

*Original article (short paper)*

## Comparison of different ways of expressing creatine kinase concentration of soccer players during a competitive season

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**Abstract**—The aim of this study was to analyze the responses of creatine kinase [CK] expressed in different forms to the training load of professional soccer players during a competitive season. Twelve players (age,  $24 \pm 4$  years) participated in the study. [CK] was analyzed before the pre-season (Pre), after the pre-season (Post), and in the competitive mesocycles (M1, M2, and M3). Results showed [CK] in the Pre, Post, M1, M2, and M3 phases in absolute values ( $181.3 \pm 58.7$ ,  $416.4 \pm 155.7$ ,  $526.4 \pm 268.0$ ,  $403.8 \pm 137.0$ , and  $442.5 \pm 212.3$  U/L, respectively), relative values ( $16.3 \pm 4.6$ ,  $39.5 \pm 19.1$ ,  $47.8 \pm 20.1$ ,  $37.5 \pm 14.2$ ,  $40.1 \pm 17.4$  %CK<sub>max</sub>, respectively), and values relative to the variation delta ( $19.9 \pm 3.6$ ,  $48.1 \pm 26.5$ ,  $57.0 \pm 23.2$ ,  $45.1 \pm 17.7$ , and  $48.3 \pm 22.0$  % $\Delta$ CK<sub>max</sub>, respectively). [CK] was lower only during the Pre phase compared to the other phases ( $p < 0.05$ ). [CK] was expressed as %CK<sub>max</sub> or % $\Delta$ CK<sub>max</sub> may be more specific to monitor training. In addition, the individualization of this biomarker optimizes the athletes' performance as muscle injuries can be prevented.

Keywords: physical exertion, training, performance

### Introduction

Competitive soccer requires great physical effort, including jumps, ball disputes, sprints, braking, accelerations, and changes of direction<sup>1,2</sup>. Moreover, a competitive season is marked by a large number of high-intensity games<sup>3</sup> and with more than one competition occurring simultaneously; therefore, the time available for athletes to recover is not always sufficient<sup>4</sup>.

Periods of intense and lengthy workouts can trigger a process called overtraining that decreases sportive performance<sup>5</sup> and leads to the athlete's temporary (burn-out) or permanent (drop-out) retirement from the sport<sup>6</sup>. Thus, monitoring the training status of athletes to identify whether they have already recovered before being subjected to a new stimulus is of fundamental importance for gaining adequate control of the training load<sup>7,8</sup>.

Taking this into consideration, several physiological indicators of physical stress have been investigated, namely creatine kinase (CK)<sup>4,8-13</sup>. Specifically, CK is an energy metabolism-related cytoplasmic enzyme that, when released into the extracellular medium, is indicative of muscle microtrauma<sup>3,4,12,14,15</sup>. An elevation of serum CK level [CK<sub>serum</sub>] is usually observed one to four days after physical activity<sup>16-19</sup> and is an indicator of the stress imposed on the skeletal muscle following exercise<sup>13,20,21</sup>. Recently, CK has also been used as a monitoring marker of training load<sup>22-24</sup>, especially in soccer<sup>5,25</sup>, since its measurement can be performed quickly and conveniently.

However, the CK response to exercise shows great individual variability<sup>9,26,27</sup> because it is influenced by personal factors, such as gender, race, muscle mass, training status<sup>23</sup>, and genetic predisposition<sup>28</sup>. Therefore, the same training stimulus can cause different individuals to be classified as having a high or low response to increased [CK<sub>serum</sub>]. However, little is known about the response of CK in the reality of Brazilian soccer<sup>3,4,13</sup>, a collective sport with an intermittent, high-intensity pattern and short periods of preparation and recovery in which more than one competition may occur simultaneously.

Therefore, the aim of this study was to analyze the responses of [CK<sub>serum</sub>] expressed in different forms to the training load of professional soccer players during a competitive season.

## Methods

This study was approved by the ethics in research committee of the Federal University of Minas Gerais (ETIC-291/09) and complied with all the norms set by the National Health Council (Res. 196/96) involving human research. Written informed consent was obtained from the volunteers prior to their participation in the study after they were given the opportunity to raise any questions or concerns they may have had.

### Participants

Professional players from a club in the first division of the Brazilian soccer that participates in the national and international competitions organized by the Brazilian Football Confederation and the South American Football Confederation comprised the sample for this study.

All athletes were monitored during the months of the state and South American championships, which occurred concurrently between January and April 2010, for 18 games in total. Ultimately, twelve players participated in this study (age, 24 ± 4 years; height, 181.1 ± 7.0 cm; weight, 77.6 ± 3.8 kg; body fat, 9.1 ± 1.3%; and VO<sub>2max</sub>, 66.0 ± 4.5 mL·kg<sup>-1</sup>·min<sup>-1</sup>). The inclusion criteria of the athletes were as follows: participation in all the CK collections described below and participation in > 60% of all training activities. The athletes who did not fulfill the above criteria, or who suffered any injury during the study, were not included. A total of 21 athletes were eligible when the study began.

### Procedures

The climatic conditions monitored during the training phase in this study were: 24.65 ± 3°C and 61 ± 7% relative humidity.

[CK<sub>serum</sub>] was determined during presentation of the players prior to their first training session; this was designated as the concentration at the beginning of the pre-season (Pre). At the time of this sample collection, the athletes were returning from vacation and thus, had not performed any previous systematic physical activity.

The HR<sub>max</sub> was determined by the highest value found in one of two situations: 1) specific field test (Yoyo Endurance Test Level II) to determine the players' maximal aerobic capacities, and 2) training sessions.<sup>29</sup> During the pre-season, CK samples were collected on the 4<sup>th</sup>, 10<sup>th</sup>, and 15<sup>th</sup> day. The average of these values corresponded to the time after pre-season (Post). The blood collections in pre-season were conducted at least 14 hours following the last training session.

The competitive period was divided into three mesocycles, each representing a total of approximately 30 days of training. The average of the CK measurements after each game within a mesocycle was then defined as the representative value for that specific mesocycle; the first, second, and third mesocycles were designated M1, M2, and M3, respectively. During this period, CK samples were collected 32–36 hours after the games from those players who participated in at least 75 minutes of that game. Blood samples were analyzed for CK immediately after collection.

During the competitive phase, the levels were monitored after the games since higher concentrations of CK were expected at these times as a result of post-game stress. Moreover, this was the average period of the presentation of the players after the games. Since the samples were collected 2–3 times per mesocycle, which was the number of games used as inclusion criteria in the study, the mean values of these measurements represented the mesocycle monitoring. All of the samples were collected in the morning prior to the day's training session.

Players who did not play in a sufficient number of games were excluded from the study. As the average number of athletes in a team throughout a season is 30, it was not possible to have a control group of players that only trained and did not play. CK monitoring was performed in three ways: 1) absolute values, 2) values relative to the maximum concentration of CK observed throughout the season in the athlete (%CK<sub>max</sub>), and 3) values relative to the maximum variation delta in CK concentration observed during the season (%ΔCK<sub>max</sub>). To calculate this last form of expression, the resting CK (Pre) value was subtracted from the exercise CK value, and this was then divided by the maximum delta of variation [(CK<sub>monitored</sub> - CK<sub>pre</sub>)/ΔCK<sub>max</sub>] × 100.

After the site was cleaned with 70% ethanol, a puncture was performed using an automatic lancet and the blood was drained into a heparinized capillary tube (Cat No. 955053202 Reflotron®; Boehringer Mannheim, USA). The blood (32 μL) was immediately pipetted onto a CK test strip (Cat No. 1126695 Reflotron®) and placed in the Reflotron Analyser® (Boehringer Mannheim), as performed in previous studies<sup>3,30</sup>. The monitoring of the training load during the microcycles was performed via heart rate (HR) measurement represented in absolute values, the percentage of maximum heart rate (%HR<sub>max</sub>), and the measurement of the

distance covered in the training sessions. To measure and record HR, a set of heart monitors was used (Polar Electro Oy®; Polar Team System, Finland). To assess the distance covered, a portable GPS device (Forerunner® 405; Garmin, USA) was used; the sum of the monitored values is presented.

*Statistical analysis*

Initially, the Kolmogorov-Smirnov test was applied to verify the normality of the data. Since all data showed normality (absolute CK values,  $p = .60$ ; %CK<sub>max</sub>,  $p = .204$ ; %ΔCK<sub>max</sub>,  $p = .093$ ), parametric statistics were used. To compare the different phases, an analysis of variance with one-factor repeated-measures (one-way ANOVA with repeated measures) and the post-hoc Bonferroni test were used when necessary. Values are showed as mean and standard deviation. Furthermore, the statistic power ( $\omega$ ) of analysis of variance and Partial Eta Squared ( $\eta^2_p$ ) to demonstrate the magnitude of the differences between each measure (small < .01, mean = .06, extensive > .14)<sup>31</sup> were calculated. The significance level was set at  $\alpha < .05$ . The Statistical Package for the Social Sciences for Windows 14.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data.

**Results**

Table 1 shows the characteristics of the monitored period.

The one-way ANOVA with repeated measures for absolute values CK [U/L] ( $F_{4,44} = 8,93$ ;  $p < 0,001$ ;  $\eta^2_p = 0,45$ ;  $\omega = 0,99$ ) showed significant differences between Pre and other measures (Post,  $p = 0.001$ ; M1,  $p = 0.012$ ; M2;  $p = 0.004$ ; M3,  $p = 0,015$ ) (Table 2). No significant differences were found in the remaining comparisons ( $p > .05$ ).

Similarly, the one-way ANOVA with repeated measures for percentages of CK<sub>max</sub> [%CK<sub>max</sub>] ( $F_{4,44} = 8,37$ ;  $p < .001$ ;  $\eta^2_p = .43$ ;  $\omega = .98$ ) showed only differences between Pre and other measures (Post,  $p = .005$ ; M1,  $p = .004$ ; M2;  $p = .004$ ; M3,  $p = .006$ ) (Table 3). No significant differences were found in the remaining comparisons ( $p > .05$ ).

For percentages of delta CK variation [%ΔCK<sub>max</sub>] analysis, the one-way ANOVA with repeated measures ( $F_{4,44} = 7,88$ ;  $p < .001$ ;  $\eta^2_p = .41$ ;  $\omega = .99$ ) indicated only differences between Pre and other measures (Post,  $p = .009$ ; M1,  $p = .003$ ; M2;  $p = .004$ ; M3,  $p = .006$ ) (Table 4). No significant differences were found in the remaining comparisons ( $p > .05$ ).

Table 1. Description of the elapsed time of each type of training, and the distance and intensity (%HR<sub>max</sub>) for each phase of training (training meso-cycle). Absolute and relative values (%) for each phase are shown.

Microcycles	Post		M1		M2		M3	
	Absolut	%	Absolut	%	Absolut	%	Absolut	%
Tr.G. Des. (min)	300	19.92	230	9.64	110	5.41	180	6.5
Tr. G. Rec. (min)	290	19.26	400	16.77	390	19.16	535	19.31
Tr. E. Sim. (min)	450	29.88	520	21.8	405	19.9	630	22.74
Tr. E.Compl. (min)	195	12.95	290	12.16	330	16.22	355	12.82
Tr. E.Compe. (min)	270	17.93	945	39.62	800	39.31	1070	38.63
Σ micr. (min)	1506	100	2385	100	2035	100	2770	100
Dis. Per. (km)	82.8	-	146.7	-	126.2	-	198.8	-
HR (%HR <sub>max</sub> )	76.1±5.2	-	71.9±4.4	-	70.7±3.5	-	71.9±6.4	-

Classification of exercises according to Frisseli and Montovani<sup>27</sup>. Development general training (Tr.G. Dis.); Recovery general training (Tr. G. Rec); Simple Specific Training (Tr. E. Sim.); Complex specific training (Tr. E. Compl); Competitive specific training (Tr. E. Compe); Sum of the time in each micro-cycle (Σ micr.); Time after the pre-season (Post); Mesocycle 1 (M1); Mesocycle 2 (M2); Mesocycle 3 (M3), minutes (Min); Type of training (Tr.); Heart rate (HR); and percentage of maximum heart rate (%HR<sub>max</sub>).

Table 2. Creatine kinase (CK) values of players during a competitive soccer season, expressed in absolute terms.

Phases/volun	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8	V-9	V-10	V-11	V-12	X	SD	CV
<b>Pre</b>	110	207	180	199	186	265	212	102	158	137	292	128	181,3	58,7	32,4
<b>Post</b>	467	651	371	568	249	336	339	408	221	697	435	256	416,4*	155,7	37,4
<b>M1</b>	861	273	206	700	764	725	955	395	390	205	565	280	526,4*	268	50,9
<b>M2</b>	507	351	274	744	439	361	467	295	330	289	289	502	403,8*	137	33,9
<b>M3</b>	779	476	270	706	362	394	836	325	229	318	370	246	442,5*	212,3	48

Values are presented in an absolute values U/L (12 players), mean (X), standard deviation (SD), and coefficient of variation (CV). \*Difference compared to the Pre phase ( $p < 0.05$ ). Values at rest (Pre), after the pre-season (Post), and at the end of mesocycles 1 (M1), 2 (M2), and 3 (M3).

Table 3. Creatine kinase (CK) expressed in values relative (%) to the maximum concentration of CK-U/L during a competitive soccer season

Phase/volun	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8	V-9	V-10	V-11	V-12	X	SD	CV
Pre	8,5	27	14,4	14	13,3	17,4	18,3	13,4	14,2	20,9	17,3	18	16,3	4,6	28,3
Post	35,9	85	29,7	40	17,8	22,1	29,2	53,6	19,9	49,8	55	36	39,5*	19,1	48,3
M1	66,2	35,6	16,4	78,9	54,6	47,7	82,3	51,8	35,2	40,4	25,9	39,3	47,8*	20,1	42,1
M2	39	45,8	21,9	52,4	31,4	23,7	40,2	38,7	29,7	20,6	36,5	70,5	37,5*	14,2	37,7
M3	59,9	62,1	21,6	49,7	25,8	25,9	72,1	42,8	20,6	26,4	40,2	34,6	40,1*	17,4	43,4

Values are presented (12 players) in relation to the percentage of the maximum concentration of CK (%CK<sub>max</sub>), mean (X), standard deviation (SD) and coefficient of variation (CV). \*Difference compared to the Pre phase ( $p < 0.05$ ). Values at rest (Pre), after the pre-season (Post), and at the end of mesocycles 1 (M1), 2 (M2), and 3 (M3).

Table 4. Creatine kinase (CK) expressed in values relative to the delta maximum variation in the concentration of CK (% $\Delta$ CK<sub>max</sub>) throughout a competitive soccer season

Phase/volun	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8	V-9	V-10	V-11	V-12	X	SD	CV
Pre	9,2	37	16,8	16,3	15,3	21,1	22,4	15,5	16,6	26,4	20,9	21,9	19,9	3,6	55,5
Post	39,2	117	34,6	46,5	20,5	26,8	35,7	61,9	23,2	62,9	66,6	43,9	48,1*	26,5	54,9
M1	72,4	48,8	19,2	91,7	62,9	57,7	101	59,9	41	51	31,3	47,9	57,0*	23,2	40,7
M2	42,6	62,7	25,6	60,9	36,2	28,7	49,2	44,7	34,6	26,1	44,2	86	45,1*	17,7	39,3
M3	65,4	85,1	25,2	57,8	29,8	31,4	88,2	49,4	24,1	33,4	48,6	42,2	48,3*	22	45,5

Values are presented (12 players) in relation to the percentage of the delta variation of the CK (% $\Delta$ CK<sub>max</sub>), mean (X), standard deviation (SD) and coefficient of variation (CV). \* Difference compared to the Pre phase ( $p < 0.05$ ). Values at rest (Pre), after the pre-season (Post) at the end of mesocycle 1 (M1), at the end of mesocycle 2 (M2) and at the end of mesocycle 3 (M3).

## Discussion

The aim of this study was to analyze the responses of [CK<sub>serum</sub>] expressed in different forms during a competitive soccer season. The main contributions of this study were presentation of the values of each athlete individually and of two more expressions [CK<sub>serum</sub>] in addition to the absolute values. As observed (Tables 2–4), CK concentrations were higher in the Post, M1, M2, and M3 periods than in the Pre period, regardless of expression method. These results correspond to a response to a demand imposed by training loads and the competitive period, as expected. As the competitions began, there was a decrease in the general training load and an increase in the specific competitive training load. Despite this, there were no significant differences in serum CK concentrations. To explain this finding, we may speculate about a possible muscular adaptation to training.

Zoppi<sup>13</sup> used CK as a parameter for chronic monitoring of 21 sub-20 soccer players over five months of the Brazilian championship. CK concentrations were determined every month. The first determination was performed at the end of the pre-season and before the competitions and terminated when the team was disqualified from the championship. The authors found values of 350–400 U/L throughout the season, similar to those found in the present study; however, pre-competition values or the individual relativized values were not observed in this study. Moreover, the training load was also not shown. It should be emphasized that the players evaluated in the abovementioned study only participated in one competition during the study period, and not in two simultaneous competitions like the players in the present study.

Lazarim<sup>4</sup> evaluated 128 professional players at different times during the Brazilian soccer championship and found mean resting

values of  $493 \pm 315$  U/L. Although these values showed high variability, they were similar to our results and are values expected for active individuals, athletes, and particularly soccer players. In this same study, the authors performed a monthly evaluation of 29 players during the national championship, which lasted for five months. In this analysis, the values obtained were similar to those in the present study, but they varied between the evaluated months. Additionally, in the second month of the championship, high CK values were observed, which then normalized in the following months. Additionally, the authors did not report data relating to the training load throughout the championship.

To monitor the training status of rugby athletes and identify a possible state of overtraining, Coutts<sup>25</sup> monitored a team of 18 players over a six-week preparation period. The team was divided into two groups; the second group performed the same training as the first group, but with greater volume and intensity. The authors found that the testosterone/cortisol ratio decreased over time (for both groups) and increased in the post-training period for the second group. The VO<sub>2max</sub> of athletes in the lower intensity group was higher in the post-training period, which indicates that the group that trained at a higher intensity had adaptation difficulties. CK concentration values increased steadily over the weeks and were higher in the higher intensity group, reaching values of  $1,402 \pm 1,107$  U/L in the sixth week, which were almost double the values of the normal training group. The values obtained in this study were higher than those obtained in the present study, which may be attributed to the fact that different sports were analyzed. The applicability of CK as a biochemical monitoring marker for training status was confirmed in that study; CK concentration responded to increase in volume and intensity of physical activity, as in the present study.



Nunes<sup>25</sup> analyzed enzymatic, psychometric, and hematological parameters during a microcycle within a pre-season of professional soccer to determine which variable is more sensitive to training loads. Results showed that there was a significant increase in the CK and LDH enzymatic variables, as well as a reduction in hematological variables, erythrocytes, hemoglobin, and hematocrit in response to the evaluated training load. According to the authors, CK was the variable that best responded to the training load, whereas the other studied variables could be used as a complement while monitoring the acute effects of soccer-training load.

Overall, CK monitoring shows great individual variability<sup>27</sup>, even values for individuals who perform similar training and the values of a group of athletes rarely show a normal distribution<sup>27</sup>, which may compromise the interpretation of the mean data of a team, especially in sports like soccer. Thus, as in the case of other physiological variables used for monitoring physical activity, the presentation of individualized values that are relative to the maximum values CK data is of interest for establishing parameters for interpretation and intervention in the training period. Moreover, in a secondary analysis, we found that athletes with higher basal CK levels also presented with a higher CK<sub>max</sub> ( $r=0.60$ ,  $p<0.05$  – unpublished data). Thus, the use of the relative values of CK, instead of absolute values, is an alternative method to interpret the CK responses to physical exertion on an individual basis.

Thus, by stipulating a maximum value of [CK<sub>serum</sub>], we obtained the stress threshold that a player can reach. As a result, training can be conducted individually with a load compatible with each athlete's biomarkers, preserving those most vulnerable, thus preventing muscle injuries and optimizing the athletes' performance. The training of high-performance athletes should be based on the progress of evidence in sports science to improve the physical use and obtain the lowest possible organic exhaustion<sup>32</sup>.

As observed in the results, the coefficient of variation (CV) of CK expressed as  $\% \Delta \text{CK}_{\text{max}}$  is higher than the other expressions of concentration. The CV is the mean divided by the standard deviation and indicates how the measure varies; in this case, the more this measure varies, the more individualized it should be. This aspect reveals that by using this data expression method, the variation between the individuals results increase, indicating a greater need to individually monitor each athlete's training.

With this goal, Lazarim<sup>4</sup> established percentages to determine the CK concentration parameters that aid in the interpretation of the physical demands imposed by training, as well as the risk of muscle injury. However, even considering that a representative sample (128 players) was assessed and considering the difficulty of accessing athletes at this competitive level, the collection of more samples per athlete during the various periods would be important for individual monitoring. This fact is important due to the high intra-individual variability in the CK responses to exercise.

Moreover, with an aim to individualize exercise-induced stress monitoring and to protect susceptible athletes from overtraining, Pimenta<sup>33</sup> evaluated hormonal responses and muscle microtrauma in professional soccer players in relation to genetic variability in the alpha-actinin-3 (ACTN3-577R/X). The players were grouped into the RR, RX, and XX genotypes. The XX group that does not

express alpha-actinin-3 showed higher concentrations of CK after a field test of muscle demand than the other groups. Thus, there is a need to adjust the training load based on genetics since it requires different adaptation times for training and muscle recovery.

This study and those that were previously presented, corroborate the ideas of Urhausen and Kindermann<sup>34</sup>, who claimed that CK concentration represents exercise-induced mechanical wear due to the exercise performed in previous days and is linked to training intensity and volume. Hence, for the development of a training program, it is imperative to assess an athlete's muscular stress using indicators, such as CK, considering that the results can guide the type of specific training required for each player in order to prevent overtraining and ensure that the training will improve, or at least maintain, performance.

Therefore, CK can be considered an important tool for monitoring the status and training load of athletes. Furthermore, our results suggest that CK concentrations expressed in values relative to percentage of the maximum concentration or its maximum delta variation should be individualized and may be more specific for monitoring the training load in team sports, such as soccer.

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