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original article

Assessment of acute physiological demand for soccer

Avaliação da demanda fisiológica aguda no futebol

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Abstract – Soccer is a sport practiced worldwide, on all continents. It is considered an intermittent activity of high intensity and long duration, in which movements that require great strength and speed, such as jumps and sprints, result in high levels of muscle microtrauma, hampering athletes' training and recovery. The present study aimed to evaluate the magnitude of changes in different markers of physiological demand resulting from a soccer match in healthy individuals. Ten healthy male physical education students participated in the study and were evaluated in two matches: the semi-final and final games of the college tournament at the federal university where they studied. Blood samples were collected from each volunteer pre- and post-match. Cortisol, IL-6 and CK concentrations were increased after the match ($p < 0.05$). Testosterone and alpha-actin concentrations did not change. Our results indicate that changes in some of the acute response markers evaluated in players before and after competitive soccer matches provide important information for planning training or recovery, as well as nutritional strategies for improving performance.

Key words: Biological markers; Creatine kinase; Soccer; Testosterone.

Resumo – *O futebol é um esporte de abrangência mundial praticado em todos os continentes. É considerada uma atividade intermitente, de alta intensidade e longa duração, na qual as ações de grande força e velocidade como saltos e sprints implicam altos níveis de microtrauma muscular, atrapalhando o treinamento e a recuperação dos atletas. O propósito deste trabalho foi avaliar a magnitude das alterações de diferentes marcadores da demanda fisiológica em indivíduos saudáveis decorrentes de um jogo de futebol. Participaram do estudo, dez homens considerados saudáveis, estudantes de Educação Física. Os indivíduos foram avaliados em dois jogos, sendo a semifinal e a final do torneio universitário da universidade federal onde estudavam. A amostra sanguínea foi retirada de cada voluntário nos momentos pré e pós-jogo. Resultados: As concentrações de Cortisol, IL-6 e CK, apresentaram aumento pós-jogo ($p<0,05$). As concentrações de Testosterona e alfa-actina não se alteraram. Pode-se concluir que as alterações em parte dos marcadores das respostas agudas avaliados em jogos competitivos de futebol fornecem informações importantes para o planejamento de métodos de treinamento, recuperação ou estratégias nutricionais para o aperfeiçoamento do esporte.*

Palavras-chave: *Creatina quinase; Futebol; Marcadores biológicos; Testosterona.*

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INTRODUCTION

Soccer is a sport that is practiced worldwide, on every continent[1]. It is an intermittent activity of high intensity and long duration[2] in which movements requiring great strength and speed, such as jumps, block tackles and sprints, are performed during a match[3].

Hormonal and biochemical markers have been examined to investigate the physiological demand imposed on players participating in a soccer match[4-6]. When physical exercise creates significant stress, the testosterone/cortisol ratio changes[7,8]. A decreased testosterone level after the activity indicates a catabolic state. The increased production of cytokines is also a response to the magnitude of stress and is a metabolic response to exercise[6,9].

Activities with high intensity and high demand on the musculoskeletal system, such as soccer, result in high levels of muscle microtrauma[6,10]. Microtrauma can be evaluated by measuring the blood levels of muscle alpha-actin[11,12] and creatine kinase (CK)[13], which come from the sarcoplasm.

Monitoring acute response to exercise, especially in specific, real-life situations such as competitive soccer matches, provides important information for further understanding the demand imposed on the body. This information can be used to plan training and recovery methods as well as to implement nutritional strategies for improving the sport.

In view of the above-mentioned factors, the present study aimed to evaluate the magnitude of changes in different markers of the acute physiological demand resulting from soccer practice in healthy individuals.

METHODS

The present study was approved by the Ethics Research Committee (Comitê de Ética em Pesquisa - COEP) of the federal university where the study was conducted (ETIC-291/09) and complied with all of the standards established by the National Health Council (Conselho Nacional da Saúde, Res. 196/96) for research on human beings. Each volunteer gave their informed consent before participating in the study and had the opportunity to seek clarification of any questions or concerns they had after reading the informed consent document.

Sample

Ten healthy male physical education students at the university where the study was conducted participated in the study (Table 1). The volunteer group was determined by judgment sampling based on their participation on a soccer team, as individuals who participate in sports generally declare themselves to be healthy. Individuals who presented some sort of lesion or discomfort when blood was collected were excluded from the sample. Regardless of these aspects, the sample calculation is presented below.

Procedures

Initially, anamnesis was conducted, and questionnaires for risk stratification

(physical activity readiness [PAR-Q] and coronary risk factors) were administered. To measure the maximum O_2 consumption (VO_{2max}), the Bruce treadmill test was performed (Quinton® Med-Track ST65, Pennsylvania, USA) at an initial speed of 1.7 miles/hour with a 10% slope. Each stage lasted three minutes; at the end of each stage, the speed was increased by 0.8 miles/hour and the slope was increased by 2% relative to the previous stage. The subjects' VO_{2max} was measured using the open-circuit spirometry method (BIOPACSystem®, GasSys2, Goleta, California, USA), and the device was calibrated prior to each collection. This device records the oxygen consumption at each respiration.

The volunteers were members of the outdoor soccer team at their university when the study was conducted. They were evaluated in two matches, the college tournament semi-finals and finals of the federal university where they studied, and the matches occurred one week apart. Individuals who participated in at least 75% of one match (67.5 min) were selected. This tournament ran with a match schedule of four groups, and the top two teams of each group participated in the quarter finals, semi-finals and finals. All players had at least 36 hours of rest before the games in which they were evaluated.

The volunteers' heart rate (HR) was measured and recorded during the games with a set of heart rate monitors (Polar Electro® Oy, Team System, Kempele, Finland), and the data collected were analyzed using Polar Precision Performance SW 3.0 software. The device records the heart rate using telemetry without using a wrist monitor because wrist monitors are prohibited in official soccer matches because of the possible risks they present to the integrity of the athletes, their teammates, and their opponents. A 5-second sampling rate of the heart rate was used.

An 8-mL blood sample was collected from each volunteer pre- and post-match. The blood was collected at an appropriate location set up in the changing room of the club where the games occurred, right next to the field. The pre-match blood sample was collected immediately before the warm-up activities, when the athletes were already dressed for the match. The post-match blood samples were collected immediately after the match, including the time required for the players to go from the field to the changing room. Thus, the blood samples were collected approximately 10 to 20 minutes after the match, and the post-match collection followed the same procedure that was used in for prematch collection.

The samples were centrifuged, and the serum was fractioned and stored at -20 °C until the laboratory analyses were performed. The samples were collected by trained researchers via venipuncture of the antecubital vein using sterile disposable material.

The highest individual HR found during the matches or the treadmill test was used as the maximum heart rate (HR_{max}) to relativize the effort as $\%HR_{max}$ [14].

Physiological variables evaluated

Serum CK was analyzed using the MPR3 CK Nac-ativado kit (Boehringer Mannheim, Mannheim, Germany).

Plasma cortisol and testosterone levels were determined using the chemiluminescence enzyme immunoassay (ADVIA Centaur Siemens, Eschborn, Germany).

Interleukin 6 (IL-6) plasma levels were measured using the ELISA method with a high-sensitivity kit (Quantikine® HS, R&D Systems Minneapolis, MN, USA).

The alpha-actin was measured using the ELISA method, in which plasma samples were diluted in an adequate buffer (coating buffer; g/L composition: Na_2CO_3 1.59; $NaHCO_3$ 1.93; pH 9.6). A 100-µL volume of the diluted samples was used to sensitize 96-well plates for 12 hours at 4 °C. After this period, all of the liquid was removed from the plates, which were gently washed with washing buffer (NaCl 0.9% + Tween-20 0.05%). Then, nonspecific binding sites were blocked by adding 100 µL of 2% skim milk (Molico) diluted in PBS to each well for 1 hour at 37 °C. After this period, the plates were washed again with washing buffer, and anti-alpha-actin primary monoclonal antibody (Sigma, St. Louis, USA) diluted 1:1000 in PBS-T (PBS + Tween-20 0.05%) was added. After incubation for 1 hour at 37 °C, the primary monoclonal antibody was removed, the plate was washed again, and secondary polyclonal anti-mouse antibody diluted 1:3000 in PBS-t + 2% milk was added. The plate was washed two times, and the substratum of the reaction was added (OPD 0.2 mg/mL in citrate buffer 5.2 g/L, pH 5.0). After 20 minutes, the reaction was stopped with 20 µL of 4N H_2SO_4 and read in a microplate reader at 492 nm. There were negative (coating buffer only) and positive (10, 5, 1, and 0.5 µg alpha-actin) controls.

Statistical analysis

Pre- and post-match variables were compared using the Student's t test for paired samples after confirming the normality of the data with the Kolmogorov-Smirnov test. A 95% confidence interval, an 80% statistical power, and the highest coefficient of variation (CV) among the variables analyzed were considered. In this case, cortisol and N were determined in 10 subjects.

Data regarding sample characteristics are presented as the mean and standard deviation. The values for CK, cortisol, testosterone, testosterone/cortisol ratio, IL-6, and alpha-actin are shown as the mean and the standard error of the mean. The significance level adopted was $p < 0.05$.

RESULTS

The characteristics of the study participants are presented in the table below.

Table 1. Sample characteristics. The values are shown as the mean and standard deviation.

	Age (years)	Body mass (Kg)	Fat percentage (%G)	Height (cm)	HR (bpm)	VO_{2max} (mLO_2/Kg/min)
N (10)	22.0 ± 2.8	68.9 ± 7.5	14.1 ± 3.3	177.5 ± 2.4	173.5 ± 12.5	42.3 ± 4.0

The mean HR determined for this match was 173.50 ± 12.50 bpm or 87.33 ± 3.44 $\%FC_{max}$.

The values for testosterone (T), cortisol (C), and T/C ratio are shown in Table 2. The testosterone levels did not change, but the cortisol levels increased post-match. The T/C ratio was decreased post-match ($p < 0.05$).

Table 2. Testosterone, cortisol, and testosterone/cortisol ratio (T/C) pre- and post-match moments. The values are presented as the mean and standard deviation.

Match phase	Testosterone (NG/DL)	Cortisol (UG/DL)	T/C
Pre	581.2 ± 38.8	14.2 ± 1.3	40.9 ± 6.1
Post	620.5 ± 61.8	20.5 ± 2.0	30.2 ± 6.7
p value	0.36	0.01	0.02

Figure 1 shows that the CK plasma concentration was increased post-match.

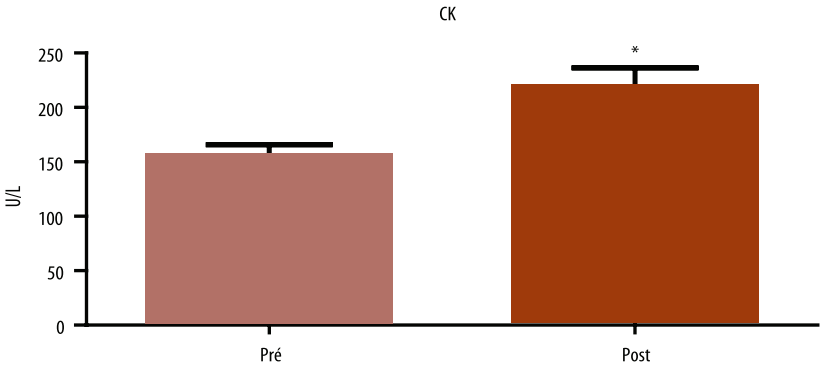

Figure 1. Pre- and post-match CK plasma concentration. *Different from the pre-match measurement ($p < 0.05$).

Figure 2 shows that IL-6 values were also increased post-match.

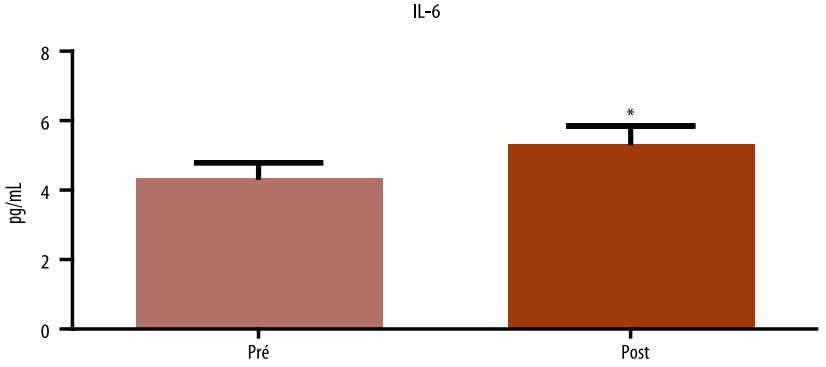

Figure 2. Pre- and post-match IL-6 plasma concentration. *Different from the pre-match measurement ($p < 0.05$).

Figure 3 shows that only alpha-actin values did not show a significant increase post-match.

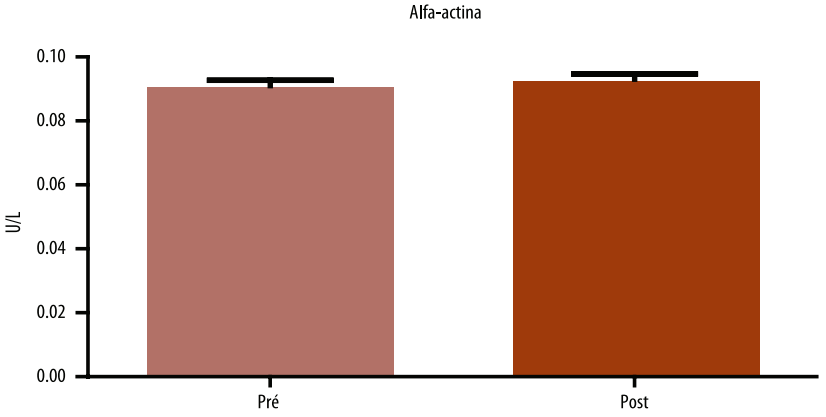

Figure 3. Pre- and post-match alpha-actin plasma concentration.

DISCUSSION

The main observation of the present study was that participation in a soccer match significantly changed some of the monitored physiological parameters, indicating a significant, acute physiological demand imposed on the main biological systems.

Like other hormones, testosterone is used to monitor the fitness status and the physiological demand of certain acute and chronic activities. Its decrease after sport events identifies a less anabolic state that must be evaluated in relation to other hormones, such as cortisol, which is a catabolic hormone[15,16].

Strenuous activities like soccer induce greater hormonal responses[17,18], especially because of sprints[19], which are inherent to this sport. Such activities require high testosterone production, most likely by increasing adrenalin and lactate concentrations, which influence gonadal activity[19].

Nevertheless, a significant change in post-match testosterone concentrations was not identified in the present study ($p = 0.36$). This fact corroborates the findings of Ispirlidis et al.[6], who did not find a significant increase in testosterone concentrations after a soccer match. In this case, even though the players evaluated by Ispirlidis et al.[6] were professional players and had most likely adapted to the physical stimulus of a soccer match, the effort intensity was similar to that found in the present study and caused similar stress to the volunteers in the present study.

Changes in cortisol concentrations can also be used to evaluate the stress caused by a sports season[8,16]. Cortisol is a catabolic hormone secreted by the adrenal cortex in response to physical and psychological stress. Exercise yields averages above 70% VO_{2max}; exercise with weights or maximum intensity and short duration[21] and high- and moderate-intensity/long duration activities[22] are considered stressing factors that can cause increased cortisol concentration[21,22]. The effects of these activities also occur after exercise, during the recovery period[20].

In the present study, post-match cortisol concentrations were increased, corroborating the results found by Ispirlidis et al.[6], Cunniffeet al.[23], and Cormack et al.[7]. Those studies also showed a significant increase in cortisol concentrations after a game of rugby, a sport with a movement pattern similar to that of soccer.

Changes in the post-exercise and rest anabolic-catabolic hormonal balance (testosterone/cortisol ratio) indicate an individual's anabolism/catabolism state as determined by exercise or during rest[7,18].

The acute hormonal status in response to training and the hormonal status throughout training is a factor should be identified for planning training loads and the recovery time between them. In the acute condition, T/C ratio decreases greater than 30% compared to the resting ratio indicate a catabolic profile that, if it persists at rest, indicates overtraining syndrome[15]. In the present study, the T/C ratio decreased to 10.7 ± 7.2; this represents a decrease of approximately 26% compared to rest, which

would still not indicate an acute catabolic state. For research purposes, data processing results in average values, and some players may present greater changes, resulting in a greater decrease in the T/C ratio. In addition, later hormonal changes were not checked for possible changes in the athletes' hormonal profile, and it is possible that a catabolic state may have been found later. These aspects can be considered as limiting factors of the present study.

Similar to the present study, Cormack et al.[7] identified higher post-match cortisol concentrations when assessing hormonal responses after a rugby match. Those authors also found a decreased T/C ratio, as did Uchida et al.[18], who also determined post-strength training catabolic states.

The study by Ispirlidis et al.[6] also shows a decreased T/C ratio after a soccer match, indicating an acute catabolic state in the athletes. Combined with muscular microtrauma indicators, such as the enzymes CK and lactate dehydrogenase (LDH), as well as muscle pain and decreased performance in jumps and sprints among soccer players (all of which were identified in the study), this finding indicates the high physical demand of a soccer match and the need for recovery periods greater than the ones that are currently used.

High-intensity activities and with high demand on the muscle skeletal system, such as soccer, result in high levels of muscle microtrauma and the release of inflammatory and muscle damage markers[6,10]. Microtrauma and damage can be determined by measuring IL-6[6,10], CK[13] and alpha-actin[11,12], all of which are present in the blood and result from muscle rupture.

In the present study, IL-6 showed a significant post-match increase, representing the metabolic response to the activity performed[9]. The production of IL-6 is related to intracellular calcium signaling. Long-duration activities are characterized by calcium availability support over time, which is a favorable scenario for IL-6 production.

Therefore, and not coincidentally, IL-6 and IL-6 mRNA production occurs mainly in the muscles, with a greater predominance of Type I fibers[24,25] in intense long-duration and intermittent activities, which are typical of soccer[26], and in situations with high demand and high intracellular calcium gradient, both of which trigger the production of IL-6.

Similarly to the present study, Ispirlidis et al.[6] identified significant changes in post-soccer match IL-6 concentrations. The authors also verified that 24 hours after the match, IL-6 levels had already returned to baseline values. Moreover, Pimenta et al.[10], who also identified significant changes in IL-6 concentrations in soccer players after a battery of plyometric jumps, verified that IL-6 values returned to baseline 2 hours after the activity. The same kinetic behavior of increased post-activity IL-6 was observed in female soccer players[4].

Nonetheless, muscle damage markers are used to analyze the damage caused by physical exercise, and they can be determined using direct and indirect measures. Direct measured include the analysis of muscle samples or magnetic resonance imaging.

Indirect methods for analyzing muscle damage are more commonly

used in studies because they are easy to obtain and are inexpensive compared with direct methods. CK, LDH, myosin heavy chain, troponin-I, and myoglobin are markers of muscle damage. This occurs because these are cytoplasmic molecules and are not able to cross the sarcoplasmic membrane barrier.

For this reason, the increased serum concentration of these molecules is used as an indicator of damage to the muscle membrane and other tissue structures. These markers have been reported as not being the most adequate in terms of sensitivity, reproducibility, and specificity, and muscle alpha-actin has been recommended as a specific, easily identifiable marker of this process[11,12]. This is because these authors identified higher concentrations of alpha-actin and CK in athletes when compared to sedentary individuals.

In the present study, no differences were found in post-match alpha-actin concentrations. CK, however, showed an increased post-match concentration. Changes in CK concentrations after training or soccer matches have been shown by several authors[5,6,10,27,28]. The present study disagrees with other studies, such as the one by Pimenta et al.[10], which found changes in alpha-actin concentrations in soccer players, but evaluated them after a sequence of standardized eccentric muscle actions to simulate the demands of the game.

The absolute post-match CK values found in the present study were lower than the average values normally found in soccer and futsal[28] players, which are between 350 and 400 U/L[5,6,27]. These values correspond to the normal daily physical demand of soccer players throughout a season, but they are considered high for nonathletes, as studied by Mougios[29].

The smaller absolute values found in the present study most likely result from the smaller strength and speed magnitudes generated by college athletes compared with professional players, even with intensity values represented as similar HR[6]. This difference was not detected in the present study, and it may be considered a limitation.

CK plasma concentrations have been used as an indicator of the stress imposed on skeletal muscles after activity[6,13] and as a factor for monitoring the training load[8]. Identifying the acutely and chronically imposed stress on the muscles contributes to planning for recovery periods and indicates the possibility of using new training loads to prevent overtraining and promote the maintenance of physical abilities throughout a competition[30]. Thus, CK was proven a good marker of muscle microtrauma resulting from a soccer match in college players. Alpha-actin, however, did not show the same behavior.

Although the players evaluated in the current study were physically active, participated in a competitive soccer match with real characteristics, and presented an average relative intensity similar to that of professional soccer games, it is possible that different results would be found if professional players had been evaluated. Higher absolute intensities could be found, and this might be reflected in higher absolute response magnitudes of the markers evaluated. Because of the difficulty of matching individuals who would not undergo the match as treatment with the sample, this study had no control group. Thus, even considering the difficulties of performing

this type of field study and ensuring external validity, the sample of college players and the lack of a control group can be considered limitations of the present study.

CONCLUSION

The physiological demand that a soccer match imposes on players significantly changed the concentrations of most of the biological markers evaluated in the present study, indicating that the physical demand of a soccer game can cause significant stress in human physiological systems and that it should be considered when planning training programs and post-match recovery efforts.

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