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Analysis of Muscle Damage Through Urine Staining and Density as Prevention of Rhabdomyolysis During Physical Training

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SUMMARY

Objective. The present study aimed to analyze the variables that could serve as evidence of rhabdomyolysis, such as room temperature, global temperature, rating of perceived exertion (RPE), urine color (myoglobinuria) and urine density, clinical and physical symptoms in a military police operational course conducted in the city of Rio de Janeiro.

Methods. Forty-nine male service members were approved to start the Dog Handlers Course for Police Employment II/2019 of the Dog Action Battalion of the Military Police of the State of Rio de Janeiro. A total of 311 urine samples and 311 perceived exertion values were collected. Ambient temperature and moist globe temperature (WBGT) was controlled.

Results. During the collections, the ambient temperature was 24.75 °C and the humidity temperature was between 10 and 18.3 °C. Borg's levels of subjective perception (SPE) (CR-10) of exertion increased between Collections 1 and 8 ($p < 0.05$). There was a positive correlation between urine density and absorbance (rho = 0.561; $p < 0.001$).

Conclusions. Color analysis by spectrophotometry and urine properties was a method with potential efficiency for the detection of myoglobinuria, renal system overload and origin of rhabdomyolysis.

KEY WORDS

Urine color; urine density; muscle damage; rhabdomyolysis; military operational course.

INTRODUCTION

Rhabdomyolysis is a syndrome characterized by skeletal muscle cell injury and muscle necrosis, which can be defined by unscheduled cell death derived from external factors. With this content in the bloodstream, the organs are affected and eliminate these toxic components, through the urine (1-3).

Urine color can vary, ranging from colorless to black. These color variations can be caused by changes in hydration, diseases (metabolic, genetic, muscle injuries, stones, tumors), physical activity, and sample storage conditions. However, only the change in urine color does not indicate abnormal functioning of the body and rhabdomyolysis (4).

The measurement of myoglobin or myoglobinuria is less sensitive than the measurement of plasma creatine kinase (CK) for diagnosing rhabdomyolysis (5). However, CK is the first substance of muscular origin to increase and the first to contribute to kidney damage, which can trigger nephrotoxic acute kidney injury (5-7). Controlling these indicators is important for intensive care and maintenance of renal health, as the mortality rate can reach 59% in critically ill patients with acute kidney injury (8).

Difficulty in detection may be a factor related to high mortality rates since non-invasive tests do not provide diagnostic accuracy. The most sensitive test for detecting rhabdomyolysis is plasma CK (3). This makes individuals who undergo intense physical exertion, such as athletes, recreational practitioners, and military personnel, more susceptible to this syndrome because part of their training routine consists of long walks and tests and strenuous physical exercises (9).

According to the latest data from the U.S. Armed Forces Health Surveillance Center, among active-duty service members in 2021, there were 513 cases of exertional rhabdomyolysis, resulting in an unadjusted incidence rate of 38.6 cases per 100,000 person-years, a slight decrease of 0.2 compared to the previous year (10).

Although physical exercise, namely resistance training, reduces the risks of metabolic diseases and improves fitness levels, it is essential to understand the physiological responses of practitioners based on the prescribed dosage (11, 12). Thus, there is a concern with the military operational routine, since maximum levels of physical conditioning and perfection in the execution of activities are required for participation in military operational courses, which may include, among other skills and functions, patrolling violent urban areas with the driving of a police dog in the midst of weather adversities and geographically rugged for a long period, almost strenuously (13).

In this context, prevention becomes a crucial strategic action to avoid cases of rhabdomyolysis during a military course or operational activity. The emergence and development of the syndrome are intricately linked to the practice of high-intensity physical activity. Hence, the present study aimed to analyze the variables that could serve as evidence of rhabdomyolysis, including room temperature, overall temperature, rating of perceived exertion (RPE), urine color (myoglobinuria), and urine density, as well as clinical and physical symptoms in a military police operational course conducted in the city of Rio de Janeiro.

MATERIALS AND METHODS

Experimental design

This study is characterized as a quantitative and descriptive field research (14). The participants were recruited from a pool of 500 service members aged between 20 and 40 after undergoing a selection process, which included specific ability tests, physical fitness tests and health inspections. This made them able to start the course without any physiological marker of non-standard assessment of general health status. There were no health complications that could interfere with the results of this research. Thus, no pre-course data were collected to be used as baseline in this research.

Forty-nine male service members were approved to start the Dog Handlers Course for Police Employment of the Dog Action Battalion of the Rio de Janeiro State Military Police Secretariat. Military personnel who were discharged during the course for any reason other than related to clinical complications, those who requested dismissal of their own volition, and those who presented technical insufficiency in the tasks performed during the course were excluded from the study. The study was approved by the Research Ethics Committee Nº 95856318.0.0000.5259 – date of approval: October 4, 2018 –, with opinion Nº 2.940.992 and the participants signed the free and informed consent form in accordance with resolution 466/12 of the National Health Council.

Procedures

Eight days of data collection were carried out, spaced at least three days apart, throughout the 38-day duration of the course. The first collection took place on the second day after the beginning of the course, and the last one was two days before the end of the course. The collections were always carried out in the morning, after the practice of physical education, according to the schedule of technical and operational activities to be carried out.

In the military operational course, the activities were carried out with military personnel in uniform and with overloaded equipment. One of the few exceptions to this condition is physical education, which was conducted in the morning with appropriate clothing for exercise (shorts, shirt, sneakers, and socks). The specific activities of the course are essential for training and cause high physical stress. Among them are patrol conduct in a hostile environment, rappelling and navigating geographically rugged terrains, as well as long walks, jumps, and runs. During physical education, long-distance running activities were carried out on different terrains (average of 12 km), along with resistance exercises and military physical training consisting of physical fitness tests (PFT) which included pull-up exercises on the fixed bar, sit-ups and running.

Urine and perceived exertion scale data collection

The urine collectors were coded according to the numerical identity acquired by everyone during the course and were arranged on a white poster board divided into numbered quadrants. The collectors were removed one by one by each soldier according to their corresponding number. When returning the collected material, the service members, individually, classified their perceived exertion using the Borg Perceived Exertion Scale (CR-10) (15). All collectors with at least 25 ml of urine were arranged in such a way that two instructors selected the three collectors with the darkest urine and abnormal color. These collectors were separated for later analysis and comparison with numerical values. In the event of a disagreement, a third instructor was consulted.

Urine data analysis

The sedimentation level of urine samples was analyzed immediately after the collection, using a manual refractometer (model RHC-200/ATC, Megabrix, China), to verify the density values in Urine Specific Gravity (USG). On the other hand, a spectrophotometer for ELISA was used to measure urine color, using the Skanalt software. The measurement is measured by absorbance, with a wavelength of 350 nm to 500 nm (nanometer) in 1 nm steps without changing the filter. An object or substance absorbs all incident light except that of the wavelength range observed by vision (350 nm-750 nm). Thus, the color of a solution is complementary to the absorbed light (16). The longer the wavelength, the more quantity and value attributes there are, since color perception is determined by the frequency range received by the visual system (17). The wave value of 350 nm was used for the analyses of the present study, because it presents better performance for the high sample quantity and less comprehensive color results, which facilitates visualization, comparison, and analysis (17).

Wet globe temperature

The wet globe temperature (WBGT) was measured on all days of collection in the morning using a Wet Dry Bulb Globe Thermometer (model TGM-200, Homis, Brazil). WBGT is a type of temperature used to estimate the effect of heat stress related to humidity, in addition to heat through radiation and ambient temperature (18, 19). The risk value was calculated and classified using the risk of heat stroke due to exertion, as follows: WBGT < 18.3 °C = Low risk of heat illness; WBGT from 18.3 to 22.8 °C = moderate risk of heat illness; WBGT from 22.9 to 27.8 °C = High risk of heat illness; WBGT ≥ 27.9°C = Extreme risk of heat illness (18).

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 25, and the results were presented as mean, standard deviation, as well as minimum and maximum values. The normality of the data was assessed using the Shapiro-Wilk test. For intragroup comparisons of RPE, Friedman's test was employed, followed by Dunn's *post-hoc* test. Spearman's correlation test was utilized to analyze the associations between the research variables. The study considered a significance level of $p < 0.05$ for statistical significance.

RESULTS

By the end of the intervention, 37 service members completed the course, while 12 participants dropped out and a female military member excluded from this study to make the sample homogeneous. A total of 311 urine samples and 311 perceived exertion values were collected. The ambient temperature had an average of 24.75 ± 4.46 °C, with a low peak of 18 °C and a high of 33 °C, during the mornings, in which the collections were carried out during the mornings when the samples were collected. The WBGT consistently fell within the 10 to 18.3 °C range, categorizing it as "low risk of heat illness" based on the risk value calculated and classified per the Risk of Heat Stroke due to Stress (18).

Borg's rating of perceived exertion (RPE) (CR-10) (2000) indicated a significant increase between the first and last days of data collection. Except for collection 3, the scale exhibited values of evolution of the mean RPE during the course period (**table I**) (15).

The urine samples showed a mean density value of 1,024 ± 6.45 USG, with a minimum value of 1,005 USG and a maxi-

Table I. Evolution of the rating of perceived exertion (RPE) of military personnel completing the course.

	Cll - 1	Cll - 2	Cll - 3	Cll - 4	Cll - 5	Cll - 6	Cll - 7	Cll - 8
Average	5.45	8.69	6.08	8.62	8.49	8.08	8.26	9.10*
SP	1.29	1.51	1.22	1.13	1.06	0.98	1.01	0.62
CV	0.24	0.17	0.20	0.13	0.13	0.12	0.12	0.07

Cll: Collection; SP: standard deviation; CV: coefficient of variation; *P-value < 0.05; Collection 8 *vs* Collection 1.

mum of 1,040 USG, a variation of 3.48%. They presented a mean color value, in absorbance, of 1.287 ± 0.41 nm with a minimum value of 0.286 nm and a maximum of 2.379 nm, a variation of 731.81%.

Figure 1 illustrates the mean numerical values of density (USG) and color by absorbance in nanometers (nm) of the selected urine samples among the three with the greatest color changes on each collection day. Additionally, it includes the mean values of all collections that exhibited altered coloration. Of the 311 urine samples collected in this study, 12 exhibited an orange coloration (distinct from the usual yellow hue), 1 showed a reddish tint (**figure 2**), and 298 appeared yellow (ranging from colorless to light yellow, medium yellow, and dark yellow). The densities ranged from 1,001 to 1,012 USG (n = 17; 5.46%), 1,013 to 1,029 USG (n = 194; 62.37%), and equal to or greater than 1,030 USG (n = 100; 32.15%), with two samples having a value of 1,040 USG. The variation in numerical color values for urine samples with a density ≥ 1,030 USG was 309.46%. The collector indicated by the arrow **(A)** has lighter urine color with a density of 1,015 USG and an absorbance of 1.587 nm, while the collector marked by arrow **(B)** has darker urine with a density of 1,030 USG and absorbance of 1.812 nm.

Figure 3 visually represents the relationship between urine density and color, highlighting the collectors with discrepant numerical values. It displays the correlation observed between

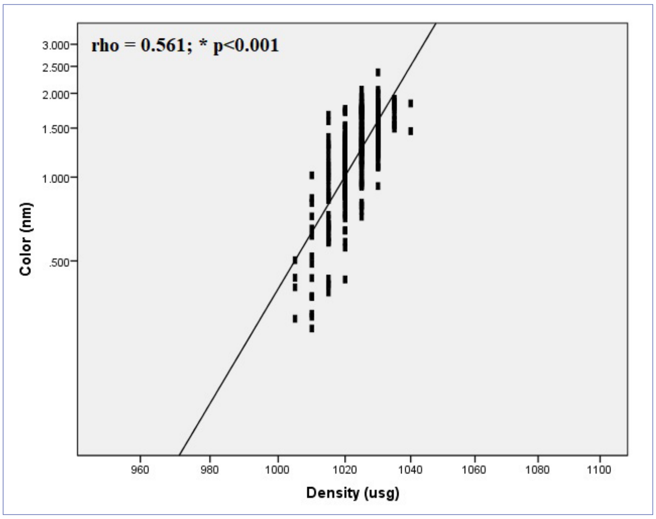

Figure 3. Relationship between absorbance and density of urine.

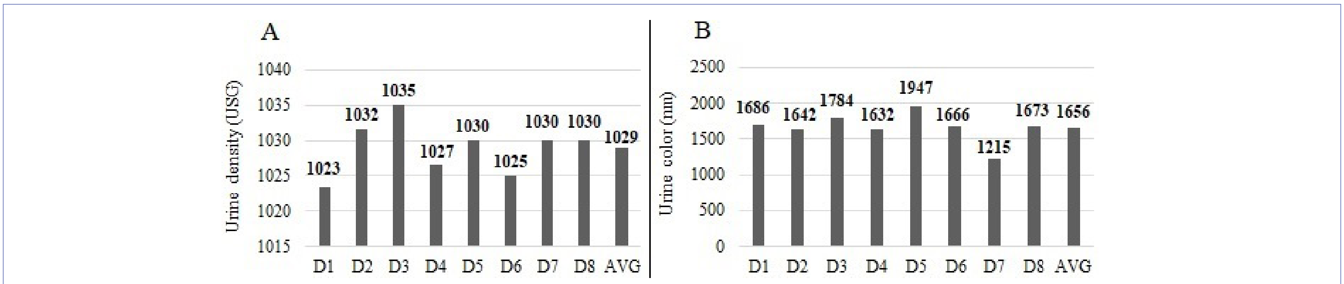

Figure 1. Urine density **(A)** and color **(B)** values by perception.

Mean urine densities: measured by refractometer that showed altered color through perception with the human naked eye - values in USG. Mean Urine Color: measured by a spectrophotometer that showed altered urine color by the naked eye - values and nm. The decimal place of the Urine Color has been subtracted for better visualization. AVG Overall mean values of urine density (USG) and coloration (nm).

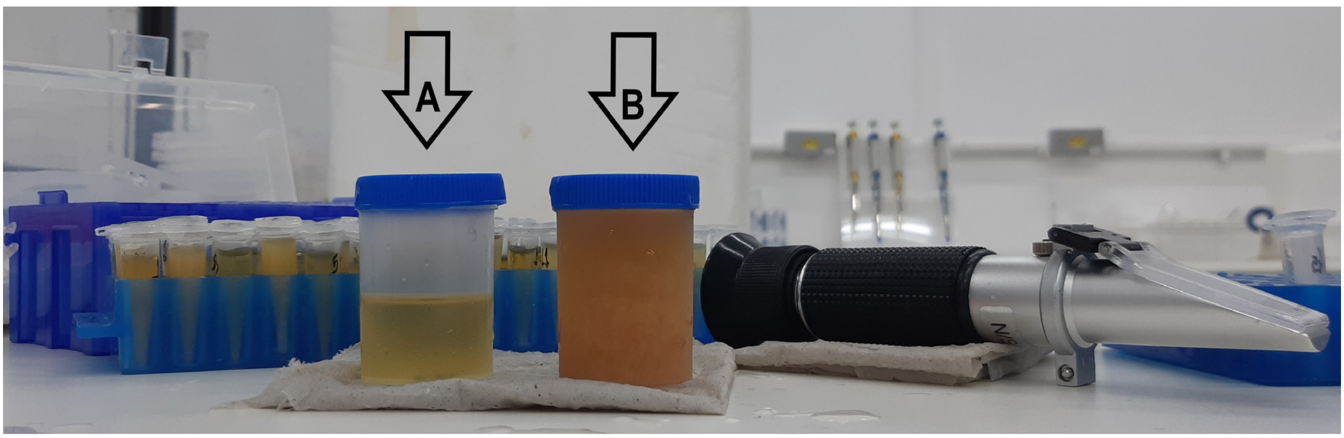

Figure 2. Visual relationship between urine color and density.

urine color (measured in absorbance in nanometers - nm) and urine density (measured in relative density - USG). A positive and statistically significant correlation was found between absorbance and density in the collected urine samples. Therefore, as absorbance values increase, urine density also increases.

DISCUSSION

The current study aimed to analyze the variables of ambient temperature, global temperature, and the rating of perceived exertion (RPE) in relation to muscle damage, as indicated by urine color (myoglobinuria) and density.

Extreme temperatures are often regarded as one of the primary contributing factors to the development of rhabdomyolysis (19). However, in the current study, ambient temperature and wet bulb globe temperature did not appear to impact the outcomes, as the recorded values during the collection period were deemed to fall within the normal range (18).

As a potential consequence of this external factor combined with intense physical exertion, the typical clinical presentation of rhabdomyolysis may manifest, characterized by the classic triad of myalgia, asthenia, and dark urine (20). Given this risk and the significant physical demands imposed throughout the operational course, the study measured the control of overall perceived exertion and muscle discomfort using the Borg Rating of Perceived Exertion (RPE) scale (CR-10) (15). The findings revealed a consistent increase during the course, which was considered a normal response to the escalating physical, psychological, and technical requirements of the training. A similar measurement was employed in a study by Jameson and Ring (21) to assess overall perceived exertion, leg muscle discomfort, knee pain, breathlessness, and heart rate intensity in cyclists.

However, when the urine samples were analyzed, changes were observed in the color of the urine seen with the naked eye when the density was compared. The spectrophotometer-based analysis of urine color provided indicators that raised concerns about dehydration, muscle damage, and the persistence of these conditions despite attempts at rehydration with water.

Military personnel are at an elevated risk of developing rhabdomyolysis during their training routines (9) because the symptoms overlap with those of heat-related illnesses during physical activities (22). Environmental temperature and dark urine are among the causes of rhabdomyolysis, with the latter being influenced by an individual's hydration status and detected through urine density (23, 24). Urine density, as determined by refractometers, offers initial insights into the kidney's capacity to selectively reabsorb essential substances and water from the glomerular filtrate. This quantification is impacted by the quantity and size of particles present in the sample (25). Normal urine typically exhibits characteristics such as a citrus yellow color, a distinct odor, clear appearance, a density ranging from 1,005 USG to 1,030 USG, and a pH between 4.5 and 7.8 (26). Consequently, these variables may be linked to the causes of rhabdomyolysis and the development of more severe cases of acute kidney injury (AKI).

Urine hydration status can be categorized based on density values as follows: Hypohydration $\geq$ 1,030 USG; Euhydrated = 1,013 to 1,029 USG; Hyperhydration = 1,001 to 1,012 USG (24). Savvides *et al*. (27) evaluated the effects of dehydration on archery athletes in a simulated competition and classified urine with a density of 1.032 ± 0.005 USG and euhydrates with a density of 1.015 ± 0.004 USG as dehydrated. Thus, if the classification used by Savvides *et al.* (27) is applied to the urine collected in the present study, 32.15% of the urine was in the condition of dehydration and 10.61% of the urine collected in the condition of euhydration, without variation and 51.76% lower than the classification of Casa *et al*. (24), respectively.

The most common aspect of urine alteration is its color, which can indicate potential causes of harm to the body. Here is a color guide: light yellow to yellow = normal; colorless or very diluted; dark yellow = highly concentrated, indicating bilirubinuria; red to brownish red = may suggest hematuria, hemoglobinuria, or myoglobinuria; greenish = could indicate bilirubinuria (26). Costa *et al.* (28) reported that ultra-endurance athletes often adjust their fluid intake to maintain urine concentration within normal parameters in terms of color and osmolarity. However, in the present study, this strategy proved ineffective in controlling hydration during the operational course, as there was a significant variety of distinct shades of yellow observed in the collected samples.

Figure 1 shows the mean values of urine density and color in absorbance (number) of the participants who had their urine selected, by the instructors, with the darkest color on each day of collection during the course. All urine colors showed values above 1,600 nm. This can demonstrate the homogeneity of the sample, or a cut-off range in which color perception visually altered to the naked eye, begins to be confirmed by color in absolute values. The difficulty in standardizing colors was reported by Armstrong *et al*. (29) when they created a scale of eight colors, which vary in scale from noticeably light yellow to brownish green. However, this scale does not evaluate other colors that may be important for identifying abnormalities in the functioning of the body. Thus, the analysis of urine color by absorbance seems to provide more accurate information to be used in the prevention of rhabdomyolysis.

In the present study, the collected urine samples exhibited a significant variation in density, even though constrained within the normal reference range of 25 units (1,005 USG-1,030 USG) (24). However, the average of the samples, more influenced by color perception on each collection day (**figure 1**), indicates that none of the urine was in a hydrated state (24).

Electrolyte imbalances, including dehydration, are significant contributors to muscle damage and, subsequently, the development of rhabdomyolysis (30, 31). Highly concentrated and excessively alkaline urine can lead to false positive results concerning altered renal function. These false results may be attributed to a range of factors, from simple dehydration to chronic kidney disease (32). The findings of this study demonstrated that all urine samples with a density falling within the range of 1,005 USG to 1,015 USG appeared diluted and exhibited a color outside the typical yellow hue, confirming the lack of precision in diagnosing electrolyte disturbances, dehydration, and muscle structure damage.

Spectrophotometric urine analysis has demonstrated its potential as a method for preventing and managing muscle damage, and consequently, rhabdomyolysis. In the urine samples depicted in **figure 2**, the urine marked as **(A)** was classified as normal based on color and euhydrated based on density. Conversely, the urine labeled as **(B)** was classified as highly concentrated in terms of color and hypohydrated based on density (24, 26). However, the absorbance values for color were quite similar.

This study revealed that not all dark or altered urine had high density, and conversely, not all light-colored urine had low density. Some sources Knochel (33) argue that myoglobinuria only occurs within the context of rhabdomyolysis. However, Khan (34) reported that myoglobinuria does not manifest without eventually leading to rhabdomyolysis. It is worth noting that visible myoglobinuria may not occur until the body has already been affected by rhabdomyolysis. This underscores the importance of effective prevention measures, as the color of urine can be influenced by the myoglobinuria process, even if it is subtle or imperceptible to the naked eye.

The relationship between the density and the color of urine in absorbance (number) is characterized by a positive correlation between these variables, because when the density increases, the color also suffers an increase in its values, but in a more detailed way. When the density increases, there is a corresponding increase in color values, albeit in a more nuanced manner. For urine samples with a borderline density indicating hypohydration at 1,030 USG, according to the classification by Casa *et al.* (24), there was a significant percentage of variation. Thus, it can be suggested that color analysis may be a more accurate, sensitive, and reliable marker. Typically, myoglobin is detected in urine when serum levels exceed values of 1,500 to 3,000 mg/ml (35). The measurement of myoglobin in serum is challenging to determine in the laboratory and is not always readily available.

No studies investigating rhabdomyolysis and its antecedent processes, such as muscle damage and myoglobinuria, through urine color analysis alone were identified. However, Mansor *et al.* (36) explored the relationship between color and dehydration, and Armstrong *et al.* (37) examined the interplay between density and color. In all these studies, color measurements were conducted using a composite color strip with eight distinct colors, which was initially introduced by Armstrong *et al.* (29) and is known as the "Urine Color Chart." Relying solely on this color strip for urine analysis may not offer a highly dependable parameter for assessing physiological changes. This limitation arises from the fact that this strip summarizes colors into just eight categories, potentially overlooking intermediate shades of color between adjacent bands on the strip. Consequently, these intermediary shades may obscure indicators of normality when values are borderline, leading to a misinterpretation of potential renal system overload.

In this study, a single instance of urine was identified with a reddish color (distinct from the typical yellowish hue) and a satisfactory density value. This occurred after a physical activity involving a 14 km run in an environment with an ambient temperature of 18 °C. The soldier who produced this urine sample reported not using any substances to enhance performance or recovery, nor was he taking any medications. Furthermore, he did not experience muscle pain or severe fatigue. The reddish or brownish discoloration of urine (indicating myoglobinuria) results from the release of myoglobin into the bloodstream and subsequent excretion through the urinary system. This pathophysiological process is initiated by physical activity and can potentially lead to rhabdomyolysis and renal failure. This cycle of renal system damage persists until interrupted by rehydration with a saline solution (38, 39).

As noted by Yu *et al.* (4), the guidelines from the Brazilian Society of Nephrology for acute renal failure advise the use of saline solution, sodium bicarbonate, and mannitol in cases involving myoglobinuria and hemoglobinuria. These interventions are aimed at reducing the occurrence and severity of kidney damage. This suggests that while increased water intake can help hydrate the body and potentially alter the color of urine, the results from this study indicate that it may not effectively interrupt the pathophysiological damage caused by insufficient or inadequate fluid intake.

The randomized study conducted by Johnson *et al.* (40) concluded that consuming approximately 1,500 mL of additional water, in addition to the average daily intake of 2,990 and 3,515 mL, was necessary to restore urine to an appropriate color within 24 hours, even after three days of restricted intake. Research by Perrier *et al.* (41) involved experiments where total daily water intake was adjusted and demonstrated that an increase of 1,110 mL was required to reduce urine color by two units on an eight-point scale strip. Both Perrier *et al.* (42) and Kavouras *et al.* (43) emphasized that in adults and children, urine has the potential to indicate hydration status through color alone. Throughout the course, the soldiers were hydrated with plenty of oral water, as a result, the light-colored urine samples should have low numerical values of density and staining through spectrophotometry. However, this did not happen with several of the urine samples collected.

A limitation of this study is the lack of an association between these urine samples and a direct marker of muscle damage or renal system overload, as hyperhydration during the rest period can alter urine color.

CONCLUSIONS

The positive correlation between urine absorbance and density suggests signs of muscle fiber destruction, which may indicate the onset of rhabdomyolysis and renal system overload in participants of the operational course, as the collected samples showed high coloration values. On the other hand, the rating of perceived exertion did not directly justify an influence on the result of urinary analysis, but it did register high values on the scale, which may support the physical discomfort perceived by the military personnel. This underscores the high physical and psychological demands imposed on military personnel during an operational course.

The analysis of urine color by spectrophotometry and urine properties has emerged as a method with potential efficiency for detecting myoglobinuria. The absolute cutoff value obtained through urine coloration can indicate massive destruction of muscle structures during the execution of strenuous exercises. However, caution is needed when assessing the association between urine color and physiological damage, as other factors such as medication use, bacterial contamination, and overhydration may influence changes in urine color, masking potential pre-existing damage that could lead to rhabdomyolysis and renal failure.

For future studies, it is suggested to investigate the association between urine color, physical symptoms, and specific markers of renal system damage, such as Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Gamma-Glutamyl Transpeptidase (GGT).

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None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

GHMD: conceptualization, methodology, formal analysis, investigation, writing – original draft, visualization. RAR: investigation, methodology, formal analysis. RAMN: investigation, methodology, formal analysis, investigation. EBN: Investigation, methodology, formal analysis. GCPSMS: formal analysis, writing - review & editing, visualization. GCL: investigation, methodology, formal analysis, investigation. RGSV: conceptualization, methodology, formal analysis, investigation, writing - review & editing, visualization.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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