

Analysis of chronic physiological demand of an annual soccer season

Análise da demanda fisiológica crônica de uma temporada anual de futebol

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Abstract – Exercise intensity monitoring has been essential for the control and planning of sports training. Thus, the aim of the present study was to assess the chronic physiological demand of soccer players during an annual soccer season using blood biomarkers. Ten professional soccer players (21.2 ± 3.7 years) participated in this study. Blood samples were collected on the day before beginning of preseason (C1); at the end of preseason and beginning of competitive calendar (C2); and at the end of the competitive calendar. Interleukin-6 (IL-6), cortisol, testosterone, testosterone/cortisol ratio, creatine kinase and alpha-actin were evaluated. Statistical analysis was performed by using ANOVA with repeated measures and the post-hoc Tukey's test. Significance level was set at P<0.05. The results showed significant differences in the following situations: testosterone – C1 higher than C2 and C3; cortisol – C3 higher than C2; testosterone/cortisol ratio – C2 higher than C1, and C3 lower than C1 and C2; creatine kinase – C2 and C3 higher than C1; alpha-actin – C3 higher than C1. IL-6 concentrations were not different between C1, C2 and C3. It could be concluded that an annual soccer season imposes high physiological demand for professional players, since relevant changes in blood biomarkers analyzed were observed.

Key words: Blood biomarkers; Competitive season; Physiological demand; Preseason; Soccer.

Resumo – O monitoramento da intensidade de esforço entre atletas tem se mostrado essencial para o controle e planejamento do treinamento desportivo. Assim, o objetivo do presente estudo foi avaliar por meio de biomarcadores sanguíneos a demanda fisiológica crônica de jogadores de futebol ao longo de uma temporada anual. Dez jogadores profissionais (21,2 ± 3,7 anos) participaram desse estudo. As coletas de sangue foram realizadas no dia anterior ao início da pré-temporada (C1), ao final da pré-temporada e início das competições (C2), e ao final do ano competitivo (C3). Investigou-se as variáveis cortisol, testosterona, relação testosterona/cortisol, creatina quinase, alfa-actina e interleucina 6 (IL-6). Para a análise estatística dos dados utilizou-se ANOVA para medidas repetidas e foi adotado nível de significância de 5%. Os resultados encontrados indicaram diferenças significativas nas seguintes situações: testosterona - C1 maior do que C2 e C3; cortisol – C3 maior do que C2; relação testosterona/cortisol – C2 maior do que C1, e C3 menor do que C1 e C2; creatina quinase – C2 e C3 maior do que C1; alfa-actina – C3 maior do que C1. As concentrações de IL-6 em C1, C2 e C3 não demonstraram diferenças significativas. Pode-se concluir que uma temporada anual de futebol impõe elevada demanda fisiológica entre jogadores profissionais, uma vez que foram observadas alterações relevantes sobre os biomarcadores sanguíneos analisados.

Palavras-chave: Biomarcadores sanguíneos; Demanda fisiológica; Futebol; Pré-temporada; Temporada competitiva.

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INTRODUCTION

The performance of soccer players is directly influenced by the physical component, as aspects such as power, speed and strength are widely requested during soccer practice^{1,2}. Especially in the high-performance competitive environment, in which victory and defeat can be decided by the smallest details, monitoring the effort intensity has proven to be essential for the control and planning of sports training in order to maximize sports performance and avoid injuries, preventing overtraining³. In this sense, several variables have been used to analyze the physical condition of athletes⁴⁻⁷.

Based on the information obtained by means of biomarkers on the balance of anabolic and catabolic processes⁸, previous studies have been focused on the investigation of hormonal and hematological parameters^{9,10}. Parameters such as interleukin-6 (IL-6), cortisol, testosterone, testosterone / cortisol ratio, creatine kinase (CK) and muscle alpha-actin⁷ have been analyzed.

However, although data currently available in literature provide important knowledge on the physiological demands of soccer, analyses of physical stress markers have been conducted only in cross-sectional studies^{9,10}. Therefore, data from longitudinal studies are still quite limited, especially on the national scenario and in high-performance collective sports. In this sense, the evaluation of blood biomarkers during a soccer season can be extremely important for physical trainers, physiologists, physical therapists and coaches for contributing to the development of intervention strategies throughout the season.

Considering that the soccer calendar of upper-division teams contains a large number of games and short recovery periods¹¹, further investigations should take into account the cumulative effect of these physical efforts in the medium and long term. The aim of this study was to assess the chronic physiological demand of soccer players during an annual soccer season using blood biomarkers.

METHODOLOGICAL PROCEDURES

Subject

Ten professional soccer players linked to a second-division Brazilian Championship team were evaluated in this study. All subjects participated in at least 75% of matches throughout the season and did all proposed blood collections. Athletes who suffered serious muscle injuries during the season and those in care of lesions at the time of any collection were excluded from the study, featuring non-probabilistic sampling.

After receiving information regarding the risks and benefits involved in the study, the subjects signed the Informed Consent Form. This study was approved by the Research Ethics Committee (COEP) of the Federal University of Minas Gerais (ETIC-291/09) and complied with all the rules established by the National Health Council (Res. 466/12) involving research with human beings.

Table 1 shows the characteristics of the study sample.

Table 1. General characteristics of the sample

Age (years)	Height (cm)	Body mass (Kg)	BF (%)	SD (m)
21.2 ± 3.7	177.0 ± 5.0	77.6 ± 5.0	9.9 ± 1.8	2182.6 ± 184.3

BF - Body fat, Distance traveled in the Yo-Yo intermittent recovery test level 1 (YYIR1). Values are presented as mean ± standard deviation.

EXPERIMENTAL DESIGN

For the analysis of hormonal and hematological parameters, three blood collections were performed throughout the season. The first collection (C1) occurred on the day before the start of preseason. The second collection (C2) occurred at the end of preseason and start of competitions, more precisely on the day before the official opening match. The third collection (C3) was performed at the end of the competitive calendar, a day before the last official match.

This study evaluated moments representing the routine of a professional soccer club that participates in state and national competitions organized by the Brazilian Soccer Confederation (CBF). This routine is characterized by a season that includes between 11 and 11.5 months of work characterized by technical, tactics and physical preparation to compete in competitions of that year.

The preparation season is planned in order to dispute competitions in the best shape of the described capabilities. All team works are described within the weekly microcycle (physical, technical, tactical, or mixed training, travels, competitive and friendly matches, participation in competitions, days off, exams, nutrition). Programming items are followed by the entire group of athletes and professionals involved. Players train on average 6 days a week, 6 training sessions lasting about 90 minutes each.

Along the competitive season (11 months), 50 official matches were recorded. Training was monitored during this period and the parameters observed are shown in Table 2. Heart rate monitors (Polar Electro Oy) were used to measure and record heart rate and the sampling rate was 5 s. To measure the distance travelled, global positioning system (GPS) with portable devices was used (Garmin Forerunner 405).

Evaluation of physiological variables

Standardized conditions were maintained during blood collections. All players remained housed in the training center's facilities with standard hours to sleep, start training and make meals. Meals were prepared by the club's nutrition department and followed individualized dietary recommendations according to the demand of each athlete.

Table 2. Training monitoring parameters throughout the competitive season

Month	DR(hrs)	HR(bpm)	%HRmax	TP (C°)	RH (%)	DT(km)
JANUARY	54.1	172±7.9	86.0±6.1	37.2±1.9	68.5±13.1	41±4.5
FEBRUARY	35.9	175±8.6	87.5±4.0	35.1±1.1	70.5±13.0	32±3.4
MARCH	32.9	176±8.3	88.0±5.6	32.4±1.3	68±12.6	26±5.2
APRIL	28.1	169±9.0	84.5±7.0	31.6±2.3	72±12.9	22±3.1
MAY	35.6	175±7.8	87.5±5.3	29.5±1.8	71±13.6	29±3.9
JUNE	26.1	162±12.5	81.0±5.4	26.8±1.9	76±14.1	19±2.9
JULY	35.5	164±9.8	82.0±6.3	25.8±2.2	73±15.0	26±2.6
AUGUST	29.8	178±13.1	89.0±3.2	27.6±1.1	65±14.6	15±4.1
SEPTEMBER	37.2	170±12.4	85.0±5.7	32.8±1.3	69±14.7	24±6.5
OCTOBER	32.4	167±11.0	83.5±4.4	36.8±1.8	74±15.0	18±3.4
NOVEMBER	42.4	158±13.2	79.0±4.8	38.4±1.4	71±14.2	25±2.2
Mean±SD	35.4	169.6±10.3	84.8±5.2	30.8±1.6	70.7±13.8	25.1±3.5
Total	390.2					277.6±27.6

DR - duration; HR - heart rate; %HRmax - percentage of maximum heart rate; TP - room temperature; RH - relative humidity; DT - distance traveled.

Blood samples were always collected in the morning, with athletes fasting and for at least 36 hours without exercise. Samples were collected by nurse experienced in blood collections in adequate room (medical department), immediately before daily training activities and the maximum period between the collection of the first and last athlete was 30 minutes.

For each blood collection, a tube was filled with a clot activator of 8 mL of blood for analysis of monitored variables. Immediately after collection, tubes were centrifuged at 1500 g for 10 min at 4°C and aliquots (0.5 ml) of serum were stored at -80°C.

Plasma cortisol and testosterone concentrations were measured by chemiluminescence enzyme immunoassay principle (ADVIA Centaur Siemens, Eschborn, Germany). The testosterone / cortisol ratio (T/C) was also determined.

Analysis of serum CK concentration was performed using the “MPR3 CK NAC-activated” kit (Boehringer Mannheim, Mannheim, Germany).

Alpha-actin was assessed by enzyme-linked immunosorbent assay method (ELISA) using monoclonal anti-alpha-actin primary antibody (Sigma, St. Louis, USA) and polyclonal anti-mouse secondary antibody⁷.

The analyses of serum IL-6 concentrations were carried out by ELISA method using the high sensitivity kit (Quantikine® HS, R & D Systems Minneapolis, MN, USA).

The maximal aerobic capacity was assessed with the application of the Yo-Yo intermittent recovery test level 1 (YYIR1). In this test, which was specifically developed for intermittent modalities as in the case of soccer, the distance traveled is directly related to the player's aerobic capacity¹².

Statistical Analysis

Data were expressed as mean \pm standard deviation. After investigating data normality by the Shapiro-Wilk test, analysis of variance (ANOVA) was adopted for repeated measures in order to compare the concentrations of blood biomarkers in C1, C2 and C3. In case of significant difference, post-hoc Tukey test was used. SPSS software was used and the significance level was 5%.

RESULTS

Figure 1 shows that testosterone concentrations were lower in C2 (542.4 ± 50.3 NG / dl, $p = 0.015$) and C3 (518.3 ± 47.7 NG / dl, $p = 0.009$) compared to C1 (647.6 ± 61.9 NG / dL). For cortisol, the values observed show significant differences between C3 (18.7 ± 1.4 UG / dl) and C2 (11.5 ± 1.0 UG / dl, $p = 0.002$). There was no difference between C1 (17.9 ± 1.0 UG / dL) and other measures. Furthermore, analysis of the T / C ratio throughout the annual season in relation to C1 (37.8 ± 2.7) showed increase at the end of preseason in C2 (53.5 ± 5.1 , $p = 0.003$) and decrease at the end of the competition calendar in C3 (28.6 ± 2.4 , $p = 0.012$).

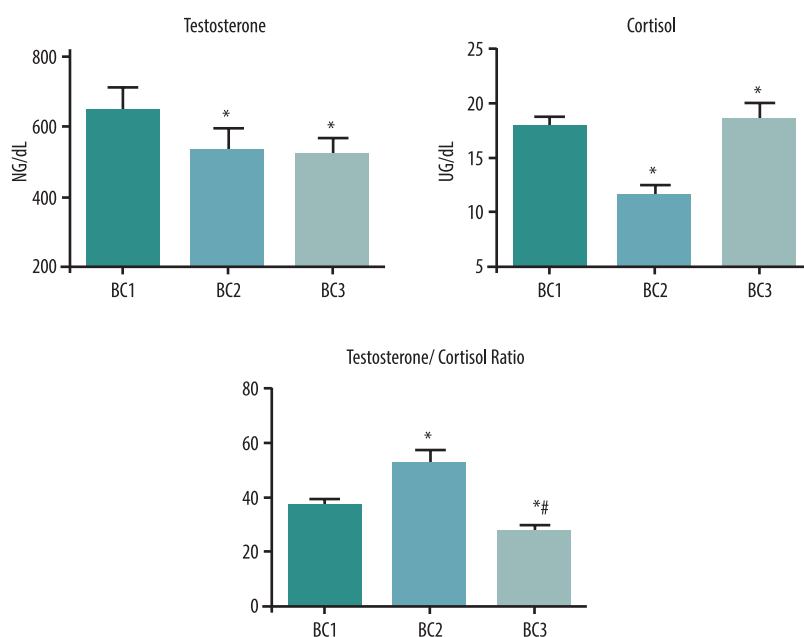


Figure 1 - Testosterone, cortisol and testosterone / cortisol (T / C) values throughout the annual season. * Difference in relation to blood collection 1 (BC 1) ($p < 0.05$). # Difference in relation to blood collection 2 (BC 2) ($p < 0.05$). Values expressed as mean \pm standard deviation.

Figure 2 shows significantly higher CK values in C2 (298.1 ± 48.7 U / L, $p = 0.024$) and C3 (347.8 ± 43.3 U / L, $p = 0.021$) compared to C1 (102.3 ± 8.7 U / L). Alpha-actin values showed significant difference only between C3 (0.107 ± 0.005 U / L) and C1 (0.084 ± 0.005 U / L, $p = 0.017$). IL-6 concentrations were not significantly different among samples (C1 = 2.6 ± 0.3 , C2 = 3.5 ± 0.7 and C3 = 3.4 ± 0.4 pg / ml).

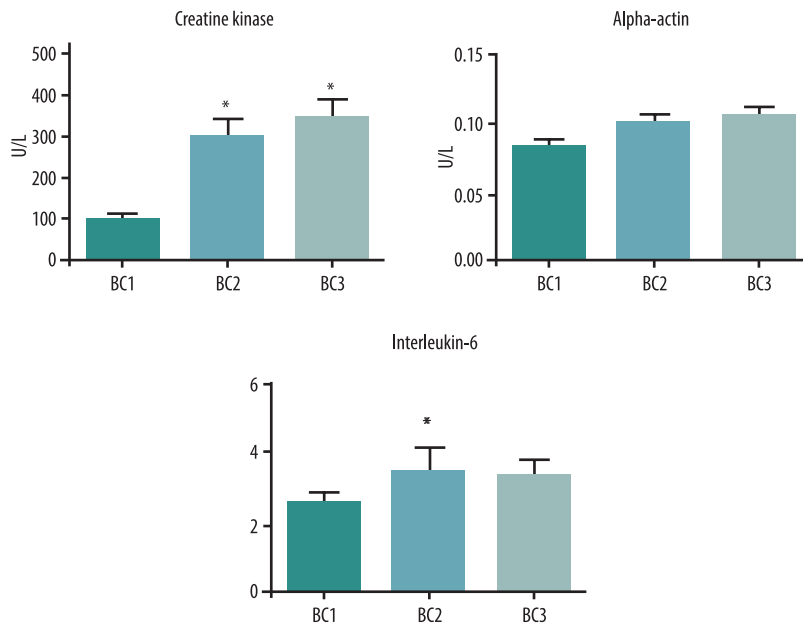


Figure 2 - Creatine Kinase (CK) and muscle alpha-actin values along the annual season. *Significant difference in relation to blood collection 1 (BC 1) ($p < 0.05$). Values are presented as mean \pm standard deviation.

DISCUSSION

The results show that an annual soccer season causes considerable chronic changes in players. This fact is represented by changes in testosterone and cortisol concentrations, reduction of more than 30% in the T / C ratio and increases in creatine kinase and alpha-actin levels, identified in this study. Collection of blood samples did not require intervening in the team planning (microcycle, mesocycle and macrocycle), and due to this fact, these findings represent actual steps of the preparatory and competitive period of a Brazilian soccer season. In addition, it is noteworthy that the volunteers were professional players and participants of the second-division National Soccer Championship.

The reduced plasma testosterone concentrations, as occurred in C2 and C3 indicate a less anabolic state^{4,13} compared to C1. In addition, the cortisol concentration, which tends to increase in response to physical and psychological stress and is characterized as a catabolic hormone, showed a significant increase at the end of the competitive period. Considering the important role of these hormones in the anabolic / catabolic balance¹⁴, reduction equal to or above 30% in the T / C ratio has also been identified as an indication of incomplete recovery, residual fatigue and impairment of competitive performance^{6,13,15}. Accordingly, reduction of 53% in the T / C ratio between C2 and C3 observed at the end of the competitive period in this study points to a possible reduction on physical performance among players evaluated.

Similar findings of reduced T / C ratio in chronic monitoring of soccer players were identified by Filaire et al.¹³. However, the authors associated hormonal markers to the application of the profile of mood states questionnaire

(POM'S), indicating that the hormone profile was also influenced by the results of matches in the championship. In this study, the results of competitions have not been presented. The reduced T / C ratio indicated in the present study may be related to the high volume of training sessions and matches, which traditionally make up the Brazilian professional soccer scenario ¹¹.

The difference in CK concentration observed between C2 and C1 corroborates the results by Coelho et al.¹⁶, who observed higher CK concentration throughout a preseason (4th, 10th and 15th day) compared to the end of the holiday period. In addition, data obtained in this study indicate increased CK concentrations at the end of the competitive period, probably due to the successive and accumulated efforts over the annual season.

In fact, the high CK values found in C3 (347.8 ± 43.3 U / L) are similar to those presented by Lazarim et al.¹¹, who also assessed the CK behavior among professional players during the Brazilian National Championship. Although these authors have also recorded CK concentrations higher than 1000 U / L during the competitive season, the most frequent CK concentration was 300 U / L, which is an average value expected for soccer athletes in activity ¹¹. These data support the idea that soccer can be classified as a strenuous exercise, with high requirement of different muscle actions and capable of causing damage to the structure of the muscle cell at sarcolemma level and to Z-discs. Under these conditions, the membrane permeability is changed and CK is released into the extracellular medium ¹⁶.

It is known that IL-6 plasma concentration is changed in response to physical exercise and returns to baseline levels within hours after physical activity, thus being considered a marker of the post-exercise acute inflammatory process ⁹. In addition, its main function during exercise is to provide energy for physical activity ¹⁷. In the present study, no significant differences were observed for the three moments analyzed. Given that IL-6 production is directly linked to muscle glycogen concentrations for the same relative effort, trained individuals have lower IL-6 production in muscles ^{17,18}. Thus, over the course of the soccer season and with better physical conditioning of athletes, the IL-6 concentrations were expected to reduce, which did not occur.

The alpha-actin concentration was higher in C3 compared to C1 (Figure 2). Other studies have evaluated the alpha-actin concentration in soccer players as marker of muscle microtrauma ^{7,19}; however, in the acute form, considering that this is an innovative analysis technology in sports. Coelho et al. ⁷ found no differences in the alpha-actin concentrations before and after a soccer match, unlike Pimenta et al.¹⁹. However, the first study evaluated college players and the second evaluated professional players, which probably interfered with the absolute intensity of the match ²⁰. The study of Pimenta et al.¹⁹ corroborates the findings of this study, which also found changes in the alpha-actin concentrations of players, however, in the chronic form.

Since alpha-actin is considered as an interesting marker for the identification of all types of myofibrillar injuries, including those related to sports situations ²¹, the values found indicate high muscle damage at the end of

the season. This, again, suggests that the rest time proposed to players is not enough to offset the volume and intensity of training during the season.

The small sample size can be considered a limitation of the present study. Twenty-five athletes agreed to participate as volunteers. However, over the training and especially the season matches, part of these players showed participation less than that required by the inclusion criteria ($\geq 75\%$), were injured seriously or were in treatment during data collection periods.

Thus, the criteria adopted were mainly aimed to ensure that the final results would represent the actual physiological demand imposed to players involved in an annual season. It is noteworthy that the intensity of actions developed by high-performance athletes is higher than among athletes from other levels ²⁰ and that therefore the physiological demand also differs. Therefore, the findings of this study may be useful for athletes and technical committees that may use information like these to develop intervention strategies throughout a soccer season.

CONCLUSION

Based on the results found, it could be concluded that an annual soccer season imposes high physiological demand for professional players, as relevant changes on blood biomarkers analyzed were observed. In a practical perspective, these data can be useful for the control and planning of high-performance sports training.

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