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## Original Research Article

## Functional and biochemical adaptations of elite level futsal players from Brazil along a training season

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## ABSTRACT

**Background and objective:** Although hard training is mandatory in elite level futsal training, few studies have proposed a biochemical follow up in futsal players during a whole season. Therefore, the aim of this study was to compare functional and biochemical markers in Brazilian elite level futsal players throughout a competition season.

**Materials and methods:** Eight players aged  $25.5 \pm 5.4$  years were evaluated at three time points: preseason (T1), immediately before the FIFA®-Intercontinental-Futsal-Cup (T2), and at the end of the season (T3), with a tapering period of 1 week before T2. Functional parameters (weight, height, body fat,  $VO_2$ max, heart rate, and distance ran) and blood sampling for cell count and lipid profile (cholesterol, HDL-C, LDL-C, triglycerides) were assessed at each time point. After, a Yo-Yo R2 test was carried out in each time point (T1, T2 and T3) and blood samples to assess skeletal muscle damage (creatine kinase [CK], lactate dehydrogenase [LDH]), inflammation (C-reactive protein [CRP]) and oxidative stress markers (ischemia modified albumin [IMA], and advanced oxidation protein products [AOPP]) were obtained before and after the tests.

**Results:** Although functional parameters did not change throughout the season, greater total number of erythrocytes ( $P \leq 0.05$ ), and hemoglobin ( $P \leq 0.05$ ) were found at T2 compared to

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T1. Similarly, lower LDH ( $P \leq 0.05$ ) and CK ( $P \leq 0.05$ ) levels were found at T2 compared to T1. CPR levels were also decreased at T2 in comparison to T1 both before and after Yo-Yo R2 test ( $P \leq 0.05$ ), while IMA and AOPP levels showed only a season effect ( $P \leq 0.05$ ).

**Conclusions:** The tapering strategy was successful considering players presented lower levels of muscle damage, inflammation and oxidative stress makers before T2, which preceded the main championship of the year. These results are of great relevance, considering the team won the FIFA®-Intercontinental-Futsal-Cup, which happened at T2. Thus, it seems that routine-based biochemical markers may be useful as training control means in this population.

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## 1. Introduction

Intensive training is a common routine for elite athletes in order to improve performance [1]. However, to optimize performance both intensive training and proper recovery are needed [2,3]. Usually, recovery takes place within 24 h following a single bout of exercise, while elite level athletes may train two, three times per day, followed by a relative short recovery period [4]. This could cause an imbalance between training, competition and recovery, which greatly impacts performance outcomes [5]. In this sense, it is well accepted that training monitoring is essential to optimize performance and reduce muscle injury risks [6], especially among high-performance futsal players [7].

Intense exercise may induce skeletal muscle damage, which is related to cytosolic enzymes appearance in the bloodstream, such as creatine kinase (CK) and lactate dehydrogenase (LDH) [8]. These biomarkers are associated with micro injuries and skeletal muscle inflammation, causing a delayed increase on inflammatory markers in the bloodstream, such as the C-reactive protein (CRP) [9], which is reported in different sports modalities, including futsal [10–12]. On the same line, new and specific oxidative stress and inflammation markers have been also studied in sports scenarios, such as the serum ischemia-modified albumin (IMA), a marker of inflammation-related myocardial ischemia and necrosis [13–15], and advanced oxidation protein products (AOPP), a potent systemic marker of oxidative stress [16–18].

Futsal is an intermittent, high-intensity sport with different levels of effort in which players may reach up to 85–90% of the maximal heart rate (HR<sub>max</sub>) and 75% of maximal oxygen uptake (VO<sub>2</sub>max) [19,20] during 70–85% of the match [19,21,22]. In this sense, players may undergo more than 25% of a match on extremely high intensity efforts, which is a common routine throughout a competitive season [19]. Additionally, players are usually submitted to 2–3 matches per week during competition, which induces great physical stress, increasing injury risks and performance lost due to muscle damage, fatigue, stress and inflammation [19]. Therefore, tapering strategies, known as a planned reduction in training loads before important competitions, have been vastly used in team sports [23]. The reduction of training loads can be achieved by several means, such as reduced training frequency, volume, and/or intensity [24], which has been proven to positively

impact on performance and physiological markers of different sportsmen [23,24].

Over the last decades, scientific interest in futsal has considerably grown [19,25]. Although few reports have pointed out a futsal match-induced change on biochemical markers [25], the current state of art does not provide practical methods to control stress conditions and recovery during a futsal season. This is still a significant limitation on the field, considering that high intensity efforts and recovery time during futsal clearly indicates that players are prone to muscle damage and inflammation increases [22,26,27].

In this sense, studies aiming to establish new biochemical markers for training control in elite level futsal players are necessary for technical support. This could provide a reliable feedback for training preparation to coaches and exercise performance professionals. Therefore, this study aimed to compare functional and biochemical markers related to skeletal muscle damage, inflammation and oxidative stress on elite level Brazilian futsal players during a whole season, including preseason and competition periods.

## 2. Materials and methods

### 2.1. Subjects

At first, seventeen futsal players (full time pay-job) were enrolled in the study. However, only eight outfield elite male players ( $25.5 \pm 5.4$ -years-old) with over  $6.8 \pm 5.8$  years of professional futsal experience met the inclusion criteria, and thus were included in the study. Recruited participants had to match (1) no injuries during the study, and (2) no anti-inflammatory medications or antioxidant supplements intake throughout the season. The participants were informed about the experimental procedures and possible discomforts associated with the study before signing a written informed consent. The study was approved by the Ethics Committee of the Universidade Federal de Santa Maria and carried out according to the Declaration of Helsinki and Ethical Standards in Sport and Exercise Science Research.

### 2.2. Experimental design

Experimental procedures were carried out on three time points along the season: at the end of the preseason (T1), two weeks

before the FIFA® Intercontinental Futsal Cup 2012 (T2), and at the end of the season (T3), with a tapering period of 1 week before T2. Participants performed the Yo-Yo Intermittent Recovery Level 2 test (Yo-Yo IR2) [28] in order to estimate the maximal oxygen uptake ( $\text{VO}_2\text{max}$ ) at each point. The HRmax was calculated at T1 as reference. Baseline values of functional and anthropometric parameters, and lipid and whole blood profiles were obtained before each Yo-Yo R2 test. A comparison before (pre) and after (post) each Yo-Yo R2 test was also performed for blood lactate concentration, skeletal muscle-related damage markers and oxidative-inflammatory parameters as well. Fig. 1 depicts the experimental design of the study.

### 2.3. Futsal training season

The futsal season lasted 44 weeks. Considering competitions, it was divided into one preparatory period (preseason T1) and two competitive periods (T2 and T3). Players played 36 matches between T1 and T2, which lasted 20.5 weeks. From T2 to T3 there were 22 matches, and the period lasted 17.5 weeks. Although no sampling took place after T3, it is important to mention that players played another 6 matches during the 6 weeks between T3 and season's end. In this regard, the training intensity during the competitive periods ranged from 80 to 90% of the HRmax throughout the entire periods, and was controlled during each training session through the use of heart rate monitors (Polar Team System, Polar Electro Oy, Kempele, Finland). The HRmax was calculated through a maximal oxygen uptake test performed independently by the ACBF staff before the study, as previously described [1]. Training time varied among periods, with 3900 min between T1 and T2 and 6240 min from T2 to T3 (central part of the training, warm up and cool down not included). Finally, during the week (22nd) before the International Cup players

underwent a tapering, with 50% decrease on training volume [23]. Unfortunately, due to technical limitations running distance during training could not be assessed. Overall, players participated in 64 official futsal matches throughout the season: 29 on Brazilian National Championship, 4 of the International Futsal Cup and 31 other matches divided in different championships and tournaments, with the FIFA® Intercontinental Futsal Cup as the main goal of the season.

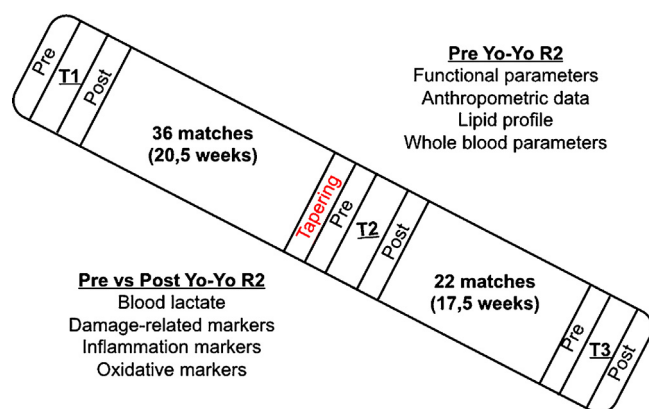
### 2.4. Anthropometric characterization, $\text{VO}_2\text{max}$ estimation test and blood lactate analysis

Participants were weighed, height measured and had body fat (%) calculated according to a previous described equation [29] with the use of calipers (Holtain Skinfold Caliper, UK). For the  $\text{VO}_2\text{max}$  estimation futsal players performed the Yo-Yo R2 test as previously described [28]. The  $\text{VO}_2\text{max}$  was estimated applying the specific equation  $\text{VO}_2\text{max} (\text{mL/kg/min}) = \text{distance (m)} \times 0.0136 + 45.3$ . Total distance covered during the test was measured and the heart rate (HR) was registered with specific monitors (Polar Team Sport System, Polar-Electro OY, Kempele, Finland). Blood for lactate analysis was collected from the index finger and analyzed before, 3 and 5 min after each Yo-Yo IR2 test (Accutrend Lactate, Roche, Basel, Switzerland).

### 2.5. Blood sampling and biochemical analysis

Blood samplings were obtained from the antecubital vein 48 h after an official match (8 am) throughout the study using appropriate collection tubes (Vacutainer®, Beckton Dickinson, Rutherford, NJ, USA). For the pretest collection, athletes were instructed to fast for 12 h (overnight) before sampling [30]. After the pretest blood sampling, all players were served an ordinary breakfast (bread, milk, butter, ham and cheese) before proceeding to the Yo-Yo IR2 test (45 min after breakfast). This methodology was applied in order to avoid antioxidant supplementation before the test, which could bias the obtained results. The posttest blood sample was taken immediately after the Yo-Yo IR2 test. The methodological approach of a pre and post data sampling of specific biomarkers lays on the basis that physiological responses to a challenge, such as an all-out test, reflect training adaptations [31].

Blood samples were centrifuged  $1500 \times g$  for 10 min for serum/plasma isolation in order to perform biochemical analysis. Triglycerides and total cholesterol were calculated by standard commercially available kits (Labtest, Lagoa Santa, Brazil). LDL cholesterol was estimated with the Friedewald equation [32]. Serum HDL-cholesterol (coefficient of variation [CV]  $\leq 3.7\%$ ), LDH (CV  $\leq 4.3\%$ ), CK (CV  $\leq 9.9\%$ ) and CRP (CV  $\leq 7.3\%$ ) levels were calculated using standard methods on a Cobas MIRA® automated analyzer (Roche Diagnostics, Basel, Switzerland). Serum IMA (CV 4.3–5.2%) and AOPP (CV 5.8–7.4%) levels were measured by a colorimetric spectrophotometric analysis as previously described [33,34]. Hematocrit (Hct), hemoglobin concentration (Hb), and total and differential counts of red (erythrocytes) and white blood cells (neutrophils, lymphocytes and monocytes) were determined using an automated hematology analyzer (Coulter Juniors JS, Coulter Electronics, Delkenheim, Germany).



**Fig. 1 – Experimental design of the study.** Participants performed a Yo-Yo R2 test in three different periods over the season: preseason (T1), two weeks before the FIFA® Intercontinental Futsal Cup 2012 (T2), and at the end of the season (T3), with a tapering period of one week before T2. Functional, anthropometric, and blood parameters were evaluated just at pre T1 (baseline). Blood lactate, damage markers, and oxidative-inflammatory parameters were evaluated before (pre) and after (post) each Yo-Yo R2 test.

## 2.6. Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 17, Ins, Chicago, IL, USA) was used for the statistical analyses. Data were expressed as mean  $\pm$  standard deviation of means (SD). The Shapiro–Wilk test was used to confirm the normality of quantitative variables. With normal data, significance was assessed by one (parameters displayed in [Tables 1 and 2](#)) – or two-way ([Fig. 2](#) and [Table 3](#)) analysis of variance for repeated measures (ANOVA), followed by Bonferroni test for post hoc comparison when appropriate. Cohen's *d* for effect size was performed, and statistical power was calculated as previously described elsewhere [35]; Pearson correlation (*r*) was also calculated. Significance was set at  $P < 0.05$  and *F* values are shown where differences were found.

## 3. Results

### 3.1. Participants characterization, lipid profile, and performance parameters

[Table 1](#) depicts the anthropometric and lipid profiles of players at each blood sampling throughout the season. As appreciated, no differences between time points were observed in this study.

Blood lactate levels revealed a significantly increased 3 and 5 min compared to basal level after each Yo-Yo IR2 test, although no changes were found between season's time [F(8,42) = 37.17;  $P < 0.05$ ; [Fig. 2](#)].

**Table 1 – Basal anthropometric and lipid profiles of elite level futsal players during a season (*n* = 8).**

Parameter	T1	T2	T3
Weight, kg	73.61 $\pm$ 4.9	74.26 $\pm$ 5.9	73.81 $\pm$ 5.9
Height, m	1.76 $\pm$ 0.04	1.76 $\pm$ 0.04	1.76 $\pm$ 0.04
Body fat, %	11.9 $\pm$ 1.5	11.84 $\pm$ 1.6	11.85 $\pm$ 1.5
VO <sub>2</sub> max, mL/kg/min	55.7 $\pm$ 2.8	56.6 $\pm$ 2.8	56.2 $\pm$ 3.5
Heart rate, bpm	184.5 $\pm$ 12.2	181.3 $\pm$ 9.5	179.5 $\pm$ 16.6
Distance run, m	764.4 $\pm$ 206.7	833.3 $\pm$ 203.1	803.3 $\pm$ 253.8
Cholesterol, mg/dL	133.9 $\pm$ 16.0	137.1 $\pm$ 9.3	165.0 $\pm$ 8.6
HDL-C, mg/dL	43.5 $\pm$ 8.2	44.8 $\pm$ 3.5	35.0 $\pm$ 6.2
LDL-C, mg/dL	74.6 $\pm$ 8.1	72.3 $\pm$ 7.2	108.2 $\pm$ 6.8
Triglycerides, mg/dL	78.9 $\pm$ 15.4	100.3 $\pm$ 22.2	109.3 $\pm$ 20.4

Values are mean  $\pm$  standard deviation. bpm, beats per minute; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

## 3.2. Whole blood parameters

[Table 2](#) presents the hematological parameters of the players throughout the season. Increases in the number of erythrocytes as well as in hemoglobin levels were found at T2 comparing to T1 and T3 time points [F(2,18) = 1.682;  $P < 0.05$  and F(2,21) = 2.248;  $P < 0.05$ , respectively]. In addition, total number of monocytes were found reduced at T2 when compared to T3 levels [F(2,21) = 3.265;  $P < 0.05$ ].

## 3.3. Biochemical markers of muscle damage

Both CK and LDH levels were unaltered when comparing pre and post Yo-Yo IR2 test levels at the same season's time points. Nevertheless, when comparing different season's time points, T2 registered both lower CK and LDH levels when compared to T1 and T3 at both pre and post Yo-Yo IR2 test [F(5,42) = 2.311;  $P < 0.05$  and F(5,42) = 28.55;  $P < 0.05$  in [Table 3](#), respectively]. Significant correlations were found at T2 time season: a positive correlation ( $r = 0.796$ ,  $P < 0.05$ ) between CK/PCR on pre-Yo-Yo IR2 tests; positive correlations between CK/LDH ( $r = 0.746$ ,  $P < 0.05$ ), PCR/LDH ( $r = 0.753$ ,  $P < 0.05$ ) and CK/PCR ( $r = 0.874$ ,  $P < 0.01$ ) on pre-Yo-Yo IR2 tests.

## 3.4. Biochemical markers of oxidative stress and inflammation

The data demonstrated a time-dependent decrease on IMA levels throughout the season. IMA levels at T2 posttest were decreased when compared to T1 posttest. Similarly, IMA levels at T3 pretest were also decreased when compared to T1 pretest. Finally, IMA levels at T3 posttest were decreased when compared to T1 posttest [F(5,42) = 10.03;  $P < 0.05$ ; [Table 3](#)].

For AOPP measurements, only a T3 pre  $\times$  posttest effect was observed [F(5,42) = 4.612;  $P < 0.05$ ; [Table 3](#)]. CRP data presented a season effect, indicated by significant decrease on T2 levels when compared to T1 at both pre and posttest measurements [F(5,38) = 5.758;  $P < 0.05$ ; [Table 3](#)].

## 4. Discussion

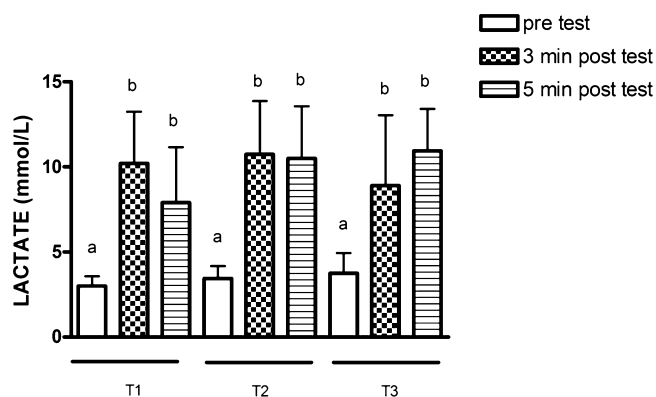
This study describes functional and exercise-related biochemical parameters during a competition season on Brazilian elite level futsal players. Although no differences were observed for the functional parameters (weight, height, body fat, VO<sub>2</sub>max, heart rate and distance run), the obtained data showed favorable biochemical adaptations throughout the

**Table 2 – Blood cells count in elite level futsal players before each Yo-Yo IR2 test performed (*n* = 8).**

Parameter	T1	T2	T3
Erythrocytes, $\times 10^6/\text{mm}^3$	4.64 $\pm$ 0.30 <sup>a</sup>	4.99 $\pm$ 0.40 <sup>b</sup>	4.69 $\pm$ 0.42 <sup>a</sup>
Hematocrit, %	44.06 $\pm$ 1.75	44.86 $\pm$ 3.53	44.21 $\pm$ 3.38
Hemoglobin, g/dL	14.29 $\pm$ 0.78 <sup>a</sup>	15.29 $\pm$ 1.09 <sup>b</sup>	14.51 $\pm$ 1.09 <sup>a</sup>
Leukocytes, $\times 10^3/\text{mm}^3$	5.70 $\pm$ 2.05	6.19 $\pm$ 1.58	5.36 $\pm$ 1.64
Neutrophils, $\times 10^3/\text{mm}^3$	3.09 $\pm$ 1.47	3.11 $\pm$ 1.29	2.79 $\pm$ 1.15
Lymphocytes, $\times 10^3/\text{mm}^3$	2.22 $\pm$ 0.58	2.73 $\pm$ 0.76	2.19 $\pm$ 0.61
Monocytes, $\times 10^2/\text{mm}^3$	1.36 $\pm$ 0.51 <sup>ab</sup>	0.81 $\pm$ 0.39 <sup>b</sup>	1.56 $\pm$ 0.40 <sup>a</sup>

Values are mean  $\pm$  standard deviation. Different letters indicate significant differences.





**Fig. 2 – Blood lactate levels before and after (3 and 5 min) Yo-Yo IR2 tests throughout different time points of the season. Values are means  $\pm$  SD ( $n = 8$ ). Means without a common letter differ significantly ( $P < 0.05$ ).**

season herein seen as skeletal muscle damage (CK and LDH), oxidative stress (IMA and AOPP), and inflammation markers (CPR). In this line, the biochemical markers analysis reflected a planned training schedule according to the main goal of the team along the season, indicating positive training adaptations. To our knowledge, this is the first study to analyze multiple exercise- and damage-related biochemical markers on elite level futsal players throughout an entire season.

Tapering strategies are vastly used in a wide range of sports in order to enhance performance edge over competitions [23,24]. In this sense, taper's main goal is to decrease intense training-related fatigue while maximizing physiological adaptations, which positively impacts on performance outcomes [23]. For that purpose, tapering has been pointed out as a suitable strategy in team sports to optimally prepare before an international championship [36] similarly to the observed adaptations herein seen at T2, a week before the FIFA® Intercontinental Futsal Cup 2012. An interesting meta-analysis pointed out that a reduction of 41–60% on training volume was associated with maximal performance gains and such reduction should be applied on sessions' duration [23]. In the present study, training volume was lowered by 50% before the main competition of the year, which was associated with decreases in relevant biochemical markers in sports, such as CRP and CK. Similarly, Coutts et al. [37] applied a 55% decrease on training time of professional rugby players within the same age range and  $VO_{2max}$  of the futsal players herein described. Tapering in the study by Coutts et al. also lasted 7 days and biochemical markers, such as CK, were positively adapted as well.

The anthropometric profile did not change throughout the season, and similar values for  $VO_{2max}$  and HR were previously described for similar populations [38]. On the same line, a slight erythrocyte count and hemoglobin levels were increased at T2 despite stable hematocrit levels throughout the season, showing a positive effect of the tapering strategy. Surprisingly, no changes on estimated  $VO_{2max}$  were found on T2, apart from the well-known relationship among erythrocytes, hemoglobin and oxygen uptake in sports [39,40]. Although recent studies have shown that Yo-Yo IR2 test is reproducible and correlates well with match performance [28,41], we have not

found articles that compared estimated Yo-Yo R2 test  $VO_{2max}$  values with erythrocytes and/or hemoglobin levels in futsal players. In this regard, it is important to mention that changes on red blood cells are important in order to enhance  $VO_{2max}$ , although several other factors are also relevant, such as capillary density and peripheral diffusion gradients [42]. Considering that highly trained individuals adapt more slowly during the season [43] and are able to maintain  $VO_{2max}$  despite the imposed stress [44], we hypothesized improvements on erythrocytes and hemoglobin have afforded advantages upon other physiological parameters also relevant to exercise performance, such as red blood cell mediated vasodilation and oxygen unloading to exercising muscles [40].

The understanding of specific skeletal muscle-related damage markers during exercise may help on increasing the training control feedback, assisting coaches and physicians during the long and tough training season on elite level modalities. The serum cytosolic enzymes CK and LDH reflect the muscle damaged caused by exercise, which may be detected in the bloodstream [8]. Strenuous exercise damages the muscle cell structure [45] and results in increased total CK in the serum [46] while increases on LDH levels during exercise rely on intensity and duration of the effort [47]. In this sense, direct muscle damage may be assessed by serum cytosolic enzymes CK and LDH which reflect the functional status of the tissue [48]. In fact, both CK and LDH serum levels have been shown to increase in response to a futsal match due to muscle damage and inflammation [25].

In this study, no significant changes were observed in CK or LDH between before and after Yo-Yo IR2 tests, at any given time point of the season. However, a season effect was found for CK levels when comparing T2 and T1 pre-test and T2  $\times$  T3 pre and post-test, indicating overall positive effect of the tapering strategy applied on the week before the FIFA® Intercontinental Futsal Cup 2012. On a similar trend, LDH levels were decreased at T2 comparing to T1 and T3, which also indicates a positive adaptation to the tapering. The decrease on circulating cytosolic enzymes has long been attributed to exercise training adaptations [49]. In addition, a positive correlation between CK and LDH was observed in T2, reinforcing the effectiveness of the tapering.

**Table 3 – Biochemical markers of muscle damage, inflammation and oxidative stress before (pre) and after (post) Yo-Yo IR2 tests throughout the season in elite level futsal players during a season (n = 8).**

Parameter	T1			T2			T3		
	Pre	Post	Effect size	Statistical power	Pre	Post	Effect size	Statistical power	Effect size
IMA, UABS	0.69 ± 0.35 <sup>a</sup>	0.67 ± 0.23 <sup>a</sup>	0.054	0.052	0.51 ± 0.09 <sup>ab</sup>	0.34 ± 0.11 <sup>bc</sup>	1.809	0.952	0.28 ± 0.08 <sup>bc</sup>
AOPP, µmol/L	24.14 ± 10.85 <sup>ab</sup>	25.5 ± 8.52 <sup>ab</sup>	0.125	0.058	38.09 ± 16.91 <sup>ab</sup>	52.7 ± 28.28 <sup>ab</sup>	0.864	0.409	22.48 ± 5.66 <sup>a</sup>
CRP, mg/L	4.75 ± 3.15 <sup>a</sup>	5.15 ± 3.4 <sup>a</sup>	0.127	0.058	0.6 ± 0.32 <sup>b</sup>	0.62 ± 0.4 <sup>b</sup>	0.079	0.073	2.26 ± 2.02 <sup>ab</sup>
CK, U/L	446.5 ± 192.3 <sup>ac</sup>	493.1 ± 200.6 <sup>ac</sup>	0.678	0.273	271.3 ± 212.9 <sup>b</sup>	413.6 ± 358.5 <sup>ab</sup>	1.593	0.891	754.8 ± 569.2 <sup>cd</sup>
LDH, U/L	359.6 ± 40.39 <sup>a</sup>	388 ± 41.38 <sup>a</sup>	0.703	0.306	175.3 ± 26.07 <sup>b</sup>	232.6 ± 65.57 <sup>b</sup>	2.198	0.996	395.3 ± 73.39 <sup>a</sup>

Values are mean ± standard deviation. IMA, ischemia modified albumin; AOPP, advanced oxidation protein products; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase. Different letters indicate significant differences.

Regarding inflammation status, it has been proposed that blood lactate levels mediate the exercise reduction in IMA during exercise [50]. The mechanism proposed by the authors associated changes on lactate concentrations ranging between 3 and 11 mmol/L with reduced IMA values [51]. Similarly, in this study increases on lactate levels 3 min after the Yo-Yo IR2 tests were approximately 11 mmol/L, and IMA values progressively decreased throughout the season. Other studies have also pointed out decreases on IMA values after different types of exercise [52,53]. More recently, IMA kinetic values on plasma after a Bruce treadmill protocol also shown reduced values immediately after exercise [54]. In this regard, Apple and coworkers [15] did not find IMA values to increase 30 min a marathon, but 24–48 h post exercise similarly to data described by Middleton and colleagues [50]. According to authors, IMA values are not likely to increase after exercise partially due to skeletal muscle ischemia produced by the effort [15]. These are important data once exercise-related IMA increases have been proposed to reflect an insult to the cardiac tissue [14]. Despite the lack of data on chronic exercise effects on IMA levels, the results herein found are interesting considering that increased IMA levels may be also linked to oxidative stress [55].

Similarly, AOPP is one of the most reliable by-products of ROS-mediated protein oxidation, and may be related to both hypoxia [56], and inflammation [34]. Accordingly, AOPP was proved to be increased after exercise during hypoxia, which was accompanied by augmented oxidative stress [57]. Corroborating the obtained IMA results, reduced AOPP plasma levels were also found at T3 pretest. Together with IMA data, this result may indicate reduced oxidative stress levels at the end of the season, possible due to adaptation mechanisms as previously reported in different scenarios [30]. Currently, it is well accepted that exercise training decreases oxidative stress through an upregulation in the antioxidant systems, thereby halting the overproduction of oxidants and enhancing the antioxidant defenses [16]. Considering training in top level modalities may induce oxidative stress due to high working loads [58], the results of IMA and AOPP corroborate the tapering strategy applied before the FIFA® Intercontinental Futsal Cup 2012. In this sense, the relevance of these results may be reflected on the current knowledge that 35% of muscle damages during a futsal world cup were reported to be caused by non-contacting activities [59].

Additionally, it is well known that increased levels of oxidative stress are correlated with muscle damage [60], which is highly associated with inflammation. The CRP is a classic acute-phase protein, which plasmatic concentrations greatly increase during the inflammation course, such as after an intense exercise bout [61]. In this study, CRP values showed a season effect reflecting a positive adaptation to the tapering. Similarly, decreased CRP levels were found after a 9 months marathon training in male runners [9], and have been also described in well-trained athletes in comparison to control subjects [10]. This CRP reduction may be linked to the anti-inflammatory role of regular exercise [62], herein characterized by the well-planned training season. In this context, CRP levels along the season indicate that players were adapted to the imposed training load throughout the year. In fact, a positive correlation between CPR and CK levels was observed, corroborating that players coped well with training load. In

addition, we also found a reduced monocyte count at T2. Considering acute and intense exercise increases the number of monocytes and inflammation status, recent studies have suggested that training reduces the monocytes production and infiltration, which has been postulated to partially explain the anti-inflammatory role of regular exercise [63].

## 5. Conclusions

The tapering strategy applied a week before the most important championship of the year succeeded from a biochemical perspective, considering most markers were positively adapted. Interestingly, these players won the Intercontinental Futsal Cup 2012 a week later. We are all aware that success on team sports depends on several traits (e.g., technical and tactical skills, resilience, etc.), for what only a casual association may be established from these data. Thus, we hypothesized that observed biochemical changes herein described have also contributed to winning the tournament considering physical condition plays a decisive role in futsal as well.

A greater number of participants could have increased the statistical power of the discussed results. However, professional futsal players at this level (these were actually the worlds' best players during 2012) are very difficult to access; also futsal is a team sport with few participants. Additionally, considering blood sampling was performed, inclusion criteria were very strict in order to avoid major data bias; within this line, injured players were immediately ruled out of the study for the same reasons. As such, future studies with more futsal players and a broader range of inflammation, oxidative stress and damage-related markers are of interest to draw a more accurate map of the biochemical control of training load for futsal.

## Conflict of interest

The authors report no conflicts of interest.

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