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Muscle Damage Biomarkers for Exertional Rhabdomyolysis Inmilitary Personnel during Intense Training in the Amazon Jungle

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Abstract: Background: Diagnosing rhabdomyolysis has usually defined at least five or ten times the upper limit creatine kinase level; but it is conservative for Exertional rhabdomyolysis. The aim was to analysis the biomarkers during a military operation in an extreme environment with high physical demand. **Material and Methods:** Seventeen male military personnel (age: 27.83 ± 2.96 years; weight: 76.31 ± 7.67 kg; and height 174.92 ± 5.91 cm) participated for this investigation. The creatine kinase (CK); blood urea nitrogen (BUN); creatinine (Cr); sodium (Na); potassium (K); aspartate amino transferase (AST) and alanine amino transferase (ALT) were analyzed. Urine specific gravity (USG) the hydration status was monitored. The Generalized Linear Models for repeated measures and the Friedman's two-way test were used to comparative moments. **Results:** The BUN; Cr; Na; and K mean values showed a nonsignificant. The statistical significance of the test was found in CK and AST. There was considerable variability of CK between subjects during the operation. The moderate correlation between CK and Cr was found during study. **Conclusion:** Elevated CK levels after the operation were characterized as physiological pattern. These results reinforce that CK values are not predictors of renal function in activities with intense and continuous physical demand.

Keywords: exertional rhabdomyolysis; biomarker; acute kidney injury; military training

1. Introduction

Rhabdomyolysis is a syndrome caused by the rupture of muscle cells, resulting in extravasation of intracellular content into the circulatory system, such as electrolytes (potassium, calcium, and phosphate), enzymes (creatine kinase, lactate dehydrogenase, aspartate transaminase, and aldolase) and proteins (myoglobin)[1].This syndrome occurs with a broad spectrum of signs and symptoms, ranging from a thoroughly asymptomatic increase in Creatine Kinase (CK) to severe changes in electrolytes, disseminated intravascular coagulation, and increases in enzyme activity associated with acute kidney injury(AKI)[2].

The most significant effort in treating rhabdomyolysis is directed to the prevention of AKI, which was observed in 10% to 60% of patients who presented rhabdomyolysis, and 10% of AKI were attributed to rhabdomyolysis[3].Although some biochemical markers are used, no single tag or

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predictive model has been able to assess the risk of AKI reliably. This difficulty can be explained by the multifactorial nature of the kidney injury, with rhabdomyolysis being only one of the contributing factors and the heterogeneity in the causes of rhabdomyolysis[4].

Four mechanisms can cause muscle damage: hypoxic, chemical, biological, and physical. Patients may have multiple factors or not have an identified cause [5,6]. Exertional rhabdomyolysis (ER) is clinically identified by severe acute pain due to strenuous or regular exercise in extreme circumstances. Although the exact mechanism that damages the cells is unknown, the extravasation of cellular constituents increases the serum levels of CK, creatinine (Cr), and myoglobin, which may be associated with acute renal failure (ARF) due to tubular obstruction, oxidative injury, and vasoconstriction. Myoglobin assessment has been criticized in the ER diagnosis due to its low sensitivity after exercise and rapid renal clearance[7]. The most convenient and sensitive marker is the serum CK level. To diagnose rhabdomyolysis, some investigators have defined a serum CK elevation > 5 or 10 times the upper limit of standard[6,8]. However, there is evidence showing significantly elevated CK levels without impaired renal function in high-intensity events[7–11], and CK levels were inversely correlated with serum Cr levels in 30 cases for ER[12].

These CK parameters are assumed to be conservative for the ER and do not consider gender, ethnicity, and type of activity performed. ER is considered physiological when the individual has high CK levels with muscle pain expected for specific circumstances without any other evidence of clinical sequelae. Nonetheless, it becomes clinically relevant when pain is typically disproportionate to what would be expected for the level of exertion performed, and it usually increases the risk of clinical complications while combined with confounding variables such as sickle cell trait, dehydration, medication use, dietary supplements, myopathies and excessive environmental heat stress[13].

On the medical spectrum, there is the physiological end (sports medicine /military medicine) that is familiar with the symptoms and high CK levels from ER, and the clinical end (internal medicine, neurology, and neuromuscular medicine),which concentrates on the general causes of rhabdomyolysis to define the diagnosis. These facts can lead to the expulsion or removal of military personnel during special operations specialization or training of some agencies due to physiological and not clinically relevant ER[14,15].

The purpose of this study was to collect normative biomarker samples, including CK and Cr, during a military operation in the extreme environment of Amazon jungle, with high physical demand, during the Brazilian Army Pathfinder course.

2. Methods

2.1. Design

This study prospectively analyzed biomarkers during the Jaguar (*Onça* in Portuguese) Operation of the Brazilian Army Pathfinder Course, held on May 2021, in the Brazilian amazon rainforest.

An informed consent form, containing this research information, along with risks, benefits, and purposes, was voluntarily and individually signed by all candidates before being included in the study sample. Each author certifies that they had no competing interests while conducting this study. Patients or the general public were not involved in the planning, execution, reporting, or distribution of this study.

2.2. Participants

A final convenient sample of 17 adult male military parachutists enrolled in the Brazilian Army Pathfinder Course (Age 27.83 ± 2.96 Years, weight 76.31 ± 7.67 kg, and height 174.92 ± 5.91 cm) was selected for this study. They had no significant past medical history that would prevent them from attempting to complete the course. Only one of the 18initially enrolled participants, voluntarily withdrew from the military course for reasons unrelated to the study procedures, remaining 17 for final analysis. The research was approved by the Ethics Committee of the Salgado de Oliveira University with the number 3.846.052.

2.3. Setting

The Jaguar Operation (or *Onça-Op*.) is a military special forces airborne infiltration and exfiltration training course performed in extreme conditions of temperature and humidity. The environment is the highly dense, flora-diverse jungle of amazon rainforest.

Initially, all subjects received technical instructions at the Brazilian Army Jungle Warfare Training Center, at Manaus City, Brazil. For three days (T1, T2, and T3), the subjects underwent specific survival training in a jungle environment, performed day and night. It included orientation through the jungle, march with equipment load, obtaining water, making camp and shelter, fishing and hunting.

On the fourth day (T4), the subjects started the*Onça-Op*. by parachuting into a body of water in the region of the Puraquequara River (Amazon, Brazil). After regrouping, they started the fluvial movement along the Puraquequara River to apre-determined control point. Then, they began the first inlandmarch (infiltration phase) through the jungle. They carried a load of approximately 30kg and collective material (stretcher, chainsaw, MAG machine gun, and collective radio) during 11 hours of exhausting hiking deep into the jungle. On the fifth day (T5), they started the second infiltration day, lasting 8 hours, to reach the objective location. Finally, an exfiltration operation was carried out. During the operation, there was no deprivation of food or water.

2.4. Procedures

Before the operation starts, a basal blood collection was performed in the morning of the first training day (T1). On days 2, 3 and 4, blood samples were drawn in the morning before starting the planned activities (T2, T3, and T4). On day 5, the last day of the operation, due to strict compliance with the operation protocol, blood collection was performed at 14hs, or 2pm (T5), 2 hours after the conclusion of whole military operation. Trained professionals collected blood samples through peripheral venipuncture in the antecubital region, using a vacuum system, following conventional clinical protocols[14,15].

Due to the high temperature and humidity (RH) in an Amazon jungle environment, the hydration status of the subjects was monitored using the Urine Specific Gravity (USG), twice a day (morning and night), using a hand-held refractometer (Instrutherm model RTP-20ATC, São Paulo, Brazil). Individuals who presented a state of dehydration, or values above 1.030, were immediately instructed about the intake of water or electrolytic replenishers[16].

Blood samples were centrifuged, immediately after collection, for 10 minutes at 3000 rotations per minute (rpm) to separate the serum and subsequent analysis of the following biomarkers: creatine kinase (CK), blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). All concentrations used 5ml tubes (Labor Import, São Paulo, Brazil) without anticoagulant, containing separating gel. The automated liquid chemistry analyzer CMD 800i (Wiener Lab, Rosario, Argentina) was used. All analyses were performed at 37ºC and followed the recommendations of the specific commercial kit (Wiener Lab, Rosario, Argentina).

A MacBook Pro Notebook (Apple Inc., Cupertino, California, USA) was used for data storage and processing. It was outfitted with the Microsoft Office 365 (Microsoft, Redmond, Washington, EUA) software suite, and IBM's Statistical Package for Social Sciences (SPSS) (IBM Corporation, Armonk, NY, USA).

2.5. Data Analysis

A descriptive data analysis was initially conducted with average and standard deviation (mean ± SD) and range. The Shapiro–Wilk was utilized to verify the normality of the age, weight, height, CK, BUN, Cr, Na, K, AST, and ALT data at the initial evaluations. Table 1 displays all the sample's demographic and anthropometric information.

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Table 1. Demographic and anthropometric data of the whole military personnel sample.

Sex	Age	Height	Weight	BMI
Male (n=17)	27.83 ±2.96	174.92 ±5.91	76.31 ±7.67	25,21 ±3.27

The Generalized Linear Models (GLM) for repeated measures and the related samples Friedman's two-way analysis of variance by ranks test were used to compare biomarker parameters throughout timepoints (days), for parametric and non-parametric distribution, respectively.

The Bonferroni correction and the Dunn-Bonferroni post-hoc test were applied to assess differences in pairwise comparisons according to parametric distribution. The significance level was assumed for $\alpha = 0.05$.

3. Results

Seventeen subjects completed the operation and did not show signs or symptoms of pain disproportionate to what would be expected for the level of physical effort performed that would prevent them from continuing the course.

The results of the biochemical variables are listed in Table 2. Despite showing variations throughout the study, the mean values of renal function markers (BUN, Cr, Na, and K) remained within their corresponding reference ranges when compared to their baseline values. However, muscle damage markers CK and AST had mean values above the respective reference ranges throughout the study, with high variability among participants and a significant increase after the last day of the operation.

Table 2. Comparison among the biochemistry variables on each timepoint.

Variable	Reference Range	Day1	Day2	Day3	Day4	Day5
CK (U/L)	(24 – 195)	752.17±401.05 (92 – 7146)	587.39±193.72 (154 – 3658)	492.67±97.04† (190 – 1944)	494.06±88.89† (175 – 1448)	2515.22±399.07‡ (610 – 6215)
BUN (mg/dL)	(10 – 50)	36.06±1.70 (24 – 48)	31.56±1.21** (23 – 42)	34±1.22 (24 – 44)	44.56±2.02* (35 – 67)	43.44±1.73 (30 – 54)
Cr (mg/dL)	(0,8 – 1,4)	1.049±0.03 (0.9 – 1.2)	0.87±0.02† (0.8 – 1)	0.95±0.02‡ (0.8 - 1.1)	1±0.03 (0.8 - 1.3)	0.99±0.03 (0.8 - 1.2)
Na (mEq/1)	(134 – 145)	141.97±0.34 (138.2 - 144.4)	136.94±0.36* (132.9 – 138.8)	139.94±0.26* (138.3 - 141.8)	136.34±0.34* (133.2 - 138.4)	137.42±0.43* (133 - 139.8)
K (mEq/1)	(3,6 – 5,5)	4.42±0.06 (3.9 - 4.9)	9.18±0.25* (7.6 – 11.7)	4.67±0.07 (4.3 - 5.1)	5.88±0.10* (5.2 - 6.7)	4.64±0.06** (4.2 - 5.2)
AST (U/L)	(0 – 38)	50.22±13.32 (23 – 265)	58.22±8.38‡ (24 – 177)	57.39±6.41‡ (24 – 120)	48.89±4.81 (24 – 90)	112.23±13.70† (40 – 266)
ALT (U/L)	(0 – 42)	62.67±12.17 (25 – 260)	73.06±11.63 (22 – 242)	83.28±11.73† (25 – 233)	77.56±10.38 (23 – 195)	91.06±11.86† (33 – 218)

Notes:*p < 0.001 (GLM Repeated Measure); **p < 0.001 (GLM Repeated Measure); †p < 0.001 (Friedman); ‡p < 0.05 (Friedman)

Before starting the operation, about 11% of the subjects had CK levels > 10 times the upper limit of normal. During the military operation, there was considerable variability between subjects, with values ranging from 610 to 6215 U/L on day 5, being 50% > 5 times and 44% > 10 times (Table 3).

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Table 3. Percentage (%) of individuals with CK values of ordinary, >5 times (5X) and >10 times (10X) reference range of normality.

	Day 1	Day 2	Day 3	Day 4	Day 5
CK normal	88.89% (92 - 565)	88.89% (154 - 830)	94.44% (190 - 865)	83.33% (175 - 524)	5.56% (610 - 610)
CK >5x	0.00% (-)	5.56% (1356 - 1356)	5.56% (1944 - 1944)	16.67% (1074 - 1448)	50.00% (1130 - 1814)
CK >10x	11.11% (2689 - 7146)	5.56% (3658 - 3658)	0.00% (-)	0.00% (-)	44.44% (2000 - 6215)

Correlations were explored to identify a relationship between CK levels and renal function (BUN, Cr). The CK peak on day five did not correlate with Cr. A moderate correlation with Cr was found only on day 2. Concerning BUN, CK showed a moderate correlation after day 5, although the mean BUN remained within the reference range of normality (Table 4).

Table 4. Percentage (%) of individuals with CK values of ordinary, >5 times (5X) and >10 times (10X) reference range of normality.

		Day 1		Day 2		Day 3		Day 4		Day 5	
		BUN	Cr	BUN	Cr	BUN	Cr	BUN	Cr	BUN	Cr
CK	**Day 1**	0.297 (0.231)	0.298 (0.229)								
	Day 2			0.082 (0.747)	0.514# (0.029)						
	Day 3					0.445 (0.064)	0.339 (0.168)				
	Day 4							0.339 (0.169)	0.153 (0.543)		
	Day 5									0.504* (0.033)	0.282 (0.257)

During the five days of military operation, the average temperature was 26.9°C with 92% RH. The individuals had average urine density values close to the hydration status threshold (USG = 1.030)[16], with a maximum variation of 1.040 on day 4 (Table 5).

Table 5. Urine specific gravity (USG) during the five days.

		Day1	Day2	Day3	Day4	Day5
Period	**Morning**	1.026 (1.012-1.031)	1.020 (1.010-1.031)	1.016 (1.006-1.029)	1.028 (1.008-1.036)	1.022 (1.014-1.034)
	Afternoon	1.029 (1.022-1.035)	1.026 (1.014-1.035)	1.027 (1.018-1.032)	1.031 (1.010-1.040)	1.031 (1.018-1.037)

4. Discussion

As the RH is high in the Amazon jungle environment, during physical activity in this biome, the percentage of sweat evaporated from the body is lower, making it difficult to dissipate heat. Thus, most of the sweat secreted by the sweat glands runs through the skin, representing a loss of useless water by the body, contributing to dehydration and hemoconcentration, which can generate an increase in biochemical markers per unit of volume and, consequently, renal overload [17–20]

While this is not the first study of its kind to note levels of CK following long-endurance military operation[14,15], it is the first that the authors could find prospectively assessing normative values before, during, and after the military operation in extreme environments monitoring individuals' hydration status.

Monitoring the hydration status during the operation proved effective because the subjects were always at the threshold of the hydration state, despite carrying out intense and continuous activities

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with high RH, the use of combat uniforms, and personal equipment [21–24]. The Na and K concentrations remained within the reference ranges for normality throughout the study, avoiding the possibility of hypernatremia or hyponatremia [25,26].

In addition, punctual hydration strategies with water and/or hydro-electrolytic replenishers in dehydrated subjects contributed to the maintenance of renal function, as evidenced by BUN and Cr values within the reference range for normality throughout the study[27].

Before starting the operation, 11% of the subjects already had CK values above the cutoff point to diagnose rhabdomyolysis (CK >10 times the upper limit of normal). These high values were probably due to activities carried out in the Pathfinder Course the previous week, considering that the peak of CK extravasation is 72 hours [28–31].

Nevertheless, before starting the operation, elevated CK values did not correlate with AKI and the subjects did not have any clinically relevant signs or symptoms of rhabdomyolysis that would prevent them from starting the operation. Our results corroborate that high CK levels do not imply disease but a "muscle state"[29,32].

Elevated CK levels after the operation (day 5) were considered physiological due to the ER, as the soldiers presented muscle pain expected for the specific circumstances, without any other evidence of clinical sequelae or AKI. Cr levels remained within the reference ranges for normality and showed a low correlation with the high levels of CK after the operation. BUN levels showed a moderate correlation with CK after the military operation, although they also remained within the reference ranges for normality. These results reinforce that CK values are not predictors of renal function in activities with intense and continuous physical demand[14,29,32,33].

Other studies also observed elevated CK values associated with ER, which did not correlate with acute kidney injury. In a study with healthy individuals, high CK values were noted to average 6,420 mg/dl four days after performing 50 maximal eccentric contractions of the elbow flexor muscles. There were no significant increases in any measure of renal function. In Shumway et al.'s study with Air Force special operation candidates, CK values were noted to average 6,481U/L with a range (146 – 28.453) after intense physiological training and high CK values associated with ER have not correlated with acute kidney injury[14].Another military study described thirty hospitalized cases with ICD-9,a code of 722.88 (rhabdomyolysis) as the primary diagnosis from 2010 to 2012 in the tertiary care military hospital in Honolulu, Hawaii. The mean admission CK was 61,391 U/L (range697-233,180 U/L);paradoxically, CK levels were inversely related to serum Cr levels[12].

These results reinforce the concept that the parameters adopted for diagnosing rhabdomyolysis in clinical medicine (CK > 5 or 10 times the upper limit of normal) are conservative for ER and, when used in this context, military personnel can be excluded from military training and operations as a function of the physiological ER. CK analyzed alone is not a predictor of renal function during intense physical activity. A systematic review with meta-analysis noted that CK could accurately predict the occurrence of AKI in crush-induced rhabdomyolysis but not in ER[34].

Clinically relevant ER should be diagnosed by a physician based on the identifying symptoms and clinical findings, especially when pain is typically disproportionate to the expected level of exertion. The risk of clinical complications is increased when ER is combined with confounding variables such as sickle cell trait, dehydration, medication use, dietary supplements, myopathies, and excessive environmental heat stress[13]. In this context, muscle damage markers should not be used for predictive purposes but rather to help define the diagnosis after clinical analysis of the patient.

It is essential to have a defined protocol to diagnose individual susceptibility to ER, as reported by some authors[35] mainly for military members of the Tactical Operations Group who will be subjected to vigorous and prolonged physical exercise in extreme environmental conditions. Using such a protocol might identify risk groups prior to exposure to factors that induce ER.

Finally, adequate fluid intake before, during and after intense and continued physical activity is a prevention strategy. This will help dilute the urine and facilitate the elimination of myoglobin that has been released from the muscles. Furthermore, it should be borne in mind that some factors that contribute to clinically relevant rhabdomyolysis are modifiable, such as physical conditioning, body composition, duration and intensity of physical activity, and inappropriate use of drugs and supplements.

5. Conclusions

Although the physiological elevation of the CK concentration has been shown to be expected in high-intensity military training, this study points out that monitoring the hydration status during the performance of the activity contributes to the maintenance of renal function, avoiding the risk of acute renal failure, most serious complication of ER, showing a low incidence with the isolated increase in CK.

Therefore, the main focus should be on individualized preventive medicine for those who are at constant occupational risk of developing ER and CK levels should not be used for prediction or cornerstone of diagnosis purposes, but rather to aid medical diagnosis after identification and analysis of signs and symptoms of military personnel who present limitations or health problems during training.

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Institutional Review Board Statement: University Salgado de Oliveira Human Research Ethics Committee fully approved the study with the protocol number #1550437. The ethics committee of the University Salgado de Oliveira approved the study protocol. The datasets generated and analysed during this study are available from the corresponding author on reasonable requests from peer researchers.

Informed Consent Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed Consent was obtained from all individual participants included in the study

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