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BIOELECTRICAL PHASE ANGLE, MUSCLE DAMAGE MARKERS AND INFLAMMATORY RESPONSE AFTER A COMPETITIVE MATCH IN PROFESSIONAL SOCCER PLAYERS

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Abstract
Introduction. The purposes of this study were 1) to evaluate changes from baseline levels in bioelectrical phase angle (PhA) and markers of muscle damage and inflammation in professional players 36 h after a soccer match, and 2) to analyze the relationships between PhA and markers of muscle damage and inflammation in order to investigate if PhA might be a useful parameter to monitor recovery. ***Material and methods.*** Eighteen male professional soccer players participated in this study. Plasma lactate dehydrogenase (LDH) and creatine kinase (CK) activities, plasma C-reactive protein (CRP) and interleukin-6 (IL-6) concentrations, and PhA were measured before and 36 h after a competitive match. ***Results.*** Changes in LDH and CK 36 h after the match were greater than their reference change values (RCV). Changes in CRP and IL-6 were, however, lower than their corresponding RCV. 36 h after the match, significant correlations were observed between PhA and LDH ($r = 0.714$, $p = 0.001$), PhA and CK ($r = 0.787$, $p = 0.000$), and PhA and CRP ($r = 0.554$, $p = 0.017$). ***Conclusions***. Although IL-6 and CRP have been traditionally analyzed together to monitor inflammation after intense exercise, since 36 h after the match they have already returned or started to return to baseline levels, the use of them alone is not a good option to monitor inflammation throughout recovery. PhA might be used as a predictor of muscle damage and inflammation, but further studies covering the whole recovery period are warranted.

Key words: soccer, phase angle, muscle damage, inflammation, recovery

Introduction

Strenuous physical exercise is one of the most common causes of muscle damage and it occurs when muscles produce more physical work than they are used to producing [1]. The high level of physical exercise evokes mechanical disruption to sarcomeres and triggers an inflammatory response which is part of muscle repair and regeneration [2, 3]. This inflammatory response accentuates the loss of force production caused by the direct muscle damage and increases muscle soreness, which has a negative impact on exercise performance and delays recovery [4, 5].

Biochemical markers of muscle damage and inflammation are usually monitored to study and optimize recovery after exercise. Creatine kinase (CK) and lactate dehydrogenase (LDH) are two of the most common markers of direct muscle damage [1, 6, 7] and, according to Nowakowska et al. [8], CK and LDH together with aspartate aminotransferase and creatinine levels could constitute a useful set of markers for monitoring recovery periods in soccer players. Among the large number of biochemical markers of inflammation, interleukin-6 (IL-6) and C-reactive protein (CRP) are two of the most frequently used in sport [5, 6, 7].

Eccentric contractions cause more muscle damage than concentric contractions, especially at longer muscle lengths, when the muscle generates excessive strain during lengthening, and extensive myofibrillar disruption occurs [4]. Soccer is an intermittent sport with a powerful eccentric component [9] due to the high number of accelerations, decelerations and changes of directions [10], as well as jumps and other explosive activities [11]. It is precisely the high repetition of eccentric contractions during a competitive match that leads to greater levels of muscle damage and inflammation than other team sports [12].

There exist different methods to accelerate recovery after maximum exercise [13]. Previous investigations have reported high levels of muscle damage and inflammation up to 72 hours after a soccer match [14, 15], but in soccer it is not uncommon to have only 3-4 days between consecutive matches, which may not be enough time to full recovery [14]. This is the reason why monitoring useful markers of muscle damage and inflammation is essential to investigate the efficiency of the different strategies to accelerate recovery of muscle function.

Bioelectrical impedance analysis (BIA) is an inexpensive and non-invasive method for the assessment of body composition and nutritional status. BIA measures electrical parameters, such as resistance, reactance, bioelectrical phase angle (PhA) and impedance, which are called raw electrical impedance variables. These parameters are introduced into population-specific equations to calculate body composition, but these equations are only accurate under certain circumstances [17]. Raw variables are not, however, influenced by the equations that may affect body composition compartments [18].

BIA devices submit the human body to a weak, alternating current at one or more frequencies, detect the drop in voltage

as the current flows, and calculate and record the raw parameters [19]. Among those parameters, PhA has been widely used in clinical studies as an indicator of cellular health and cell membrane integrity [20, 21], and it is supposed that the higher the PhA value, the better the cell function [22]. PhA is obtained from the arctangent of the reactance to resistance ratio. Resistance is the decrease in voltage reflecting conductivity through ionic solutions and reactance is the delay in the flow of current due to cell membranes and tissue interfaces [21].

In sports, PhA has been recently used as an indicator of physical activity and nutritional status [20, 23]. In middle-distance athletes, PhA was positively associated with athletic performance, although it was not demonstrated whether PhA was adequate to monitor improvements in running performance [24]. In male soccer players it was observed that, compared with the elite level, players of a lower performance level had lower phase angles [25].

Since PhA is considered an indicator of cellular health and cell membrane integrity, it is reasonable to presume that there might be a relationship between PhA and markers of muscle damage and inflammation after intense exercise. However, little research has been carried out on this subject. Only Tomeleri et al. [26] studied the correlations between changes in PhA and changes in tumor necrosis factor alpha (TNF-α), CRP, IL-6 and IL-10 in older women after 12 weeks of resistance training. They found significant negative correlations between changes in PhA and changes in TNF-α and CRP, and a significant positive correlation between changes in PhA and changes in IL-10. No significant correlation was found with changes in IL-6. However, the authors did not measure any direct marker of muscle damage and measurements were not made after strenuous physical exercise.

Monitoring muscle damage and inflammation is particularly important in soccer, and PhA may be an appropriate variable to estimate muscle damage and inflammation with a quick, non-invasive and relatively inexpensive method. Therefore, the purposes of this study were 1) to evaluate changes from baseline levels in PhA and markers of muscle damage and inflammation in professional players 36 h after a soccer match, and 2) to analyze the relationships between PhA and markers of muscle damage and inflammation in order to investigate if PhA might be a useful parameter to monitor recovery.

Material and methods

Participants

The study was performed by the Department of Nutrition of an Italian first division soccer team and the Research Group of a Spanish University. Eighteen male professional soccer players (age 26.44 ± 3.03 years; height 185.07 ± 4.37 cm; weight 84.87 ± 7.29 kg) from an Italian first division soccer team participated in this study. The participants were evaluated before and 36 h after a competitive match, over a period of about one month during the first half of the season (matches 7-10). The players were included in the analysis only if they played for more than 45 minutes during the match, and goalkeepers were excluded. None of the players had suffered severe performance-limiting injuries from the beginning of the season.

This study was approved by the University Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki regarding the use of human subjects. All the players provided written informed consent to participate in the study.

Biochemical analyses

The blood extraction was performed between 8 a.m. and 10 a.m., after an overnight fast of at least 8 hours, in the morning of the competitive match and 36 h after the match. Blood samples were collected from an antecubital arm vein with the subject in a seated position. Plasma CK and LDH activities and concentrations of CRP and IL-6 were determined by the same laboratory (Synlab Italia S.R.L.). The samples were withdrawn into lithium-heparin tubes, using EDTA as anticoagulant, then centrifuged at 3000 rpm for 10 min to separate the plasma and stored at -20ºC until analysis.

LDH activity was assessed with a colorimetric kinetic method using a commercial test kit from Randox (REF 90.29.2). CK activity was determined by an IFCC kinetic optimized method with a commercial test kit from Randox (REF 90.15.4). An immunoturbidimetric method was used to measure CRP concentration with a specific test kit from Randox (REF 90.72.3). IL-6 was quantified with a chemiluminescence method using a specific test kit from Randox (REF 90.70.2). The coefficients of variation were 1.54% for LDH, 4.55% for CK, 3.0% for CRP and 7.2% for IL-6.

PhA assessment

BIA was performed with a body composition analyzer (Tanita MC-780 MA, Tanita Corp., Tokyo, Japan), by the same professional. The evaluation was undertaken between 8 a.m. and 10 a.m., after an overnight fast of at least 8 hours, in the morning of the competitive match and 36 h after the match. The subjects had refrained from moderate or intense physical exercise in the previous 24 h. Before performing the assessment, participants urinated and were instructed to remove metallic elements from their bodies. Players were measured while standing erect with bare feet on the foot-pads and holding the handgrips making contact with the four hand-pads. The device emitted an alternating sinusoidal electric current of 900 μA operating at 5, 50 and 250 kHz (multi-frequency). Standard measurements were performed according to the manufacturer guidelines. PhA was calculated from the arctangent of reactance to resistance ratio of the whole body, at a frequency of 50 Hz [20].

Statistical Analysis

Statistical analysis was performed using SPSS software v. 22.0 (SPSS Inc., USA). Means and standard deviations of all variables were calculated, before and 36 h after the match. Using the analytical and within-subject coefficients of variation provided by the European Biological Variation Study (EuBIVAS) [27, 28], reference change values (RCV) were calculated for LDH, CK and CRP. As variability of IL-6 has not been established by EuVIBAS, we took the data reported by Aziz et al. [29]. RCV were used to assess significance of the changes 36 h after the match with respect to pre-match values, and were determined as: $RCV = 2^{1/2} \times 1.96 \times (CV_A^2 + CV_I^2)^{1/2}$, where CV_A is analytical variation and CVI is within-subject biological variation [30].

Shapiro-Wilk test was applied to test data normality. As this condition was always fulfilled, Pearson's r correlation coefficients were calculated to evaluate the possible relationships between PhA and the rest of variables measured, before and 36 h after the match. They were interpreted according to the following criteria: $r < 0.1$, trivial; $0.1 < r \leq 0.3$, weak; $0.3 < r \leq 0.5$, moderate; $0.5 < r \leq 0.7$, strong; $0.7 < r \leq 0.9$, very strong; and $r > 0.9$, almost perfect [31]. If a significant correlation was found, linear regression was performed to predict changes in the dependent variable from changes in PhA. Coefficients of deter-

mination (R^2) were used to represent the goodness of the regression equation. The statistical level of significance was set as $p < 0.05$ for all analyses.

Results

Means and standard deviations of all the variables measured, before and 36 h after the match, are presented in Table 1. Changes pre-post-match and RCV are also presented in Table 1. Changes observed in LDH and CK were statistically significant because they were greater than their corresponding RCV. However, changes produced in CRP and IL-6 were not statistically significant.

No significant correlations between PhA and the rest of the study variables were found before the match. However, the present study found important relationships 36 h after the match between PhA and most biochemical markers measured (Tab. 2). Very strong correlations were observed between PhA and LDH ($r = 0.714$, $p = 0.001$) and between PhA and CK ($r = 0.787$, $p = 0.000$) and a strong correlation was found between PhA and CRP ($r = 0.554$, $p = 0.017$). Figure 1 shows coefficients of determination and regression equations between PhA and markers of muscle damage, 36 h after the match.

Table 1. Means and standard deviations of the study variables before and 36 h after the match. Changes pre-post-match and reference change values.

Variables	Pre-match	36 h post-match	Changes pre-post-match (%)	RCV (%)
	mean ± sd	mean ± sd		
PhA (º)	7.69 ± 0.38	7.61 ± 0.39	-1.0	----
LDH ($U \cdot L^{-1}$)	243.83 ± 58.83	399.17 ± 70.32	63.7*	15.6
CK ($U \cdot L^{-1}$)	384.28 ± 96.53	669.39 ± 297.76	74.2*	44.4
CRP ($mg \cdot dL^{-1}$)	0.11 ± 0.02	0.18 ± 0.04	66.2	85.3
IL-6 ($pg \cdot mL^{-1}$)	1.77 ± 0.17	2.41 ± 0.79	36.2	66.2

PhA – phase angle; LDH – lactate dehydrogenase; CK – creatine kinase; CRP – C-Reactive protein; IL-6 – interleukin 6; sd – standard deviation; RCV – reference change values; * – significant differences between post-match and pre-match values ($p < 0.05$).

Table 2. Pearson correlation coefficients between PhA and biochemical markers 36 h after the match.

	LDH	CK	CRP	IL-6
PhA	0.714**	0.787***	0.554*	0.172

PhA – Phase Angle; LDH – lactate dehydrogenase; CK – creatine kinase; CRP – C-Reactive protein; IL-6 – interleukin-6; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$.

Discussion

The first purpose of this study was to assess changes from baseline levels in PhA and markers of muscle damage and inflammation in professional soccer players 36 h after a soccer match. Changes in markers of muscle damage were greater than their respective RCV, which means that they were due to the muscle damage induced by the match, and not to biological variations. Change in CK activity 36 h after the match (Tab. 1) is in good agreement with that found by Beattie et al. [32], who measured plasma CK activity during the season, in a group of 18 elite soccer players, one day before a competition match and 48 h after the match. They found that 48 h after the match, CK activity was of 648 $U \cdot L^{-1}$, which was approximately 50% higher than their baseline value. Silva et al. [15], also assessed CK activity in a group of 7 male professional soccer players before, 24 h after, 48 h after and 72 h after the last match of the championship. However, they observed that CK activity peaked 24 h after the match and decreased to 560.6 ± 62.1 $U \cdot L^{-1}$ 48 h after the match, which was 86.5% higher than baseline values. The fact that they performed measurements after the last match of the championship and the high biological variations of CK are probably the reasons for these differences.

Change in LDH activity 36 h after the match (Tab. 1) is a bit lower than those observed in previous investigations with professional soccer players [12, 33], probably because our players started from higher baseline levels. These differences in LDH activity before the match may be due to the fact that participants from those studies were measured at the beginning of the season [12] or off the season [33], and their LDH levels were, therefore, lower than ours.

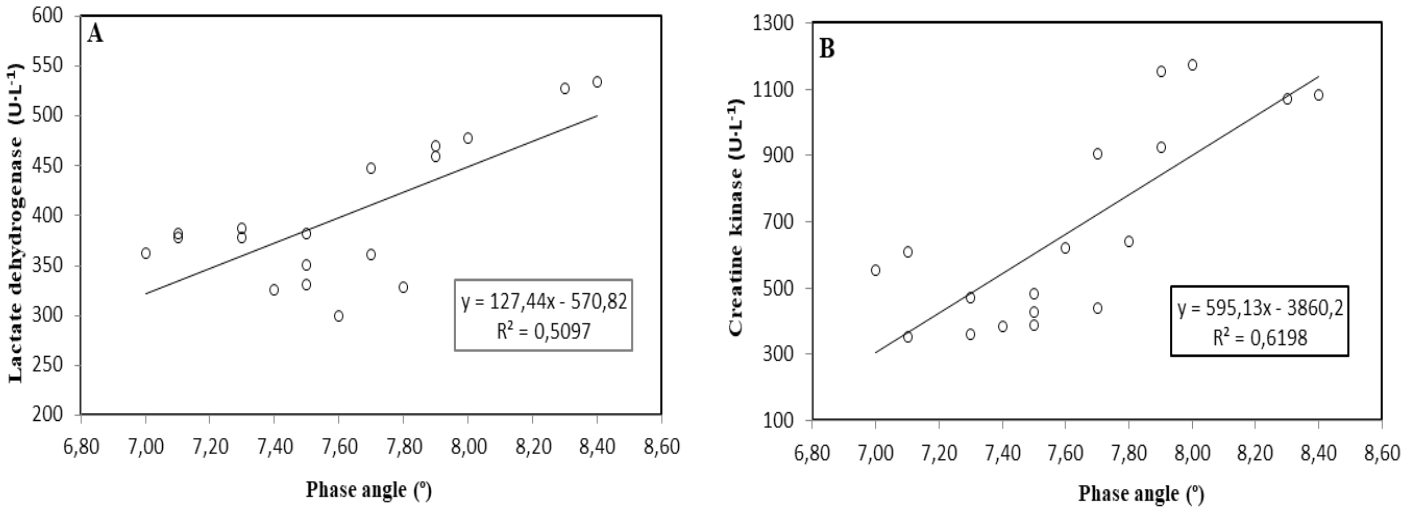

Figure 1. Correlation of phase angle with (A) plasma lactate dehydrogenase activity and (B) creatine kinase activity

With regard to markers of inflammation, changes from baseline levels 36 h after the match in CRP and IL-6 were lower than their corresponding RCV, which might indicate that the competitive match did not induce enough inflammation. After intense exercise, muscle damage triggers an inflammatory response, resulting in an increase in IL-6 concentration which usually peaks immediately after exercise and returns to baseline levels within 24 h [12, 33, 34]. Increased circulating IL-6 concentration stimulates the synthesis of acute-phase proteins such as CRP, which frequently peaks 24 h after exercise [15, 33, 34].

Therefore, in agreement with previous investigations, our results do not indicate that the competitive match did not induce inflammation but that 36 h after the match, CRP and IL-6 values had already returned or started to return to baseline levels. Thus, even if IL-6 and CRP have been traditionally analyzed together to monitor inflammation after intense exercise, the use of them alone is not a good option to monitor inflammation during the whole recovery period.

Changes in PhA were negligible (1%), which means that the match did not induce a change in PhA or it returned to baseline levels before 36 h. These findings cannot be compared with previous results owing to the lack of investigations studying the changes in PhA during the recovery period after a soccer match. However, our average value in the morning before the match (7.69 ± 0.38^0) is consistent with the result obtained by Levi-Micheli et al. [25]. They studied a group of 219 elite players of Italian first and second divisions, and obtained an average value of 7.7 ± 0.6^0, measured before a midweek training session in the first half of the season.

The second purpose of this study was to analyze the relationships between PhA and markers of muscle damage and inflammation, and to investigate if PhA may be useful to monitor recovery. It was expected to find negative correlations between PhA and markers of muscle damage and inflammation, because PhA is considered an indicator of cellular health and cell membrane integrity [20, 21].

No significant correlation was found between PhA and the biochemical markers measured the morning before the match. Nevertheless, we obtained strong positive correlations between PhA and LDH or CK and a moderate positive correlation between PhA and CRP (Tab. 2). These results are not in line with those obtained by Nescolarde et al. [35] and Francavilla et al. [36], who concluded that PhA decreased with increasing muscle injury severity in soccer players. However, they used a localized bioimpedance and, in addition, they measured injured lower limbs. The present study evaluated PhA of the whole body and, even if our soccer players probably suffered from exercise-induced muscle damage, they were not really injured. Therefore, our findings cannot be compared with theirs.

To the best of our knowledge, just two studies analyzed the correlation between PhA and markers of muscle damage or inflammation. Tomeleri et al. [26] found a very strong negative correlation between increases in PhA and increases in CRP, but those increases were not calculated before and after intense exercise, but before and after a 12-week resistance training, and measures were carried out a minimum of 48 h since the last physical exercise session. Beverashvili et al. [37] found a small/moderate negative correlation between changes in PhA over time and changes in IL-6 in patients on maintenance hemodialysis, but those results are not comparable to ours, either.

A possible explanation for our unexpected results could be the total distance covered by the players during the match, but no significant correlation was found with PhA or any of the biomarkers measured (data not shown). Moreover, significant correlations with total walking distance, total running distance or distances covered at different speeds during the match were not observed. The reasons why we obtained those positive significant correlations are then unclear but the fact that they exist indicates that 36 h after the match, the greater the PhA, the higher the muscle damage and inflammation.

The main limitations of this study are the consequences of the difficulty to evaluate several times in 48/72 h players from European First Division soccer teams. It would have been more interesting to analyze all the variables measured before the match, and to observe their changes during the whole recovery period. Notwithstanding that, our greatest strength is that this is the first study to correlate PhA with markers of muscle damage and inflammation before the match and at a certain point of recovery, and may serve as a reference for future studies evaluating different time points of recovery or even several consecutive matches throughout the season.

Conclusions

A soccer match induces a marked rise in biochemical markers of muscle damage and inflammation, particularly in CK, LDH, CRP and IL-6. IL-6 and CRP have been traditionally analyzed together to monitor inflammation after intense exercise but, since 36 h after the match they had already returned or started to return to baseline levels, the use of them alone is not a good option to monitor inflammation during the whole recovery period. The moderate/strong positive correlations observed between PhA and LDH, CK and CRP, 36 h after the competitive match suggest that after intense exercise, PhA is inversely related to cell membrane integrity in healthy soccer players, and might be used as a predictor of muscle damage and inflammation throughout recovery.

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