvements in physical fitness and health (Posnakidis and colab., 2020; Tornero-Aguilera and Clemente-Suárez, 2019). In this context, CO aims to improve cardiopulmonary and neuromuscular qualities, based on physical

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valences indispensable to the execution of military tasks such as endurance and aerobic power, explosive and static/dynamic strength, localized muscle resistance, and static/dynamic balance (Brasil, 2020).

In general, physical exercise, especially when performed at high intensity, damages the muscle fibers, a process which is characterized by alterations on a cellular level (Clarkson and colab., 2006). Serum dosage of proteins that are physiologically intramuscular is an indirect way to assess muscle damage, since these proteins spill over into the bloodstream after damage. Therefore, these proteins are considered indirect markers of muscle damage after exercise (Baird and colab., 2012). The analysis of multiple biomarkers, such as creatine kinase (CK), myoglobin (Mb) and lactate dehydrogenase (LDH), is a relevant tool in the acute assessment of muscle impairment due to physical training and may also provide support for assessing the necessary recovery time (Banfi and colab., 2012).

CK and Mb change in serum in response to different exercise protocols and intensity levels. In addition, these concentrations vary according to age, gender, race, muscle mass, fitness level and climatic condition (Szabó and colab., 2003). The kinetics of CK appearance in serum can range from 24 to 72 hours (Bessa and colab., 2008). On the other hand, Mb is considered a more sensitive biomarker, increasing faster its serum levels (about three to four times immediately after exercise), reaching peak concentration in around three hours and returning to basal concentrations after about 24 hours (Lippi and colab., 2008; SILVA and colab., 2018).

The rapid renal clearance of Mb makes this biomarker a good fit for the analysis of the response in the first 24 hours after a exercise stimulus (Cervellin and colab., 2017), which is relevant, since, in many exercise programs, another exercise session would be performed after this time, leading to accumulated muscle damage and, in more severe cases, even to renal failure (Spada and colab., 2018). LDH appears in the bloodstream earlier than CK and persists for a longer duration (ARAÚJO and collab., 2016). Although LDH is not specific to skeletal muscle, its presence in many tissues means that when analyzed alongside other musculoskeletal biomarkers, it can more accurately indicate muscle damage (Houston, 2008).

3 Methods

3.1 Participants

The sample included 24 male recruits, aged between 18 and 26 years (Table 1).The following inclusion criteria were considered: 1) negative PAR-Q; 2) recruits categorized as “excellent” in the push-up test and the 95% best (“superior”) in the 12-min race test, according to criteria published by

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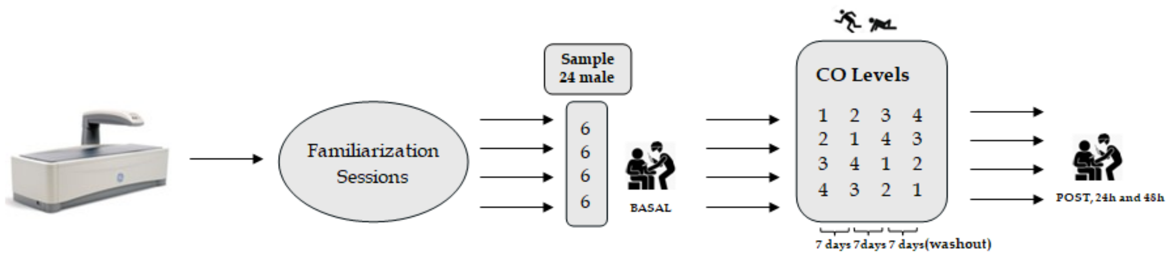
the American College of Sports Medicine (American College Of Sports Medicine, 2017). Similarly, the following exclusion criteria were considered: 1) not completing one of the CO series; 2) history of osteomyoarticular diseases or pain; 3) use of anti-inflammatory drugs or pharmacological ergogenic resources within seven days before and during the study.

The volunteers were informed about objectives and procedures and signed the informed consent form, according to CNS/MS Resolution No. 466, of 12/12/2012. This study was approved by the Research Ethics Committee of the Army Physical Training Center (CAAE: 14943119.9.0000.9433) and did adhere to the ethical policies of IJES (Navalta and colab., 2019).

3.2 Protocol

The volunteers performed an evaluation of body composition using the x-ray double emission equipment (model Lunar iDXA, GE, USA), for the purpose of characterizing the sample. They performed three sessions of CO familiarization, under guidance of physical education professionals. The recruits were then randomly divided into four subgroups to perform the four CO levels, according to a cross-design, in which each subgroup performed the series in a different order (crossover), with a seven-day washout between sessions (Figure 1).

Figure 1: experimental design



Fonte: the author

As recommended in the “Caderno de InstruçãoTreinamento Rústico Operacional – Cross Operacional” (Operational Rustic Training Instruction Notebook – Cross Operational) (BRASIL, 2020), the CO consisted of 12 isotonic and isometric exercises, performed after every 200 meters of running, on an athletics track. In each CO level, the exercises vary depending on the way of execution, execution time or number of repetitions. The recruits performed the 200 m race in levels 2, 3 and 4, as fast as 70%, 80% and 90% of the average speed for the 12-min race test. At level 1, the pace of the race was controlled through the subjective perception of exertion, seeking to reach the mild to moderate intensity. Each CO session, considering the warm-up, lasted approximately 35 minutes.

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Venous blood samples were collected in the morning. Samples of 8.0 mL venous blood were collected from a vein of the antecubital region in a tube with separator gel, without anticoagulant (Vacuplast, China). Then, the samples were centrifuged for 12 min, at 2800 rpm to obtain the serum. The first blood collection (basal) occurred on the day before the execution of each CO serie and the second immediately after each session. The third and fourth blood collections occurred 24 and 48 hours after each session. The individuals did not practice physical exercises in the 48 hours prior to the training sessions and between the blood collections. The samples were analyzed immediately after the pre-analytical phase. In the biochemical dosages of CK and LDH, an automatic BT3000 analyzer (Wiener Lab, Argentina) was used, at 37ºC, with specific reagent kits. Mb was dosed using the immunoenzymatic method with final fluorescence detection, Mini Vidas autoanalyzer (Biomerieux, France), according to manufacturer's recommendations. Controls and calibrators used were provided by the kit manufacturers.

3.3 Statistical Analysis

The Shapiro-Wilk test was used to analyze the normality of anthropometric data and biomarkers. Regarding inferential statistics, the biomarkers presented a non-normal distribution. Thus, statistical analysis was performed using Friedman's nonparametric test, with Bonferroni's post-hoc. Outliers were excluded from statistical analyses. The significance level $p \leq 0.05$ was considered and the statistical software SPSS, version 20.0, was used. The sample size is compatible with a 95% statistical test power, paired data within factors, effect size f of 0.25 (Beck, 2013).

4 Results

Table 1 shows the confirmed normality of the data used to characterize the sample.

Table 1: anthropometric, aerobic performance and body composition characteristics.

Variable	Mean ± SD
Body mass (kg)	69.6 ± 9.4
Height (m)	1.76 ± 0.07
BMI (kg/m²)	22.82 ± 2.34
Body fat percentage	15.40 ± 4.80
Lean body mass (g)	55.18 ± 6.95
Average Speed Cooper's Test (m/s)	4.21 ± 0.80

Results expressed as mean ± standard deviation. BMI = body mass index

Table 2 shows the results of CK, in which it is observed that, in all CO levels, there was an increase ($p \leq 0.05$) in the 24h collection, in relation to the basal collection. In levels 2 and 4 there was

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an increase (p=0.001 and p=0.031, respectively) in the collection immediately after, but the peak concentration occurred in the collection 24h (p=0.000 and p=0.001, respectively). There was a significant reduction in the 48h collection only at levels 3 and 4 (p=0.012 and p=0.001, respectively).

Table 1: serum creatine kinase levels at baseline, immediately after exercise, 24 and 48 hours later after the four series of Cross Operational, expressed in U/L.

SERIES		CK BASAL	CK POST	CK 24h	CK 48h
1		394.5	437.0	405.0 *	379.0
	Q1	252.0	283.5	299.0	224.0
	Q3	506.0	513.0	661.5	520.0
2		269.5	369.5***	533.0*	451.5 ** a
	Q1	191.0	257.0	348.5	291.0
	Q3	368.5	567.5	786.0	592.5
3		297.5	400.0	539.5*	341.0 #
	Q1	164.5	289.0	330.0	259.0
	Q3	493.5	1701.0	842.5	689.0
4		258.0	348.0***	708.5*	518.0 # a
	Q1	174.0	297.5	475.0	325.5
	Q3	381.5	524.0	1203.0	699.5

Data expressed as median and first (Q1) and third (Q3) quartiles. CK Basal = analysis of the basal sample (before the CO session); CK Post = analysis of the sample collected immediately after the CO session; CK 24 h = analysis of the sample collected 24 hours after CO; CK 48 h = analysis of the sample collected 48 hours after CO. * represents an increase with significant difference between the basal and 24 h moments; ** represents an increase with significant difference between the basal and 48 h moments; *** represents an increase with significant difference between the basal and post moments; # represents a reduction with a significant difference between the 48 h and 24 h moments; a represents a significant difference in relation to baseline.

Table 3 shows that, at all CO levels, serum Mb concentrations were significantly higher ($p<0.05$) in the collection immediately after compared to the baseline collection. At CO level 4, Mb concentrations remained high (p=0.000) 24 hours after exercise. At levels 2, 3 and 4, a reduction (p=0.001, p=0.002 and p=0.015, respectively) of Mb is observed in the 24h collection. Such a

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reduction has already provided a return to baseline values at all CO levels, except for level 4, in which the return occurred in the 48h collection.

Table 2: serum myoglobin levels at baseline, immediately after exercise, 24 and 48 hours later after the four series of Cross Operational, expressed in µg/L.

SERIES		MB BASAL	MB POST	MB 24h	MB 48h
1		17.5	33.5*	19.0#	18.5##
	Q1	14.5	25.5	16.0	13.0
	Q3	24.0	42.0	24.0	23.0
2		19.5	51.0*	21.0#	20.5##
	Q1	14.0	33.0	15.0	15.0
	Q3	23.0	69.0	36.0	24.0
3		17.0	39.0*	22.0#	16.5##
	Q1	13.5	30.5	17.0	14.0
	Q3	25.5	73.5	36.0	24.0
4		15.5	51.0*	26.0** #	19.5##
	Q1	11.0	44.0	21.0	13.5
	Q3	19.5	66.0	35.0	22.5

Data expressed as median and first (Q1) and third (Q3) quartiles. Mb Basal = analysis of the basal sample (before the CO session); Mb Post = analysis of the sample taken immediately after the CO session; Mb 24 h = analysis of the sample collected 24 hours after CO; Mb 48 h = analysis of the sample collected 48 hours after CO. * represents an increase with significant difference between the basal and post moments; ** represents an increase with significant difference between the basal and 24 h moments; # represents a reduction with significant difference between the 24 h and post moments; ## represents a reduction with a significant difference between the 48 h and post moments;

Table 4 shows the serum levels of the LDH marker, which, at all CO levels, were significantly higher ($p<0.05$) in the collection immediately after, compared to the baseline collection. At level 1, there was a reduction ($p=0.043$) in the 24-hour collection and, at level 3, although there was no significant reduction, there was also no difference in relation to baseline values. At levels 2 and 4, the reduction ($p=0.001$ and $p=0.000$, respectively) occurred 48 hours after CO.

Table 4: serum LDH levels at baseline, immediately after exercise, 24 and 48 hours later after the four series of Cross Operational, expressed in U/L.

SERIES		LDH BASAL	LDH POST	LDH 24h	LDH 48h
1		304.0	361.5*	325.5##	293.0#
	Q1	267.0	303.0	278.0	269.0
	Q3	351.0	398.0	381.0	353.0
2		302.0	349.5*	316.5**	320.5#
	Q1	260.0	319.0	288.0	272.0
	Q3	338.0	397.5	393.0	359.5
3		304.5	358.5*	341.0	332.0#
	Q1	265.5	311.0	307.5	294.0
	Q3	355.0	405.0	352.5	352.0
4		296.0	370.5*	316.5**	320.5#

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	Q1	263.0	331.0	288.0	272.0
	Q3	338.0	406.5	393.0	359.5

Data expressed as median and first (Q1) and third (Q3) quartiles. LDH Basal = analysis of the basal sample (before the CO session); LDH Post = analysis of the sample taken immediately after the CO session; LDH 24 h = analysis of the sample collected 24 hours after CO; LDH 48 h = analysis of the sample collected 48 hours after CO. * represents an increase with significant difference between the basal and post moments; ** represents an increase with significant difference between the basal and 24 h moments; # represents a reduction with significant difference between the 48 h and post moments; ## represents a reduction with a significant difference between the 24 h and post moments.

5 Discussion

This study aimed to investigate the behavior of indirect markers of muscle damage after different series of CO. The results showed that a single session of each CO series, with different difficulty levels and duration of approximately 35 minutes, was able to increase the serum concentration of the evaluated biomarkers, as expected. Myoglobin and LDH showed peak concentration immediately after each session, whereas the increase in serum CK levels occurred 24 hours after CO. Regarding recovery, 48 hours were sufficient to recover LDH and Mb levels in all CO levels, but not the CK levels, whose concentrations returned to baseline only at the lowest difficulty level (level 1).

Therefore, the analysis of these biomarkers is a relevant tool for assessing training progression and estimating the recovery time required for the subsequent session, in addition to being useful in prescribing exercises before and after CO, in order to understand the responses physiological. The CK peak value measured 24 hours after the execution of CO levels 2, 3 and 4 (533.0; 539.5 and 708.5 U/L) are comparable to the CK activities observed by Tibana and colab (2019), who performed serum measurements 24, 48 and 72 hours after a CrossFit™ competition and showed CK elevations with peak concentration (698.7 U/L) in the 24 hour collection and a reduction in the 72 hour collection. Howatson and Milak (2009) investigated another training method, HIIT with repeated sprints, and identified increases in muscle damage and pain, with the CK peak also 24 hours after exercise (776.0 U/L) and return to baseline levels after 72 hours . In the present study, after 48 hours of rest, CK serum levels were also still above baseline values in CO levels 2, 3 and 4. Knowing that this marker presents high inter-individual variability due to the existence of high and low responders (Machado and Willardson, 2010), the prolonged CK response may be an indicator for prescribing subsequent exercise sessions, in order to avoid extreme muscle damage.

These results indicate that CK has possibly changed as a result of the CO levels with higher metabolic and/or muscle demand. The study by Wiewelhove and colab (2016) showed higher CK

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plasma levels after sprint protocols when compared with lower intensity protocols, attributing muscle damage to the highest amount of eccentric contractions performed. Each CO level presents a certain increasing number of sprints in the last exercise, which may have influenced the CK leakage into circulation. CK levels may also have been influenced by the difference in the number of repetitions of plyometric exercises, three of the 12 CO exercises, which reflects in the greater susceptibility of damage to type II, fast twitch, muscle fibers (Macaluso and colab., 2012). Therefore, it is reasonable to assume that muscle damage after CO was comparable to other similar training methods.

Regarding Mb serum levels, the study showed an increase immediately after the end of all CO levels, reducing after 24 hours to concentrations similar to baseline (Table 3). Even being a more acute elevation biomarker than CK, at level 4 high values were observed even in the 24h collection, indicating a more pronounced damage as a result of a circuit of greater metabolic demand. Similar results were found in the study by Spada and colab (2018), in which an increase in Mb was observed two hours after the performance of high-intensity resistance interval training and a reduction after 24 hours of rest. Bartolomei and colab (2016) also observed that, after 30 minutes of protocols with resistance exercises, Mb levels increased significantly. Additionally, Cipryan (2017) performed a 30-min running HIIT intervention and also observed an increase in Mb after cessation of training, but to a lesser extent in well-trained individuals when compared to moderately or untrained individuals.

As for LDH, serum levels increased in all CO levels in the test immediately after the exercise, reaching peak concentration, and lowered down at the 48 hours test. This increase agrees with the results of the study by Bartolomei and colab (2016), in which similar increases were observed after a high intensity resistance training. The results of the present study also meet Cipryan's study (CIPRYAN, 2017), which evaluated the influence of three different race HIIT protocols on muscle damage markers, showing that LDH activity was higher immediately after the protocols. In the study by Timón and colab (2019), which evaluated individuals after two different CrossFit™ sessions, the peak concentration of LDH occurred within 24 hours and returned to baseline values within 48 hours, partially corroborating the recovery findings of this study.

Based on the observed results, it is concluded that the peak concentrations, as well as the recovery kinetics, present different behaviors among the markers. The high level of physical conditioning of the military personnel, who regularly practice physical exercises, sometimes intense, seems to have had a protective effect against muscle damage at levels 1, 2 and 3, the same behavior was not observed at level 4 of the CO, in that all biomarkers remained elevated. In healthy individuals, this increase in indirect markers of muscle damage is explained as a physiological response to exercise and the inflammatory cascade triggered after the damage, in tissue repair and remodeling, thus, level

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4 of CO seems to more efficiently provoke the development of the physical conditioning of individuals with the characteristics of this sample, for causing more metabolic stress.

This study was limited to using only male volunteers, physically well conditioned and in a narrow and young age group. This sample selected for convenience limits the interpretation of results to the mentioned conditions. Another relevant limitation was the impossibility of carrying out more collection moments, for a longer period. According to the latest guideline (Cadegiani and Kater, 2019), of the potential biomarkers for the assessment of overtraining syndrome, the elevation of CK stands out, therefore, since in the present study CK remained above baseline values in the 48-hour collection of levels 2, 3 and 4, it would be relevant to assess the serum levels of this marker at later times.

6 Final considerations

It is worth noting that this is a pioneering study evaluating broad and fine adjustments to CO, a training method recently developed by the Brazilian army. This evidence of acute changes in indirect markers of cell damage, like those observed by other methods already consolidated, such as CrossFit™ and HIIT, suggest that CO can help to improve physical training. A training program using CO must progress according to the individual load prescription in order to avoid extreme muscle damage. Therefore, the analysis of these biomarkers is a relevant tool to evaluate training progression and estimate the recovery time required for the subsequent session. Longitudinal studies are needed to identify chronic adaptations, with research subjects with different physical characteristics, to consolidate CO implementation in the different military organizations in Brazil. Besides, this dynamic method, with exercises not exclusively related to military tasks, can be used in physical training programs in the civil sphere, contributing as an attractive method of health promotion.

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