



Rev Bras Futebol 2020; v. 13, n. 3, 17 - 28

**CONCENTRAÇÕES DE LACTATO, CREATINAQUINASE E LACTATODESIDROGENASE EM JOGADORES DE FUTEBOL AMADOR EXPOSTOS A CRIOTERAPIA POR IMERSÃO E EFICIÊNCIA DA PERCEPÇÃO SUBJETIVA DO ESFORÇO.**

**LACTATE, CREATINE KINASE, AND LACTATE DEHYDROGENASE CONCENTRATIONS IN AMATEUR SOCCER PLAYERS EXPOSED TO IMMERSION CRYOTHERAPY AND EFFICIENCY OF THE SUBJECTIVE PERCEPTION OF EFFORT.**

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**CONCENTRAÇÃO DE LACTATO, CREATINA QUINASE E LACTATO DESIDROGENASE EM JOGADORES DE FUTEBOL AMADOR EXPOSTOS A CRIOTERAPIA POR IMERSÃO E EFICIÊNCIA DA PERCEPÇÃO SUBJETIVA DE ESFORÇO.**

## RESUMO

**Introdução:** O futebol é predominantemente caracterizado como uma prática aeróbica, no entanto, considerando algumas etapas específicas, a amplificação exponencial da velocidade é necessária para aumentar a probabilidade de sucesso. Dessa maneira, a recuperação irregular aumenta a probabilidade de efeitos negativos no desempenho.

**Objetivo:** Analisar os efeitos da crioterapia nos parâmetros de lactato, creatina quinase (CK) e lactato desidrogenase (LDH) no período de recuperação após uma partida de futebol amador e identificar se a percepção subjetiva do esforço (PSE) é um bom marcador de intensidade.

**Métodos:** Inicialmente participaram vinte jogadores com idades entre 20 e 36 anos, no entanto, quatro foram excluídos das coletas de lactato e cinco das coletas de CK e LDH. Totalizando então, 16 participantes para coleta de lactato e cinco 15 para coleta de CK e LDH. Os jogadores foram submetidos a 10 minutos de crioterapia final da partida em um tambor de 250 litros com água a uma temperatura de  $5^{\circ} \pm 1^{\circ} \text{C}$  até a espinha ílica ântero-inferior.

**Resultados:** Não foi observada diferença estatística em relação ao fator de crioterapia nos marcadores lactato ( $p = 0,143$ ), CK ( $p = 0,74$ ) e LDH ( $p = 0,71$ ). Nas análises do PSE, foi encontrada uma correlação entre o PSE médio 6-20 ( $13 \pm 1,11$ ) realizado no final da primeira ( $12,13 \pm 1,12$ ) e a segunda metade ( $13,86 \pm 1,19$ ) com o PSE 0-10 aplicado por 30 minutos após o final do jogo ( $3,73 \pm 1,16$ ).

**Conclusão:** A crioterapia por imersão não tem efeitos moduladores nas concentrações de lactato, CK ou LDH em jogadores de futebol amador, e que tanto o PSE 6-20 quanto o PSE 0-10 podem ser utilizados como uma ferramenta para identificar a intensidade do exercício.

**Palavras chaves:** Creatina quinase, Lactato desidrogenase, Futebol.

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AMATEUR SOCCER PLAYERS EXPOSED TO IMMERSION CRYOTHERAPY AND EFFICIENCY OF  
THE SUBJECTIVE PERCEPTION OF EFFORT.**

**ABSTRACT**

**Introduction:** The football is predominantly characterized as an aerobic practice, however, considering some specific steps, exponential amplification of velocity is necessary to increase the probability of success. In this way, irregular recovery increases the probability of bad effects on performance.

**Objective:** To analyze the effects of cryotherapy on parameters of lactate, creatine kinase (CK), and lactate dehydrogenase (LDH) in the recovery period after an amateur soccer match, and to identify whether the subjective perception of effort (SPE) is a good marker of intensity.

**Methods:** Initially, twenty players aged between 20 and 36 years participated, however, four were excluded from lactate collections and five from CK and LDH collections. Then totaling, 16 samples for lactate collections and 15 for CK and LDH collections. The players were submitted to 10 minutes of cryotherapy in a 250-liter drum with water at a temperature of  $5^{\circ} \pm 1^{\circ}$  C up to the anterior inferior iliac spine at the end of the match to evaluate the concentrations of the studied biochemical parameters.

**Results:** No statistical difference was observed regarding the cryotherapy factor on the markers lactate ( $p= 0.143$ ), CK ( $p= 0.74$ ), and LDH ( $p= 0.71$ ). In the PSE analyses, a correlation was found between the mean PSE 6-20 ( $13 \pm 1.11$ ) performed at the end of the first ( $12.13 \pm 1.12$ ) and second halves ( $13.86 \pm 1.19$ ) with the PSE 0-10 applied 30 minutes after the end of the game ( $3.73 \pm 1.16$ ).

**Conclusion:** It is concluded that immersion cryotherapy does not have modulatory effects on the concentrations of lactate, CK, or LDH in amateur soccer players, and that both the PSE 6-20 and PSE 0-10 can be used as a tool to identify the intensity of exercise.

**Keywords:** Creatine kinase, Lactate dehydrogenase, Soccer.

## INTRODUCTION

Several studies around the world have found that football is predominantly characterized as an aerobic practice, due to the great distances covered during the course of the game, ranging from 9 to 14 km. However, considering some specific steps, exponential amplification of velocity is necessary to increase the probability of success, and in this way several small anaerobic stimuli occur throughout the session<sup>1,2,3,4,5</sup>.

Although it is of the utmost importance that players improve their techniques and master the tactics developed by the team in order to obtain good results, it is also essential to carry out specific training to enable better oxygen capitation and utilization (O<sub>2</sub>) for players to be able to withstand, for as long as possible, the physiological fatigue resulting from efforts exerted in a match<sup>6</sup>.

This high-intensity fatigue is caused not only by muscle damage, but also by oxidative stress, which contributes greatly to this outcome. This fact occurs due to the large consumption of O<sub>2</sub>, which, in turn, will be determinant to increase the production of free radicals and generate great fatigue in the individual. Adequate periods of rest are paramount at this time<sup>7,8,9</sup>.

In this way, irregular recovery increases the probability of effects on performance in training, which will then be reflected in the matches. Return to acceptable stress marker values to play subsequent matches, in addition to providing greater confidence to athletes, considerably reduces the risk of injury due to this issue<sup>10,11,12,13</sup>.

Some tools, such as lactate dosages and subjective perceptions of exertion, help to identify the level of metabolic stress and to estimate an adequate period for recovery of the organism, as well as which, proper manipulation of the variables intensifies homeostatic restoration<sup>14,15,16</sup>.

During high-intensity exercise, skeletal muscle cells are responsible for causing increased lactate concentration in the bloodstream (lactacidemia), which, at high levels, leads to muscular discomfort to the individual. In contrast, the lactate released by the muscle cells is recaptured by the cells to synthesize energy for the exercise. In this way, this substrate has been used as a marker to observe the degree of intensity of the activity<sup>17,18</sup>.

In some cases recovery of the body, after great exposure to fatigue, takes up to 120 hours of restructuring to reestablish acceptable parameters regarding physical and metabolic performance<sup>19</sup>. In addition, physical performance has frequently been associated with plasma creatine kinase (CK) and lactate dehydrogenase (LDH) concentrations, since individuals with high strength in the lower limbs present a higher capacity to reduce CK in a 48-hour post-match period<sup>11,20,21,22</sup>.

In this way, players with high levels of CK and LDH may not perform well in training, which will be portrayed in their match performance, and increase the propensity for injuries<sup>23,24</sup>.

Some studies indicate that reductions in high concentrations of CK and LDH in plasma have a direct influence on the increase in player productivity. Thus, several strategies to maximize post-exercise recovery are constantly debated, with cold water immersion representing one of the possibilities<sup>25, 26, 27</sup>.

Establishing habits of exposure to cold after a soccer game can be difficult, as it will not always be possible to separate a specific time for this practice, due to the daily activities that each individual presents in their private life<sup>28</sup>.

Therefore, the objective of the present study was to analyze the efficiency of SPE and to identify the effects of immersion cryotherapy on the removal of lactate, CK, and LDH in amateur soccer players.

## **MATERIALS AND METHODS**

### **Participants**

Initially, twenty outfield players aged between 20 and 36 years, who played the last match of the first phase of the amateur championship in the city of Santo Anastácio, participated in this study. Four players were excluded from the specific lactate collections, and five from CK and LDH collections, due to the inclusion criteria. Thus, a total of 16 volunteers were included for lactate and 15 for CK and LDH, since players excluded from some tests were not totally excluded from the study, as they performed the other tests normally.

The study was approved by the CAAE ethics and research committee 07276012.1.0000.5515 and the procedures adopted in this study obeyed the Criteria of Ethics in Research with Human Beings according to Resolution n. 466/12 of the National Health Council, and the data collections were performed only after approval of the Ethics Committee together with signing of the informed consent form.

### **Division of Groups**

Regarding lactate, the volunteers were randomly divided into two groups: eight athletes in group 1 (with cryotherapy) and eight in the control group (without cryotherapy). With respect to the CK and LDH collections, group 1 was composed of seven athletes and the control group included eight.

### **Inclusion Criteria**

- Minimum of 3 years experience;
- Start and complete the 90 minutes of the evaluated game;
- Participate in all blood collections;
- Not present orthopedic problems that may interfere with the study;
- Not use substances that alter serum levels of CK or LDH.

### **Sample Characterization**

Body weight and height were reported by the interviewees, and, based on this information, the body mass index (BMI) of each volunteer was calculated.

### **Cryotherapy**

The Group 1 players were submitted to 10 minutes of submersion cryotherapy in a 250-liter drum with water at a temperature of  $5^{\circ} \pm 1^{\circ} \text{C}$ <sup>29</sup> to the anterior inferior iliac spine after the game. The temperature was adjusted with the aid of an underwater thermometer, and more ice cubes or warm water was added if necessary.

### **Analysis of blood lactate concentration**

For analysis of lactate concentration, blood samples (25  $\mu\text{l}$ ) were collected from the ear lobe in heparinized capillary tubes and transferred to 1.5 ml Eppendorf tubes, containing 50  $\mu\text{l}$  NaF (1% sodium fluoride), and stored at  $-70^{\circ}\text{C}$ . The collections were performed in the rest periods immediately after the end of the first and second halves, and five and 15 minutes after the end of the match. The homogenate was injected (25  $\mu\text{l}$ ) into the YSL 1500 STAT<sup>®</sup> Table Lactimeter (Yellow Spring Co, USA) to determine the lactate concentration.

### **Blood collection for CK and LDH analysis**

The collections were performed 24, 48, and 72 hours after the end of the match by an accredited nurse following all hygiene and asepsis care. In total, 10 mL were collected from the right antecubital vein. At the end of the daily collection, the samples were sent to a reputable laboratory for clinical analysis in the city of Presidente Prudente, which did not have information on which group the evaluated patients belonged to, enabling characterization of the study as a simple blind study.

### Determination of the serum level of LDH and CK.

The centrifuge JOUAN CR3I was used at rotations of 10,000RPM, for the solution refrigerated at 4°C. The activity of the LDH enzyme was verified using the HITACHI UV-2001 spectrophotometer and the Analyze Diagnostic KIT by the colorimetric method<sup>30</sup>.

For analysis of CK the kit "MPR3 CK NAC-ativado" Boehringer Mannheim<sup>®</sup> was used. As well as the buffer solution (2.5 mL vial), a specific reactive tablet was used, left in a water bath at 37°C until complete dissolution. Next, 50 µl of plasma was added to the reaction solution, and the mixture again left in a water bath at 37°C for one minute. Immediately after, four absorbance readings of the same sample were taken at 334 nm, with a one minute interval between readings, to obtain a  $\Delta$  value. Calculation of CK (U/L) activity in the sample was performed using the equation  $CK_p = 8252 \times \Delta \text{ absorbance/minute}$ .

### Subjective Perception of Effort Analysis (PSE)

The PSE 6-20 scale was used<sup>31</sup> at the end of the first and second halves of the match, and the PSE 0-10 scale adapted by<sup>32</sup> 30 minutes after the end of the match to check if there was a correlation between them. The scales were explained to the athletes before application.

### Statistical Analysis

As the Shapiro Wilk test detected data normality, parametric descriptive statistical analysis was used. The characteristics of the sample are described as mean and standard deviation. The ANOVA test for repeated measures was used to detect possible differences in the behavior of the lactate concentration of group 1 with the control group in the periods of five and 15 minutes after the match, as well as the CK and LDH of the respective groups in the periods of 24, 48, and 72 hours. In order to identify the differences between intensities in the first and second halves, the paired T test was used, and to characterize the homogeneity of the groups, the independent T-test was used. A significance level of 5% was adopted.

## RESULTS

The sample consisted of male amateur soccer athletes from the city of Santo Anastácio-SP, mean age  $25 \pm 4.96$  years, with 16 players included for the lactate data, and 15 for the CK and LDH (**Table 1**).

**Table 1.** T-Test results for characteristics of the groups presented as mean and standard deviation regarding age, height, weight, and BMI.

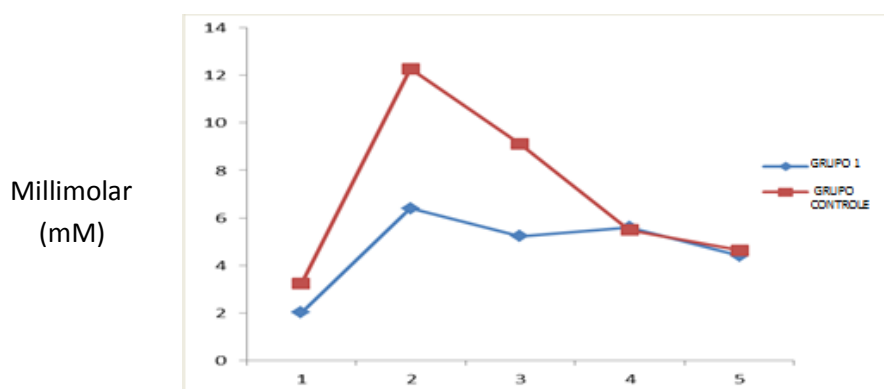
|                          | With cryotherapy | Without cryotherapy | (p value) |
|--------------------------|------------------|---------------------|-----------|
| Subjects                 | 8                | 8                   |           |
| Age (years)              | 25± 5.65         | 26± 4.62            | 0.875     |
| Height (m)               | 1.76± 0.05       | 1.79± 0.07          | 0.151     |
| Weight (Kg)              | 76.42±4.1        | 76.62± 6.61         | 0.126     |
| BMI (Kg/m <sup>2</sup> ) | 24.68± 1.74      | 23.81± 1.45         | 0.687     |

\* BMI – body mass index.

A correlation was observed between age and body weight (0.6  $p= 0.04$ ), as well as between body weight and mean PSE6-20 (0.568  $p= 0.049$ ).

Another finding was the strong correlation (0.78 and  $p= 0.01$ ) between the mean PSE6-20 (13±1.11), applied immediately after the end of the first (12.13±1.12) and second halves (13.86±1.19) with the PSE0-10 applied 30 minutes after the match (3.73± 1.16). There were no statistical differences between the PSE6-20 of the first and second halves ( $p= 0.97$ ) and lactate concentration ( $p= 0.321$ ), respectively.

Regarding the variable lactate concentration, a statistical difference was observed through repeated measures ANOVA for the time factor ( $p= 0.004$ ), while for the cryotherapy factor, no statistical difference was observed ( $p= 0.143$ ) as well as no interaction between them ( $p= 0.877$ ) (**Figure 1**).



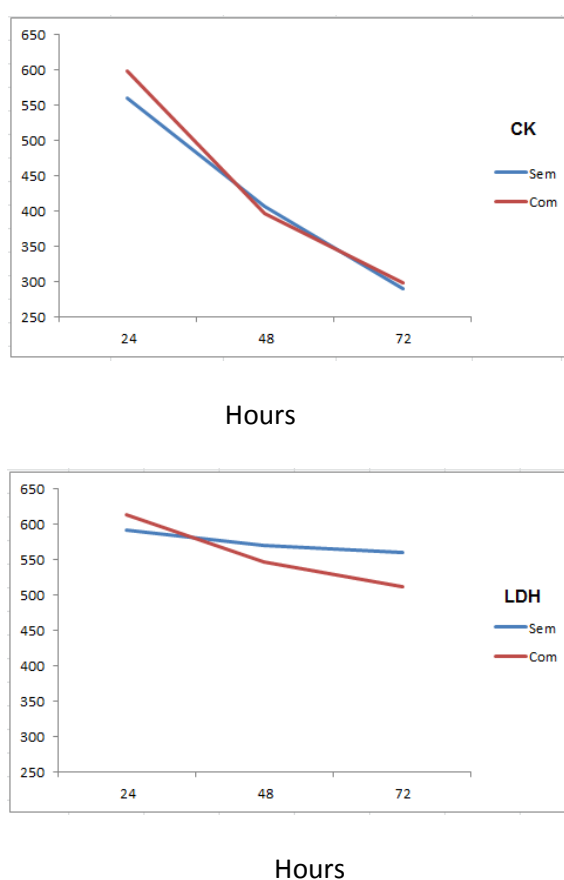
**Figure 1.** Demonstration of lactate concentration behavior over time (1 - rest, 2 – half-time interval, 3 - end of the match, 4 - five minutes of recovery, 5 - 15 minutes of recovery).



In relation to the CK variable, a statistical difference was observed through ANOVA repeated measures for the time factor ( $p= 0.001$ ), while in the cryotherapy factor, no statistical difference was found ( $p= 0.74$ ), and there was no interaction between them ( $p= 0.94$ )(**Figure 2**).

With respect to the LDH variable, no statistical difference was observed through ANOVA for repeated measures for the time factor ( $p= 0.29$ ), the cryotherapy factor ( $p= 0.71$ ), or the interaction between them ( $p= 0.78$ ) (**Figure 2**).

Millimolar (mM) -



**Figure 2.** Demonstration of CK (a) and LDH (b) behavior over time, in hours.

## DISCUSSION

Considering that all athletes participating in the study were adults and had been soccer practitioners for a period of more than three years, there were no significant differences between the characteristics of group 1 (with cryotherapy) and the control group (without cryotherapy), as shown in table 1, demonstrating the homogeneity of the volunteers.

A strong correlation was found between age and body weight ( $0.6$   $p= 0.04$ ), as well as a correlation between body weight and the mean PSE 6-20 ( $0.568$   $p= 0.049$ ), demonstrating that older players presented tendencies to be heavier, and consequently, to report higher PSE. In this way, it is possible to observe the importance of an athlete maintaining their ideal weight so as not to compromise their performance<sup>33</sup>.

Regarding the PSE analyses, a correlation was found between the mean PSE 6-20 ( $13\pm 1.11$ ) performed at the end of the first half ( $12.13\pm 1.12$ ) and second half ( $13.86\pm 1.19$ ) with the PSE 0-10 applied 30 minutes after the end of the game ( $3.73\pm 1.16$ ). In addition, no difference was observed between lactate concentrations and the PSE 6-20 of the first and second halves ( $p= 0.331$ ) ( $p= 0.97$ ), indicating that the level of the game remained the same between halves.

With respect to the subjective perception of effort, it was confirmed that even though this is not a complex tool, it provides, quickly and in a practical way, the intensity exerted by the player, contributing additional evidence on the effectiveness of the results found<sup>34</sup>.

With respect to the concentration of lactate and CK, a statistical difference was observed through ANOVA for repeated measures for the time factor ( $p= 0.004$ ) ( $p= 0.001$ ); however, in the cryotherapy test, no differences were observed in the variables ( $p= 0.143$ ) ( $p= 0.74$ ), and there was no interaction between them ( $p= 0.877$ ) ( $p= 0.94$ ).

Thus, these data corroborate the findings of Russel et al. (2017), who observed no differences between blood lactate and CK concentrations in professional soccer players exposed to cryotherapy. Therefore, this lack of difference between the trials is probably attributable to the muscle damage still present from previous training<sup>35</sup>.

Concerning the data investigated for LDH, there was no statistical difference observed through the ANOVA, as in the previous variables ( $p= 0.29$ ), as well as for cryotherapy ( $0.71$ ), and the interaction between them ( $0.78$ ). This fact demonstrates performance impairment, since inflammatory markers at high levels reduce the performance of practitioners<sup>27</sup>.

A recent study to identify the benefits of cryotherapy in restructuring muscle damage after physical exertion, identified that cryotherapy presented a similar effect to the placebo. In addition, this practice produces discomfort for individuals and requires a period to prepare and maintain the water at the ideal temperature. Therefore, according to the literature, this method is not the most appropriate for the recovery of inflammatory markers<sup>36</sup>. However, further studies with other protocols should be carried out to determine whether alterations in immersion time and water temperature produce different results.

## CONCLUSION

It is concluded that immersion cryotherapy in the parameters used does not facilitate modulating effects to the biochemical parameters analyzed after an amateur soccer match, and that both the SPE 6-20 and SPE 0-10 can be used as a tool to identify exercise intensity.

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