



Behavior of the creatinine and urea seric and urinary concentrations during a periodization developed in professional soccer players: relations with the glomerular filtration rate

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ABSTRACT

The creatinine and urea responses have been extensively used to evaluate the physical training impact. Therefore, the purpose of this study was to investigate the behavior of serum and urinary creatinine and urea concentrations during a soccer training program. Eighteen Brazilian soccer players were evaluated at the beginning (T1), in the middle (T2) and at the end (T3) of a soccer training program. The athletes had their anthropometric characteristics, aerobic capacity and alactic anaerobic metabolism efficiency assessed. Besides the measurement of serum and urinary creatinine and urea concentrations, the athletes had their creatinine clearance evaluated by three different methods. While the first method was independent from the urinary volume, the others were dependent. Anova one-way test followed by Newman-Keuls and Pearson product-moment coefficient were used to verify the responses and correlations of the data to the soccer training program. A significance level of 5% was chosen. The soccer training program led to an increase in aerobic ($p < 0.01$) and alactic anaerobic ($p < 0.01$) performances, however, the urinary volume diminished along the experiment ($p < 0.05$). The serum ($p < 0.05$) and urinary ($p < 0.01$) creatinine concentrations presented an opposite behavior during the soccer training program, in addition, there were not observed significant correlations between these parameters in any period of the study. The creatinine clearance assessed by the three different methods decreased in response to the training ($p < 0.05$). Significant correlations for all methods were observed only in T1. However, the urinary volume dependent methods were statistically correlated in T2 and T3. According to results, it can be concluded that the serum and urinary creatinine concentrations were sensitive to the training program developed, but presented opposite behaviour. This probably occurred due to the limitations of the urinary method to assay creatinine and urea.

INTRODUCTION

Soccer is a dynamic sport in which the performance maximization of the professional athlete is based on the suitable development of a set of tactics, technical, nutritional, psychological and physical factors. According to Bangsbo⁽¹⁾, more than 90% of the energy spent during a soccer game is supplied by the aerobic metabolism. Moreover, the athletes run approximately 10 km⁽¹⁻²⁾ with intensity close to the anaerobic threshold, that is, 80 to 90% of the maximal cardiac frequency. Although the metabolic basis of a soc-

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cer game is aerobic, the majority of the actions used in order to decide a game (shooting, dribbling and heading) is of anaerobic nature⁽³⁾.

The physical preparation of a soccer team may be harmed by the competitions calendar. Usually, in a soccer championship played in Brazil, a team plays an average of two games per week. Nonetheless, in order to have the athlete with a satisfactory level of competitiveness throughout the year, it is mandatory that a balance between work load (games and training) and the period dedicated to recovery exists⁽⁴⁻⁵⁾.

The appearance of sprains, total body mass reduction and dehydration may affect athletes whose recovery period is insufficient⁽⁴⁾. Therefore, the regular monitoring of certain substances such as creatinine and urea may serve as a tool in prevention of the development of the problems mentioned above⁽⁶⁻¹⁰⁾.

Creatinine is a nitrogen and non-protein organic component derived from creatine dehydration. It can be measured in the blood or urine and its concentration remains practically unchanged during 24 h. The skeletal muscle is the largest site of creatinine production; thus, variations in its production would directly indicate proportional alterations in the muscular mass⁽¹⁰⁻¹¹⁾. Reduction of muscular mass is one of the classic symptoms observed in overtraining^(4,12).

Creatinine is also extensively used in order to evaluate the renal function through the glomerular filtration rate (GFR). The GFR may be obtained through the determination of the creatinine concentration on the blood and urine; however, the greatest difficulty in GFR measurement is the determination protocol in the total urine collected during a period of 24 h⁽¹⁰⁾. Alternatively, the GFR may be estimated simply by using the blood creatinine concentration⁽¹³⁾.

Urea is synthesized in the liver by the carbon dioxide and ammonia which are formed as final products of the protein catabolism. After the synthesis, the urea is transported by the blood to the kidneys where it is filtered by the glomeruli⁽⁷⁾. Several authors have associated the increase of the urea concentrations with the increase of the protein catabolism and the gluconeogenesis in response to intense training loads^(7,14).

Great resistance from the side of directors, coaching commission and players in the soccer panorama is experienced concerning the blood collection for analysis of parameters such as creatinine and urea. Such fact occurs mainly due to the invasive character of the procedure. Thus, the utilization of urine may be an alternative and non-invasive means of evaluating these substances in soccer athletes. Moreover, as far as the authors know, no study has tried to evaluate the responses of these substances to the periodized physical training in professional soccer players.

Therefore, the main objective of the present study was to verify the behavior of the creatinine and urea concentrations determined in the serum and urine, during a periodization developed in profes-

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sional soccer players. The secondary objectives were to analyze whether the urinary method may be used as an alternative means for the determination of the creatinine and urine concentrations; to evaluate the periodized training effect in the performance parameters and in the glomerular filtration rate; and to verify the existing relations between seric creatinine and urea concentrations and the performance parameters in professional soccer players.

METHODS

Participants

18 male professional soccer players, with mean age of $22,96 \pm 2,44$ years participated in the study. The athletes, who were members of an affiliated team from the Federal Federation of São Paulo, and the coaching commission, were previously informed about the procedures which they would be submitted to. Later, they signed a free and clarified consent form approved by the Ethics in Research Committee from the Biosciences Institute of the State University of São Paulo "Júlio de Mesquita Filho"; Rio Claro Campus. The selected athletes for the present study did not make use of creatine, protein and amino acids supplementation as ergogenic device.

Experimental outline

The performance evaluations were conducted in an athletics official track and the blood samples, serum and urine analyses in the Biodynamics Laboratory of the State University of São Paulo "Júlio de Mesquita Filho" Rio Claro Campus. The athletes were evaluated in the beginning (T1, week 0), middle (T2, week 6) and end (T3, week 12) of a periodization developed in professional soccer players.

The evaluations were conducted in two days. On the first day, at 7:30 in the morning, the blood samples were collected (5 mL) at fasting for the determination of the seric creatinine and urea concentrations. After the collections, the anthropometrical evaluation was performed. On the second day, at 8:30 in the morning, the tests for determination of the lactic anaerobic performance capacity were conducted on an athletics official track. The 24 h urine collection started on the first day of evaluation.

Training protocol

During the experiment, the athletes trained 10 sessions per week added to an official game on weekends between T1-T2 and T2-T3. There was an increase of 11.36% in the average volume of each training session between T1-T2 (88 min) and T2-T3 (98 min).

The training protocol consisted of recovery training (e.g.: continuous running with intensity between 50-60% of the maximal cardiac frequency); aerobic (e.g.: 70-80% and 80-90% of the maximal cardiac frequency between T1-T2 and T2-T3, respectively); soccer specific (e.g.: game activities performed according to the athlete's position); velocity specific (e.g.: maximal efforts between 10-30 m with and without ball conduction); tactic (e.g.: activities according to the tactic scheme proposed); technical (e.g.: attack x defense on reduced fields); collective (game with the same characteristics of an official one, but played with players and substitutes in 2 sets of 30 min) and recreational (e.g.: athletes playing distinct functions from the usual ones during the collective training). Table 1 presents the summarized characteristics of the training program between T1-T2 and T2-T3.

Anthropometrical evaluation

The athletes were submitted to an anthropometrical evaluation which consisted of the height (H; cm) and total body mass (BM; kg) measurement later used for the determination of the body surface (BS; m²; equation 1).

$$BS = BM^{0,425} \times H^{0,725} \times 0,007184 \quad (\text{Equation 1})$$

TABLE 1
Characteristics of the 12 week-training program developed in professional soccer players

Training type	Session duration	Weekly frequency		Weekly frequency variation
		T1-T2	T2-T3	From T1-T2 to T2-T3
Recovery*	30 min	4	2	-50%
Aerobic*	60 min	4	2	-50%
Soccer specific*	30 min	2	4	+100%
Velocity specific*	40 min	2	4	+100%
Tactic*	30 min	2	4	+100%
Technical*	40 min	2	4	+100%
Friendly*	60 min	3	3	0%
Recreational*	60 min	1	1	0%

* All training sessions occurred after a 15 min standardized warm-up.

The fat percentage (FP; %) was obtained through the measurement of four skinfolds⁽¹⁵⁾ with the aim to quantify the lean body mass (LBM; equation 2).

$$LBM = BM - (FP \times BM) \quad (\text{Equation 2})$$

Evaluation of the lactic anaerobic system efficiency

The lactic anaerobic system efficiency of the soccer players was measured through a protocol developed by Ananias *et al.*⁽¹⁶⁾ and consisted of 5 maximal efforts of 30 meters, with a one-minute passive pause, and blood samples collections for lactacidemia analysis at the 1st, 3rd and 5th minutes after the 5 efforts ending.

The mean velocity of the 5 efforts (Vm; m s⁻¹); the blood lactate peak concentration ([Lac]_{peak}; mmol.L⁻¹) and the ratio between the blood lactate peak concentration and the mean velocity ratio ([Lac]_{peak}/Vm; mmol.L⁻¹/m.s⁻¹) for each athlete were registered as lactic anaerobic performance parameters.

Evaluation of the aerobic capacity

The athletes' aerobic capacity was obtained through the determination of the intensity corresponding to the anaerobic threshold (iTan). The used protocol was the OBLA⁽¹⁷⁾ and it consisted of the performance of 4 submaximal efforts of 800 meters with intensities corresponding to 12,4; 13,3; 14,4 and 15,7km.h⁻¹ which were controlled by sound stimuli at each 100 meters.

There were passive intervals of approximately 45 seconds between the submaximal series for the blood collection. The iTan corresponded to the lactate concentration of 4 mM and was obtained through exponential interpolation of the lactacidemic curve versus velocity.

Determination of the blood lactate concentration

25µl of artery blood were collected from the earlobe through heparinized and calibrated glass capillary tubes. The blood was placed in 1,5 mL microcentrifuge tubes with 50 µl of sodium fluoride (NaF - 1%), for later determination of the blood lactate concentration (mM) in a *Yellow Spring Instruments* Electrochemical Lactometer (YSI), model 1500 Sport.

Serum collection and analysis

The blood collections, performed with disposable materials through a vacuum system in a 05 mL tube for serology without anticoagulant (VACUETTE®), were conducted in a private laboratory, after 8 hour-fasting and with a minimum interval of 12 h after the performance of the last training session.

After the collections, the tubes were placed in water bath at 37°C during 45 minutes and centrifuged for 10 minutes at 480 g in order to obtain the serum which was stored in 1,5 mL microcentrifuge tubes at -10°C for analysis of the creatinine⁽¹⁸⁾ and urea⁽¹⁹⁾ concentrations.

Urine collection and analysis

The athletes were instructed to dispose of the first urine of the day, and from that moment on until 24 h later, all the eliminated urine was collected and stored in 2 liter-plastic bottles with the aid of a funnel. The urine volume (mL) was measured and the bottles were kept refrigerated for creatinine⁽²⁰⁾ and urea⁽¹⁹⁾ concentrations analysis.

Determination of the glomerular filtration rate (GFR)

The GFR was determined through three distinct methods:

1- Estimated GFR through the seric creatinine⁽¹³⁾ (mL.min⁻¹) – Equation 3

$$\text{GFR} = (140 - \text{age}) * \text{weight (kg)} / 72 * \text{seric creatinine (mg.dL}^{-1}\text{)}$$

2- Real GFR not corrected by the body surface⁽²¹⁾ (mL.min⁻¹) – Equation 4

$$\text{GFR}_{\text{real}} = [\text{urinary vol. (mL)} / 1440 * \text{urine creatinine (mg.dL}^{-1}\text{)}] / \text{seric creatinine (mg.dL}^{-1}\text{)}$$

3- Real GFR corrected by the body surface⁽²¹⁾ (mL.min⁻¹/1,73m²) – Equation 5.

$$\text{GFR}_{\text{real corrected}} = \text{Equation 4} * 1,73/\text{BC}$$

Statistical analysis

According to the *Shapiro Wilk's W test*, the data collection presented normal distribution and the homogeneity was verified through the *Levine's test*. Therefore, the *Anova one-way test* was used, followed by the *post hoc* test by *Newman-Keuls* whenever necessary, with the purpose to verify the response of the analyzed variables during the 12 weeks of periodization developed in professional soccer players. The *Pearson* correlation analysis was applied between the variables. The data were expressed in mean \pm standard deviation and the pre-set significance level was of 5%.

RESULTS

According to table 2, it is possible to verify that the anthropometrical parameters did not present significant alterations during the periodization.

TABLE 2
Behavior of the anthropometrical parameters during a periodization developed in professional soccer players

	T1 (n = 13)	T2 (n = 18)	T3 (n = 15)
Total body mass (kg)	72,51 \pm 7,71	72,97 \pm 7,91	72,68 \pm 8,26
Height (cm)	181,15 \pm 8,40	180,94 \pm 7,66	180,80 \pm 6,73
Body surface (m ²)	1,92 \pm 0,14	1,85 \pm 0,28	1,87 \pm 0,27
Fat percentage (%)	7,38 \pm 2,38	8,31 \pm 2,81	7,97 \pm 2,66
Lean body mass (kg)	67,09 \pm 6,74	66,85 \pm 7,02	67,57 \pm 6,82

The [Lac]_{peak} (mM) and the [Lac]_{peak}/Vm (mM/m.s⁻¹) ratio significantly decreased in T3 (4,27 \pm 1,10 mM and 0,65 \pm 0,17 mM/m.s⁻¹) when compared with T1 (6,35 \pm 1,94 mM and 0,97 \pm 0,29 mM/m.s⁻¹) and T2 (5,50 \pm 1,42 mM and 0,82 \pm 0,21 mM/m.s⁻¹). The iTan significantly increased in T2 (14,03 \pm 0,97 km.h⁻¹) and T3 (14,47 \pm 0,62 km.h⁻¹) when compared with T1 (13,05 \pm 1,03 km.h⁻¹). No significant correlations were observed between the lactic anaerobic parameters and the aerobic capacity (table 3).

It is possible to observe in table 4 that while the seric creatinine concentration presented significant increase in T3 (1,54 \pm 0,52 mg.dL⁻¹), when compared with T1 (1,14 \pm 0,31 mg.dL⁻¹) and T2 (1,24 \pm 0,26 mg.dL⁻¹), the seric urea concentration remained unchanged during the study.

The urinary volume (mL) and the creatinine concentrations (mg.dL⁻¹) significantly decreased in T2 (569,17 \pm 348,38 mL and 170,91 \pm 75,00 mg.dL⁻¹) and in T3 (705,33 \pm 406,29 mL and 112,44

TABLE 3
Behavior of the efficiency parameters of the lactic anaerobic system (Vm, [Lac]_{peak}, [Lac]_{peak}/Vm) and of the aerobic capacity (iTan) during a periodization developed in professional soccer players

	T1 (n = 13)	T2 (n = 18)	T3 (n = 15)
Vm (m.s ⁻¹)	6,52 \pm 0,20	6,68 \pm 0,19	6,60 \pm 0,18
[Lac] _{peak} (mM)	6,35 \pm 1,94	5,50 \pm 1,42	4,27 \pm 1,10**
[Lac] _{peak} /Vm (mM/m.s ⁻¹)	0,97 \pm 0,29	0,82 \pm 0,21	0,65 \pm 0,17**
iTan (km.h ⁻¹)	13,05 \pm 1,03	14,03 \pm 0,97*	14,47 \pm 0,62*

Vm: Mean velocity; [Lac]_{peak}: Blood lactate peak concentration; iTan: Intensity corresponding to the anaerobic threshold.

* Significant difference in relation to T1.

† Significant difference in relation to T2.

TABLE 4
Behavior of the creatinine and urea seric and urinary concentrations, and the urinary volume during a periodization developed in professional soccer players

Serum	T1 (n = 13)	T2 (n = 18)	T3 (n = 15)
Creatinine (mg.dL ⁻¹)	1,14 \pm 0,31	1,24 \pm 0,26	1,54 \pm 0,52**
Urea (mg.dL ⁻¹)	21,17 \pm 4,35	19,49 \pm 5,09	22,19 \pm 5,66
Urine	T1 (n = 13)	T2 (n = 18)	T3 (n = 15)
Urinary volume (mL)	1.026,15 \pm 498,61	569,17 \pm 348,38*	705,33 \pm 406,29*
Creatinine (mg.dL ⁻¹)	250,17 \pm 114,76	170,91 \pm 75,00*	112,44 \pm 30,01**
24 h creatinine (mg.kg ⁻¹ .24 h ⁻¹)	30,82 \pm 11,60	11,82 \pm 7,02*	10,72 \pm 6,94*
Urea (mg.dL ⁻¹)	2.027,49 \pm 661,54	963,05 \pm 431,48*	2.078,92 \pm 956,74†
24 h urea (mg.24 h ⁻¹)	18,26 \pm 6,07	5,03 \pm 3,50*	13,11 \pm 7,18**

* Significant difference in relation to T1.

† Significant difference in relation to T2.

\pm 30,01 mg.dL⁻¹), when compared with T1 (1026,15 \pm 498,61 mL and 250,17 \pm 114,76 mg.dL⁻¹). The creatinine concentrations were also significantly lower in T3 when compared with T2. The 24 h creatinine concentration was significantly lower in T2 (11,82 \pm 7,02 mg.kg⁻¹.24 h⁻¹) and T3 (10,72 \pm 6,94 mg.kg⁻¹.24 h⁻¹) when compared with T1 (30,82 \pm 11,60 mg.kg⁻¹.24 h⁻¹). The urea determined in the urine (mg.dL⁻¹) showed significant difference between T1 (2027,49 \pm 661,54 mg.dL⁻¹) and T2 (963,05 \pm 431,48 mg.dL⁻¹) and between T2 and T3 (2078,92 \pm 956,74 mg.dL⁻¹). The 24 h urea concentrations in T2 (5,03 \pm 3,50 mg.24 h⁻¹) and T3 (13,11 \pm 7,18 mg.24 h⁻¹) were significantly lower than in T1 (18,26 \pm 6,07 mg.24 h⁻¹), being also observed significant alteration between T3 and T2.

The estimated GFR significantly decreased in T3 (84,36 \pm 25,29 mL.min⁻¹) when compared with T1 (119,92 \pm 28,38 mL.min⁻¹). Moreover, GFR not corrected by the BS and the corrected one were lower in T2 (50,47 \pm 29,24 mL.min⁻¹ and 47,88 \pm 27,05 mL.min⁻¹/1,73m²) and T3 (34,59 \pm 18,40 mL.min⁻¹ and 30,24 \pm 15,77 mL.min⁻¹/1,73m²) when compared with T1 (148,60 \pm 77,93 mL.min⁻¹ and 134,23 \pm 71,68 mL.min⁻¹/1,73m²) (table 5).

TABLE 5
Behavior of the glomerular filtration rate (GFR), determined through 3 distinct methods, during a periodization developed in professional soccer players

	T1 (n = 13)	T2 (n = 18)	T3 (n = 15)
Estimated GFR (mL.min ⁻¹)	119,92 \pm 28,38	99,98 \pm 26,18	84,36 \pm 25,29*
GFR not corrected by the BS (mL.min ⁻¹)	148,60 \pm 77,93	50,47 \pm 29,24*	34,59 \pm 18,40*
GFR corrected by the BS (mL.min ⁻¹ /1,73m ²)	134,23 \pm 71,68	47,88 \pm 27,05*	30,24 \pm 15,77*

GFR: Glomerular filtration rate; BS: Body surface.

* Significant difference in relation to T1.

According to table 6, no significant correlations occurred between the creatinine and urea concentrations determined in the serum and urine in any of the studied periods.

TABLE 6
Correlations between the creatinine and urea concentrations determined in the urine and serum of professional soccer players during 12 weeks of periodized training

	Serum creatinine (mg.dL ⁻¹)			Serum urea (mg.dL ⁻¹)		
	T1	T2	T3	T1	T2	T3
Urine creatinine (mg.dL ⁻¹)	r = 0,17	r = -0,14	r = -0,05	x	x	x
Urine 24 h creatinine (mg.kg ⁻¹ .24 h ⁻¹)	r = 0,26	r = -0,55	r = -0,29	x	x	x
Urine urea (mg.dL ⁻¹)	x	x	x	r = 0,35	r = 0,27	r = 0,04
Urine 24 h urea (mg.kg ⁻¹ .24 h ⁻¹)	x	x	x	r = 0,14	r = 0,07	r = -0,08

According to table 7, the estimated GFR presented significant correlation with the GFR not corrected by the BS ($r = 0,70$) and with the GFR corrected by the BS ($r = 0,76$) only in T1.

TABLE 7
Correlations between the estimated glomerular filtration rate (GFR) and the glomerular filtration rate not corrected by the body surface and corrected by the body surface one of professional soccer players during 12 weeks of periodized training

	Estimated GFR (mL.min ⁻¹)		
	T1	T2	T3
GFR not corrected by the body surface (mL.min ⁻¹)	r = 0,70*	r = 0,32	r = -0,05
GFR corrected by the body surface (mL.min ⁻¹ /1,73m ²)	r = 0,76*	r = 0,25	r = -0,14

* Significant correlation.

DISCUSSION

The anthropometrical characteristics of the evaluated soccer players in the present study are according to the results found in the national⁽²²⁾ and international literatures⁽²³⁻²⁴⁾. Besides that, the fact that the anthropometrical parameters have not suffered any significant alterations during the training was also observed in French soccer players⁽²⁴⁾.

The $[Lac]_{peak}$ and the $[Lac]_{peak}/Vm$ ratio significantly decreased during the study and the obtained results in T3 ($4,27 \pm 1,10$ mM and $0,65 \pm 0,17$ mM/m.s⁻¹) were similar to the ones found in professional soccer players of the São Paulo and Brazil first division⁽¹⁶⁾ ($4,5 \pm 1,00$ mM and $0,66 \pm 0,16$ mM/m.s⁻¹).

The decrease of the blood lactate concentration accumulated in response to intermittent lactic anaerobic efforts may be explained by two hypotheses. The first one is related to the increase of the capacity of energy production provided by the lactic anaerobic system, that is, in response to the specific training, the athlete would present greater quantities of stored phosphagens (ATP-CP). Thus, the participation of the lactic anaerobic way in the energy supply for performance of lactic anaerobic efforts would be delayed, and consequently, the blood lactate production in this type of exercise would be lower⁽¹⁶⁾.

The second hypothesis is based on the premise that athletes who have high aerobic capacity remove the lactate produced in anaerobic exercises more easily. Therefore, in response to the periodized training, the athletes who present increase in the anaerobic threshold may recover the lactic anaerobic system more rapidly⁽²⁵⁻²⁶⁾.

The results of the present study show that the efficiency improvement of the lactic anaerobic system probably occurred due to an increase of the energetic reserves of ATP-CP, once no significant correlation was observed between the lactic anaerobic parameters and the anaerobic threshold in any of the evaluated periods.

A $iLan$ increased significantly between T1-T2 and T1-T3; besides that, the indices observed in T3 ($14,47 \pm 0,62$ km.h⁻¹) were similar to the findings by Silva *et al.*⁽²²⁾ ($14,28 \pm 0,62$ km.h⁻¹). However,

they were lower than the results obtained by Ananias *et al.*⁽¹⁶⁾ ($16,1 \pm 1,6$ km.h⁻¹).

The differences highlighted above may be explained by the competitive level of the evaluated athletes. The results of the present study, as well as the ones presented by Silva *et al.*⁽²²⁾, were from professional soccer players who played in the 2nd division of the São Paulo championship. On the other hand, the sample used by Ananias *et al.*⁽¹⁶⁾ included players who were in the 1st division of the championships from São Paulo and Brazil.

The main objective of this investigation was to verify the behavior of the creatinine and urea seric and urinary concentrations during a periodization developed in professional soccer players. The seric creatinine concentration significantly increased in T3 when compared with T1 and T2; however, due to the lack of significant correlations, it is not possible to relate this increase with the alterations in the aerobic and lactic anaerobic performances which were also observed in the same training period. Moreover, few studies have investigated the creatinine response to the periodized training⁽⁸⁻⁹⁾.

Lehmann *et al.*⁽⁸⁾ did not verify significant alterations in the creatinine seric concentrations in response to the increase of the training volume in long and medium distance runners. The same situation was observed by Lehmann *et al.*⁽⁹⁾ in two groups of runners, where there was volume increase in one and intensity increase in the other.

The disparity between our findings and the ones mentioned above may have occurred due to the differences between the studied modalities; however, the training period in which our athletes presented significant increase in the seric creatinine concentrations, suffered increases both in volume and intensity.

Conversely, the creatinine concentrations (mg.dL⁻¹) measured in the urine presented behavior opposite to the one observed in the seric creatinine, that is, they decreased during the experiment. In addition to that, significant correlations were not observed between the creatinine concentrations determined in the serum and urine. Paterson⁽²⁷⁾ found that the seric creatinine concentration is more constant and significant than the creatinine volume excreted in the urine during 24 h.

The methodological restrictions of the 24 h urine collection protocol previously mentioned⁽¹⁰⁾ may be used in order to explain the results discrepancy observed in the present study. The major limitations of this protocol are related to the exact amount of urine volume excreted; namely, the inclusion of the first urine in the collected volume; the incomplete emptiness of the bladder in each collection; and urine losses during shower and defecation. These are some examples of the errors most commonly made by the volunteers⁽²⁸⁾. Usually the 24 h creatinine excretion (mg.kg⁻¹.24 h⁻¹) is constant in healthy individuals. Therefore, its measurement may be used in order to confirmation whether the collection was correctly conducted. Values lower than 20 mg.kg⁻¹.24 h⁻¹ are an indication that the collection was not adequately conducted⁽¹¹⁾.

In our study, besides observing a significant decrease in the urine volume collected during the periodization, both in T2 and T3, the 24 h creatinine values were below 20 mg.kg⁻¹.24 h⁻¹. Therefore, we believe that the evaluated sample did not follow the instructions for the urine collection during 24 h correctly and probably made some of the error described above.

Although some authors have observed variations in the seric urea concentrations in response to intense training^(7,14), in the present study, as well as Halson *et al.*⁽⁶⁾ with cyclists, we did not verify significant alterations during the periodization. Moreover, the variation range of urea concentrations in all training phases remained within the reference values⁽⁷⁾ (10-50 mg.dL⁻¹).

Concerning the urea (mg.dL⁻¹) and 24 h urea (mg.kg⁻¹.24 h⁻¹) concentrations, we verified significant differences between the studied periods. Nevertheless, as mentioned before, due to the 24 h

urine collection method limitation, these results are not trustworthy and further studies are needed in order to confirm them.

All the GFR determination methods decreased in response to the periodization training; however, the percentage decreases between T1-T2, T2-T3, and T1-T3 were higher for the GFR not corrected by the BS (-66,04%, -31,46%, -76,72%) and GFR corrected by the BS (-64,33%, -36,84%, -77,47%) comparing with the estimated GFR (-16,63%, -15,62%, -29,65%).

In addition to that, significant correlations of the estimated GFR with the GFR not corrected by the BS and GFR corrected by the BS were observed only in T1. The lack of correlation in the remaining periods is probably due to the limitation in the urine collection previously mentioned. Nonetheless, data which mention the behavior of the glomerular filtration rate during physical training are still scarce in the literature.

The results obtained in the present study are extremely important to professionals who work with high level soccer, since they present a periodization that was efficient in developing the aerobic and lactic anaerobic performances. Conversely, the lack of correlation between the performance and creatinine and urea seric concentrations limits the utilization of these markers only to the training monitoring, as substances sensitive to volume and intensity changes. Another limitation verified during the study was the urine utilization as an alternative means for determination of the urea and creatinine concentrations.

Therefore, we may reach the conclusion that the creatinine concentrations determined in the serum and urine of professional soccer players were sensitive to the training program developed; nevertheless, they presented opposite behavior. Such fact is probably due to the methodological limitation of the 24 h urine collection technique which should not be used in professional soccer players as an alternative for creatinine and urea concentrations evaluation.

Moreover, the periodization developed was efficient for the aerobic and lactic anaerobic performances evolution of the soccer players and altered their glomerular filtration rate as well. The lack of correlation between the seric creatinine and urea and the performance parameters demonstrate the need of further investigation which are able to elucidate more clearly the relation between the seric creatinine and urea concentrations and the performance of professional soccer players. A possible alternative would be the evaluation of the team's global performance during a competition instead of individual evaluations.

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REFERENCES

1. Bangsbo J, Norregaard L, Thorsoe F. Activity profile of competition soccer. *Can J Sport Sci.* 1991;16:110-6.
2. Helgerud J, Engen LC, Wisløff U, Hoff J. Aerobic endurance training improves soccer performance. *Med Sci Sports Exerc.* 2001;33:1925-31.
3. Chamari K, Hachana Y, Ahmed YB, Galy O, Sghaier, F, Chatard JC, et al. Field and laboratory testing in young elite soccer players. *Br J Sports Med.* 2004;38:191-6.
4. Halson SL, Jeukendrup AE. Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Med.* 2004;34:967-81.
5. Silva ASR, Santhiago V, Gobatto CA. Compreendendo o overtraining no desporto: da definição ao tratamento. *Rev Port Cien Desp.* 2006;6.
6. Halson SL, Bridge MW, Meeusen R, Busschaert B, Gleeson M, Jones DA, et al. Time course of performance changes and fatigue markers during intensified training in trained cyclists. *J Appl Physiol.* 2002;93:947-56.
7. Hartmann U, Mester J. Training and overtraining markers in selected sport events. *Med Sci Sports Exerc.* 2000;32:209-15.
8. Lehmann M, Dickhuth HH, Gendrisch G, Lazar W, Thum M, Kaminsky R, et al. Training-overtraining: a prospective, experimental study with experienced middle and long distance runners. *Int J Sports Med.* 1991;12:444-52.
9. Lehmann M, Wiedland H, Gastmann U. Influence of an unaccustomed increase in training volume vs intensity on performance, hematological and blood-chemical parameters in distance runners. *J Sports Med Phys Fitness.* 1997;37:110-6.
10. Riehl O, Fontana KE, López RFA. Excreção de creatinina como meio de análise da massa magra corporal. *Lecturas Educación Física y Deportes.* 2004;10:1-8.
11. Burtis CA, Ashwood ER. Tietz Text books of clinical chemistry. 2nd ed. Philadelphia: WB Saunders, 1994.
12. Petibois C, Cazola G, Poortmans JB, Deleris G. Biochemical aspects of overtraining in endurance sports: a review. *Sports Med.* 2002;32:867-78.
13. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16:31-41.
14. Haralambie G, Berg A. Serum urea and amino nitrogen changes with exercise duration. *Eur J Appl Physiol.* 1976;36:39-48.
15. Durnin JVG, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr.* 1974;32:77-97.
16. Ananias GEO, Kokubun E, Molina R, Silva PRS, Cordeiro JR. Capacidade funcional, desempenho e solicitação metabólica em futebolistas profissionais durante situação real de jogo monitorado por análise cinematográfica. *Rev Bras Med Esporte.* 1998;4:87-95.
17. Mader A, Liesen H, Heck H, Philippi H, Rost R, Scharch PA, et al. Zur Beurteilung der sportartspezifischen ausdauerleistungsfähigkeit. *Sportarzt Sportmed.* 1976;27:80-8.
18. Larsen K. Creatinine assay by a reaction-kinetic principle. *Clin Chim Acta.* 1972;41:209-17.
19. Crocker CL. Rapid determination of urea nitrogen in serum or plasma without desproteinization. *Am J Med Technol.* 1967;33:361-5.
20. Bartels H, Böhmer M, Heierli C. Serum creatinine determination without protein precipitation. *Clin Chim Acta.* 1972;37:193-7.
21. Nogueira DM, Strufaldi B, Hirata MH, Abdala DSP, Hirata RDC. Exploração funcional do rim. In: Nogueira DM, Strufaldi B, Hirata MH, Abdala DSP, Hirata RDC, editores. *Métodos de bioquímica clínica.* São Paulo: Pancast, 1990;149-51.
22. Silva ASR, Santos FNC, Santhiago V, Gobatto CA. Comparação entre métodos invasivos e não invasivos de determinação da capacidade aeróbia em futebolistas profissionais. *Rev Bras Med Esporte.* 2005;11:233-7.
23. Esposito F, Impellizzeri FM, Margonato V, Vanni R, Pizzini G, Veicsteinas A. Validity of heart rate as an indicator of aerobic demand during soccer activities in amateur soccer players. *Eur J Appl Physiol.* 2004;93:167-72.
24. Filaire E, Bernain X, Sagnol M, Lac G. Preliminary results on mood state, salivary testosterone: cortisol ratio and team performance in professional soccer team. *Eur J Appl Physiol.* 2001;86:179-84.
25. Donovan CM, Pagliassotti MJ. Endurance training enhances lactate clearance during hyperlactatemia. *Am J Physiol.* 1989;257:E782-E89.
26. Mac Rae HSH, Dennis SC, Bosch AN, Noakes TD. Effects of training in lactate production and removal during progressive exercise in humans. *J Appl Physiol.* 1992;72:1649-56.
27. Paterson N. Relative constancy of 24-hour urine volume and 24-hour creatinine output. *Clin Chim Acta.* 1967;18:57-8.
28. Henry JB. Clinical diagnosis and management by laboratory methods. 17th ed. Philadelphia: WB Saunders, 1984.