

## Plasmatic and urinary analysis of muscle damage biomarkers in professional futsal athletes

### Análise sérica e urinária de biomarcadores de dano muscular em atletas profissionais de futsal

Luciano de Oliveira Siqueira<sup>1</sup> , Maria Eduarda Kegler Ramos<sup>1</sup> , Daniel Schwarzbach<sup>2</sup> ,  
Lucas Marostica<sup>3</sup> , Jose Claudio Fonseca Moreira<sup>4</sup> 

1. Universidade de Passo Fundo, Passo Fundo, RS, Brazil

2. Farmacêutico na cidade de Erechim, RS, Brazil

3. Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

4. Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

#### ABSTRACT

**Introduction:** In the view of metabolic changes resulting from sport and the consequences of overcoming individual limits in elite team sports, it is necessary to understand biochemically the phenomenon that occurs in this modality in order to minimize damage, improve athletic performance and provide protocols more suitable for every sport and individual. **Aim:** The present study aimed to analyze the metabolism of professional athletes during training by determining blood and urinary biomarkers of proteolysis, lipolysis, hemolysis and muscle microtrauma. **Methods:** The evaluation was carried out with 12 athletes participating in the Gold series of a futsal league. All were submitted to a standard training protocol and blood and urine samples were collected at rest and 15 minutes after the training session. **Results:** The statistical analysis of the results showed a significant increase ( $p < 0.05$ ) in the relative (cells /%) and absolute (cells/ $\mu$ L) counts of neutrophils, as well as in the serum concentration of uric acid, total cholesterol and HDL fractions, ALT, LDH and CK-MM. On the other hand, it also showed a statistically significant decrease ( $p < 0.05$ ) in the concentration of magnesium, glucose, urinary urea, relative lymphocyte count, absolute monocyte and eosinophil count (cells /% and cells/ $\mu$ L). **Conclusion:** The results obtained here allowed us to conclude that biochemical understanding can minimize muscle damage, improve athletic performance and offer the development of more appropriate protocols for each sport.

**Keywords:** biochemistry; sports medicine; athletic performance.

#### RESUMO

**Introdução:** Diante das alterações metabólicas advindas do esporte e as consequências da superação dos limites individuais nos esportes coletivos de elite, faz-se necessária a compreensão bioquímica dos fenômenos ocorridos nessa modalidade a fim de minimizar danos, aperfeiçoar o desempenho atlético e fornecer protocolos mais adequados para cada esporte e indivíduo. **Objetivo:** O presente estudo objetivou analisar o metabolismo de atletas profissionais durante o treinamento mediante determinação de biomarcadores sanguíneos e urinários de proteólise, lipólise, hemólise e microtrauma muscular. **Métodos:** A avaliação foi realizada com 12 atletas que participam na série Ouro da liga de futsal. Todos foram submetidos a um protocolo padrão de treinamento e amostras de sangue e urina foram coletadas em repouso e 15 minutos após a sessão de treinamento. **Resultados:** A análise estatística dos resultados mostra um aumento significativo ( $p < 0,05$ ) nas contagens relativa (células/%) e absoluta (células/ $\mu$ L) de neutrófilos, bem como na concentração sérica de ácido úrico, colesterol total e frações HDL, ALT, LDH e CK-MM. Por outro lado, apresentaram uma diminuição estatisticamente significativa ( $p < 0,05$ ) na concentração de magnésio, glicose, ureia urinária, contagem relativa de linfócitos, contagem absoluta de monócitos e eosinófilos (células/% e células/ $\mu$ L). **Conclusão:** Os resultados aqui obtidos permitem concluir que a compreensão bioquímica pode minimizar dano muscular, aperfeiçoar o desempenho atlético e oferecer formação de protocolos mais adequados para cada esporte.

**Palavras-chave:** bioquímica; medicina esportiva; desempenho atlético.

## Introduction

According to World Health Organization (WHO) physical activity promotes mental (such as reducing stress [1]) and physical health. In that sense, practicing regularly can promote several benefits [1,2]. The High Performance Sports (HPS) is an elite sports practice in which the athlete dedicates a significant amount of training time to reach the maximum performance and results, being subjected to heavy training and stress rounds [3]. Therefore, in the view of alterations from metabolic bodily activities in sports and its relevance when combined with the correct practice it is vital to comprehend the biochemistry of processes that occur during elite collective sports aiming to minimize muscular damage, improve athletic performance and provide proper protocols to each sport and individual.

The HPS practice, if based only in results, can be harmful since the athletes are submitted to heavy loads with small recovery periods with no respect for its individualities and striving to overcome its limits [4]. In intense physical exercise, a metabolic stress is created due to an increased energetic demand with a more substantial synthesis of oxidants. The high muscular activity results in an increase of oxygen reactive species that exceed the body's antioxidant capacity therefore promoting an oxidation that can generate muscular damage with reverse consequences such as strength loss and decreased athletic performance [5,6].

Plasmatic and urinary analysis of biochemical markers can contribute to finding and monitoring this overload since HPS can increase the plasmatic rate of muscle damage marker enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH); as well as signaling the tightening of muscle fibers, injuries, inflammations and adaptive micro-traumas [7]. In this context, futsal is a sport with predominant demand of the aerobic system, however, it consists of high intensity actions that demands great physical, tactical and technical level from its players with the supportive use of the anaerobic metabolism [8,9]. Thus, the occurrence of traumatic events or injuries is common during matches [10].

Sports medicine has advanced in the last decades especially for its emphasis in health, improving quality of life, injury prevention and maximization of performance of training for professional or amateur athletes [11]. Through a specialized treatment and physiological and biochemical comprehension of this athlete's routines, a multi-professional team evaluate and manages practice with specialized diets, adjusting its intensity and duration. This can produce positive results when preventing, treating and rehabilitating injuries besides maximizing the athlete's performance. That way, the athlete can reach more competitive results that, when combined with a healthy lifestyle, can contribute to its adaptation.

Based on such premises, the present study aimed to analyze the metabolism and biochemical effects of futsal sport in professional athletes during training by determining plasmatic and urinary biomarkers of proteolysis, lipolysis, hemolysis, and muscular micro-trauma.

## Methods

This is a cross-sectional, prospective and experimental study of a futsal team participating in the National Futsal League. Twelve male athletes members of a futsal team participating in the National Futsal League participated in this study. The individuals included in the experiment were starting and reserve athletes with no history of injuries within the past year; professional athletes for over 2 years; training regularly at the team for over 1 year; with a diet controlled and oriented by a nutritionist. Athletes removed because of injuries; using medication; with recent injury and with chronic illness such as diabetes and high blood pressure were excluded.

A convenience sample was used since the sample characteristics were very specific (athletes from a futsal team) and homogeneous (elite participating in the National Futsal League).

### *Ethical aspects*

This research was developed according to declarations and guidelines regarding research with human beings: the Nuremberg Code, Helsinki Declaration and the National Health Council resolution nº 466/12. This research was also approved and regulated by the Universidade de Passo Fundo Ethics Committee, with an approved CAAE 18778119.5.0000.5342, opinion nº 3.688.601.

### *Environmental conditions*

The training session was performed during the morning at the sports court with wooden floor (team's headquarters) and lasted approximately 90 minutes. The tests were conducted with 15°C of room temperature, 88% of relative air humidity (rainy day) and 783 meters above sea level. The athletes were dressed in light training clothes (dry fit).

### *Training protocol*

The training protocol included approximately 30 minutes of physical activity subdivided in 3 phases:

- 10 minutes of light run;
- 10 minutes alternating between 10 seconds of fast run and 20 seconds of light run;
- 10 minutes alternating between 30 seconds of light fast run and 90 seconds of light run.

After aerobic training the athletes performed 30 minutes of lower limbs exercises composed of three sets with 12 repetitions each and 45 seconds for recovery between machines.

### Blood samples

Blood samples were collected with antisepsis of the athlete's antecubital fossa while resting and 15 minutes after the end of the training session. A 2 mL sample was stored in flasks with 2 mg/mL of EDTA (ethylenediaminetetraacetic acid) for blood analysis. A full hemogram with platelets was carried out by impedance ABX micros 60® (ABX diagnostics, Montpellier, France) electronic counting of cells. A differential count of leukocytes was performed by microscopic analysis of 200 cells (Nikon Eclipse 600®, Nikon Corporate Instruments, Japan) in blood sheets colored with the Romanowsky (Merck®) technique. Differential analysis of cellular strains was expressed by relative (cells/%) and absolute (cells/ $\mu$ L) count.

To obtain the serum, the rest of the sample (8 mL) was stored in an anticoagulant test-tube. Then, the sample was centrifuged with 1500 rpm for 15 minutes. The serum was extracted and placed in test-tubes pre-treated with nitric acid 30% for 24 hours and rinsed five times with double-distilled water in order to spectrophotometrically measure biochemical parameters such as calcium, phosphorus, magnesium, glucose, uric acid, creatinine, urea, total proteins, aminotransferases (ALT/AST), alkaline phosphatase, lactate dehydrogenase (LDL), total creatine kinase, MB creatine kinase, triglycerides, total cholesterol and fractions using enzymatic methods in commercial kits following the manufacturer norms (Labtest® Diagnostica AS - Belo Horizonte, Brazil) with a semiautomatic equipment from Labquest® (Labtest® Diagnóstica AS, Belo Horizonte, Brazil). The sodium, potassium and chlorides were measured by the Medica EasyLite® (Medica Corporate Profile, Bedford, Massachusetts EUA) technique of selective ion electrode.

### Urine samples

A sample of approximately 50mL of urine, medium stream with previous rinsing of the genitals was collected in universal collector bottles of resting athletes and 15 minutes after the training session. The samples were collected in standard bottles that were transferred from the collection site to the laboratory under controlled temperature. Then, they were immediately processed and analyzed with a microscope as recommended by the ABNT-CB 36 (in vitro). After, samples were centrifuged with 1800rpm for 10 minutes and 1 mL of supernatant was reaped for later biochemical analysis of total urinary proteins, uric acid, creatinine and urea (Labtest® Diagnóstica S.A.).

Physical-chemical analysis was performed by visual observation of the urine aspects and color and density was measured by hand refractometer (LF® Equipamentos hospitalares, São Paulo, Brazil).

Chemical analysis was carried out with polyelectrolytes testing stripes (ComburTest Dade-Behring®) to determine blood proteins, glucose, bilirubin nitrite, pH, ketones, leukocytes, and urobilinogen within the non-centrifuged sample.

Microscopic analysis examined the urinary sediment searching for epithelial cells, crystals, leukocytes, erythrocytes, bacteria, mucus wiring.

### Statistical analysis

Results were transposed to a spreadsheet for analysis of central tendency (mean) and dispersion (standard deviation) measures. Data were analyzed for normality by the *Kolmogorof-Smirnov test*. Variables with Gaussian distribution were analyzed statistically by comparing the means with a “t” test for paired samples (parametric data). For variables without regular distribution, the *Wilcoxon-Mann-Whitney test* was applied (non-parametric data), considering  $p < 0.05$  as the minimum level of significance.

## Results

The mean age was  $25.5 \pm 4.8$  years; with  $170 \pm 4$  cm height;  $74.6 \pm 7.6$  kg body weight; and BMI of  $24.2 \pm 1.9$  kg/m<sup>2</sup>. All subjects were training for more than two years using athletic shoes provided by the team’s sponsoring company and were in great physical condition. Besides, all subjects were not using any medication or supplements that could have interfered in the analysis and outcomes of the study. All athletes were resting within 48h prior to the training session.

**Table I** – Analysis of the acute effects of training in biochemical and blood parameters of athletes while resting and 15 minutes after the training session

Markers	Resting	After exercise	P
Lymphocytes relative count (cells/%)	49.3 ± 2	35.1 ± 2	< 0.001*
Eosinophils relative count (cells/%)	4.0 ± 0.7	1.9 ± 0.4	0.001*
Segmented neutrophils relative count (cells/%)	43.3 ± 2.8	60.1 ± 2,7	< 0.001*
Segmented neutrophils absolute count (cells/μL)	2604.5 ± 224	3556.0 ± 291	0.008*
Monocytes absolute count (cells/μL)	312.2 ± 17	211.6 ± 18	< 0.001*
Eosinophils absolute count (cells/μL)	263 ± 4	117 ± 3	< 0.001*
Magnesium (mg/dL)	1.9 ± 0.1	1.2 ± 0.1	0.001*
Glucose (mg/dL)	92.7 ± 4.8	65.3 ± 3.4	< 0.001*
Uric acid	4.6 ± 0.2	6.0 ± 0.6	0.01*
Cholesterol(mg/dL)	179.4 ± 9.4	197 ± 10	0.01*
HDL cholesterol (mg/dL)	46.1 ± 3	57.2 ± 5	0.003*
Alanine aminotransferase (UI/L)	12.5 ± 0.8	19.3 ± 1.3	< 0.001*
Lactate dehydrogenase (UI/L)	126.9 ± 15	214 ± 15	0.002*
MM creatine kinase (UI/L)	251.8 ± 19	312 ± 26	0.04*
Urinary urea (mg/mg creatinine)	40.7 ± 4.2	36.3 ± 3.6	0.03*

Results are presented as ± DP. \*  $p < 0,05$  regarding the resting athletes by analysis of parametric data with “t” test for paired samples

Table I presented the analysis of acute training effects in biochemical and blood parameters of athletes while resting and 15 minutes after the training session. Results demonstrated a statistically significant increase ( $p < 0,005$ ) in relative



(cells/%) and absolute (cells/ $\mu$ L) count for segmented neutrophils, uric acid plasmatic concentration, total cholesterol, HDL cholesterol, ALT, LDH, CK-MM, that seems to be associated with a statistically significant decrease ( $p < 0.05$ ) in concentrations of magnesium, glucose and urinary urea, lymphocytes relative count, monocytes absolute count and eosinophils relative and absolute counts.

However, in parameters such as: red blood cells, hemoglobin, hematocrit, MCH, MCV, MCHC, platelets, proteins, urea and plasmatic and urinary creatinine, uric acid, AST, calcium and plasmatic chlorine, LDL and VLDL cholesterol, triglycerides, alkaline phosphatase, phosphorus, total creatine kinase and MB fraction, iron and potassium counts did not present a significant difference ( $p > 0.05$ ) between resting and after exercise (data not shown).

**Table II** – Analysis of the acute effects of training in physical and chemical parameters of urine in athletes while resting and 15 minutes after the training session

Parameters	Resting	After exercise	P
Hyaline cylinders (minimum n <sup>o</sup> )	0.0 $\pm$ 0.0	2.9 $\pm$ 3.9	0,02*
Hyaline cylinders (maximal n <sup>o</sup> )	0.0 $\pm$ 0.0	5.8 $\pm$ 2.0	0,007*
Ketones	0.1 $\pm$ 0.03	1.5 $\pm$ 1.0	0,01*
Mucus	0.7 $\pm$ 0.5	1.7 $\pm$ 0.8	0,01*

Results are presented as  $\pm$  DP. \*  $p < 0,05$  regarding the resting athletes by the Wilcoxon-Mann-Whitney test for non-parametric data

On the other hand, Table II analyzed the effects of acute training in physical and chemical parameters of urine in athletes while resting and 15 minutes after the training session. Results showed a statistically significant increase ( $p < 0,05$ ) in the amount of mucus wiring and hyaline cylinders, indicating a possible reduction in urinary flow due to the loss of renal water induced by sweating, besides an increase in urinary ketones indicating a lipolytic scenario.

In contrast, variables of physical-chemical analysis of urine, such as: color, aspect (turbidity), density, leukocyte reaction, urobilinogenin, pH, red blood cells and hemoglobin, bilirubin, glucose, epithelial cells microscopic analysis, amorphous urates, uric acid crystals and calcium oxalate crystals; did not show any significant difference ( $p < 0,05$ ) (data not shown).

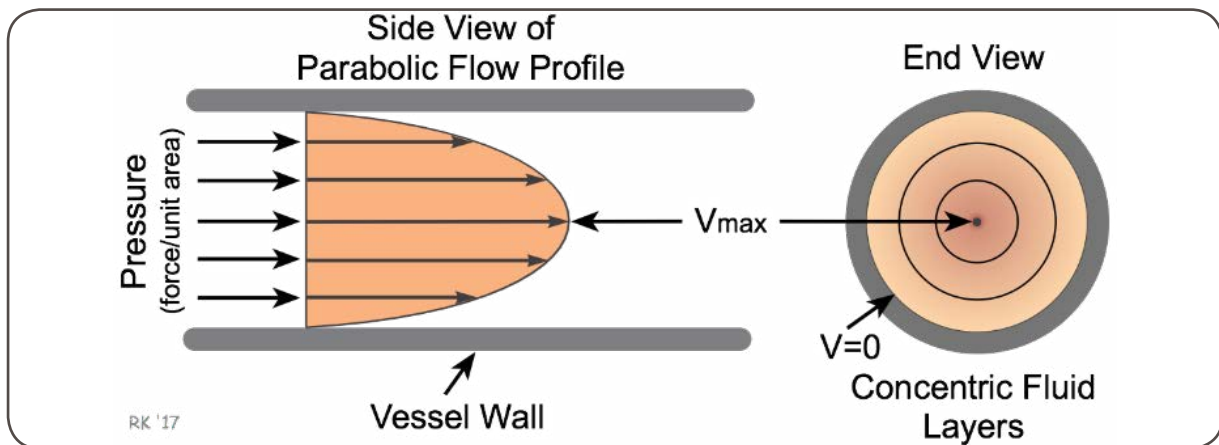
## Discussion

Sports practice generates several benefits when conducted with planning, adapting the individual's metabolism to physiological and biochemical alterations to each type, intensity and duration of training. The lack of adequacy can promote damage to the organism, such as increasing the susceptibility for muscular injuries, inflammatory diseases, reduction of antioxidant capacity, loss of muscular mass and strength, fatigue and decreased athletic performance [12-14].

The red blood cells analysis from hematologic profile did not present any significant difference in post-training when compared to resting for the following parameters: hemoglobin, erithrometry, hematocrit, MHC, MCV, MCHC and plasma-platelets in this investigation. Those findings are supported by Bezerra [12], that evaluated biochemical parameters of 42 professional male soccer athletes prior, after, 24, 48 and 72 hours after the match, and did not find significant differences in such parameters. This is a basic analysis for diagnosing anemia being an indicator for oxygenation and hydration in sports medicine. In that sense, erythropoietin, for example, is a hormone that raises the red series numbers, improving the oxidant capacity – its exogenous implementation is considered doping [15]. The hematocrit measures the relationship between plasma/cell and is roughly affected by hydration. Therefore, rises in the hematocrit value seems to be relate to dehydration and hemoconcentration [16]. The results analysis did not show any significant difference regarding the mild room temperature (15°C) in training day and adequate hydration.

Also within Bezerra [12] study, its results demonstrated that in the collection performed 24h after practice there was a small reduction of hematocrits, hemoglobin and erythrocytes that may be justified by hemodilution [16] and mechanical stress that can accentuate hemolysis. The iron indexes, an essential ion for hemoglobin synthesis and oxygen transportation, remained with no significant difference in this study.

Table I presents a statistically significant increase in relative and absolute counts of segmented neutrophils combined to a significant reduction in relative count of lymphocytes and eosinophils and absolute count of eosinophils and monocytes. Physical exercise activates the hypothalamus–pituitary –adrenal axis [17] inducing an inflammatory response characterized by leukocytosis that can be caused, among others factors, by the increase of neutrophils whom have as its function to remove undesirable elements related to tissue injuries by phagocytosis [12,18]. Blood flow has a laminar characteristic, its viscosity and cellular elements that generates friction with the blood vessel walls generating a shear force (Figure 1). Thus, smaller elements as red blood cells, run in an axial way at the center of the flow surrounded by a plasma stream where circulation leukocytes run. At the edge, marginal leukocytes are located due to the plasma's viscosity and shear force that makes them dislocate with a lower speed that the circulation pool. The catecholamines elevation promotes hemodynamic changes inducing the marginal leukocytes pool inside the blood vessel to migrate combined with the leukocytes in the middle of the vessel, justifying the development of a neutrophil leukocytosis associated with a reduction of monocytes, lymphocytes and eosinophils reported here [19].



Source: Cardiovascular Physiology Concepts [34]

**Figure 1** - Laminar blood flow

According to Table I, the protein, uric acid and creatinine urinary concentration analysis did not show any significant difference after practice. This finding responds to the study of Neto [13] that reported that the HIE (High Intensity Exercise) induced a scenario of proteinuria, cylinders and red blood cells within the urine samples. This increase was attributed to a possible vasoconstriction of the renal circulation altering the hydrostatic pressure at the glomerulus also promoting the passing of proteins and red blood cells through the glomerular basal membrane, especially when associated with a high protein diet. It can be concluded that the experimental protocol applied in this study was not intense enough to promote the alteration indicated above.

On the other hand, these results exposed a statistically significant reduction of urinary urea levels combined with an increase of the amount of mucus and urinary cylinders. Sports practice promotes an increase of non-renal hydric loss through sweating that can reduce the bodily hydric content, volemia and urinary debit promoting the gelation of Tamm Horsfall proteins within renal tubules inducing the formation of mucus and cylinders due to hydric loss [20].

The analysis of other urinary parameters: color, aspect (turbidity), density, leukocyte reaction, urobilinogen, pH, red blood cells and hemoglobin, bilirubin, glucose, epithelial cells microscopic analysis, amorphous urates, uric acid crystals and calcium oxalate crystals, did not evidence any significant difference. Those findings support the ones found by Neto [13] that also did not find any significant alterations regarding such variables after HIE. On the other hand, the proposed training promoted a statistically significant elevation in the number of urinary ketones that indicate a lipolytic scenario justified by the habit common among most athletes of training while fasting in the morning.

Among nitrogenous compounds, there was a significant increase in uric acid plasmatic concentration combined with a reduction of urinary urea. Physical activity promotes an increase in the metabolism of purine bases [21], that is biotransformed in uric acid via xanthine oxidase [22], that may act as an antioxidant. Hyperuricemia occurs when there is an increase of metabolism and/or reduction of the uric



acid elimination and can happen in disorders in homeostasis of sodium and water [21]. Plasmatic urea can result from proteolysis with a major renal excretion. The results analysis showed a decrease in urea's renal elimination, however its plasmatic concentration did not suffer significant alterations, indicating that the proteolysis scenario was incipient and that the urinary reduction may have occurred due to a volemic reduction scenario, especially when analyzed with the appearance of mucus and cylinders [23].

Regarding the lipid profile, Table I shows that the total cholesterol and HDL cholesterol had a significant increase after practice, while LDL cholesterol, VLDL and triglycerides did not. Our results correspond with existing literature, since practicing sports seems to improve the lipid profile, enhancing the muscular tissue capacity for using fatty acids and raising the activity of the lipoprotein lipase reducing the triglycerides levels and generating a balance between HDL and LDL cholesterol, being considered the first factor of protection against cardiovascular diseases [24,25].

Córdova [26] defined magnesium (Mg) as an essential mineral relevant for the energetic metabolism and for the muscular contraction and relaxation function, considered a co-factor of enzymatic reactions involved in anabolic and catabolic processes that affects the muscular performance [26,27]. In the present study, it was verified a statistically significant reduction in post-practice magnesium levels, as corroborated by Castro [14], that confirms the high loss of magnesium within blood and urine through urine and sweat in EAR. This reduction of magnesium levels associated with an elevation of creatine kinase (CK-mm) can lead to an understanding that the training protocol applied was characterized by a micro-injuries scenario. Besides, since Mg is a glycolytic route enzyme co-factor, there was also evidence that appropriate levels of Mg would indicate a fatigue scenario when associated to glycemic reduction and reports of it after training [27].

Muscular damage can be detected by analyzing the activity of muscular enzymes such as creatine kinase (total and fraction mm CK total), lactate dehydrogenase (LDH) or aspartate aminotransferase (AST) [28]. CK and LDH are heavy chain fragments from myosin and are related to muscular tissue injuries [29]. They are found in cell's cytosol and do not have the capacity to go through the sarcoplasmic barrier [28,29]. Total CK is an indirect marker of tissue damage that catalyzes the creatine transformation in phosphocreatine from adenosine triphosphate (ATP), used to detect muscular fatigue and overload. LDH is a marker of musculoskeletal injuries by muscular fibers disruption and can be understood as a depletion of strength and inflammation. Combined monitoring of CK and LDH levels shows the degree of metabolic adaptation of skeletal muscles [30].

Results analysis showed a statistically significant raise in the activity of the three enzymes. Studies from Barranco [28], Bezerra [12] and Ferreira [31] reported increasing in plasmatic concentration of CK, LDH and AST enzymes in athletes after futsal, soccer and cycling practices, respectively. The raise of muscular enzymes can signal structural alterations within muscular fibers indicating that practice is cau-

sing overload with resulting loss of muscular and athletic performance [31]. In the present study, there was a significant increase in plasmatic activity of CK-MM and LDH, while there was no significant difference in total creatine kinase, AST levels and CK-MB fraction. Such biochemical parameters can be used as strategy planning for muscular recovery of players as well as in the reduction of damages and consequent improved muscular performance.

Aminotransferases are hepatic enzymes that can suffer alterations in its levels after physical exercise, indicating hepatic, renal, cardiac, or muscular injuries [32]. AST (aspartate aminotransferase) has the higher participation in muscular lysis and elevation index related to ALT (alanine aminotransferase) and can triple after intense training. ALT is present in a higher quantity within hepatocytes; thus, its raise has a higher probability of indicating hepatic pathology or happen due to an increase in internal body temperature [31]. They increase due to excessive muscular tension, by damage or deterioration of skeletal muscles. In our study, AST levels did not show any significant differences at resting or after practice. On the other hand, ALT levels raised significantly. When there is lack of glycogen, the liver needs to produce glucose through other sources, promoting an increase in hepatic glycogenesis that uses lactate and amino acids as substrate. Therefore, due to protein catabolism, this enzymes activity can enhance [30].

Bone metabolism affects athletic performance as well as executing physical exercises and has an important role in bone mineralization, helping to maintain skeletal integrity and reduce fracture risk [33]. However, the analysis of alkaline phosphatase, calcium and phosphorus activity did not show any significant difference in this study.

The present investigation has some limitations, as the small sample of only 12 male athletes that do not represent conclusive reference values. Another limitation was that, although an anamneses regarding the use of medication and supplements, there was no diet follow-up in the days prior to the study. On the other hand, the highlight and innovation in our study was that no literature was found that presented and evaluated all the parameters combined. Besides, it is unique for its homogeneity of the futsal athletes group since it was the world futsal champions, with high physical preparation and athletic performance. Moreover, our results allow an understanding over the body biochemical functioning during sports practice and has characteristics and descriptive data with little variance that can be take into consideration for future research. Furthermore, this analysis was carried out with adapted athletes undergoing high performance training for over 2 years.

Lastly, we recognize the need to comprehend the analysis of biochemical markers corresponding to proteolysis, lipolysis, hemolysis and muscular damage during training sessions in order to prevent the low of athletic and muscular performance, as well as physical capacities, enabling the achievement of good results.

## Conclusion

Results obtained in this research allow us to conclude that the proposed training protocol induced a lipolytic scenario caused by the spoliation of carbohydrates from a low supply (fasting) e glycolytic activity from practice. However, it did not present any relevant laboratory signals of proteolysis. Moreover, the raise of enzymatic biomarkers characterized an adaptive micro-injury with no signs of muscular damage/injury due to its increase of 25% (CK-mm) and 40% (LDH). Lastly, blood alterations presented in the hemogram indicated a physiological reflex from hypothalamo-pituitary-adrenal axis characterized by neutrophil leukocytosis with lymphocytopenia. Thus, the experimental training protocol proposed was satisfactory from a biochemical point of view since the metabolic adaptations characterize an adaptation to training with no laboratory signs of damage.

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### Potential conflict of interest

No conflicts of interest with relevant potential for this article was reported.

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### Authors contribution

Research conception and design: Siqueira LO; Data collection: Siqueira LO, Schwarzbach D, Marostica LL; Data analysis and interpretation: Siqueira LO, Moreira JCF; Statistical analysis: Siqueira LO; Financing acquisition: Siqueira LO; Manuscript writing: Siqueira LO, Ramos MEK; Manuscript critical review as for relevant intellectual content: Siqueira LO, Ramos MEK, Moreira JCF.

## References

1. Oliveira VADS, Sinésio IYC, Cabral PUL, Cortez ACL, Meneses YPSF. Associação de atividade física e o estresse em pré-vestibulandos. *Rev Bras Fisiol Exerc* 2019;18(1):9-16. doi: 10.33233/rbfe.v18i1.2875
2. Organização Mundial da Saúde 2020 [Internet]. Physical. [citado 2021 Jan 15]. Available from: [https://www.who.int/health-topics/physical-activity#tab=tab\\_1](https://www.who.int/health-topics/physical-activity#tab=tab_1)
3. Soares TC, Silva LAA, Norões ARL, Medeiros SRA, Cavalcante RMS. Efeitos da suplementação de glutamina em atletas de alto rendimento: uma revisão de literatura. *Rev Brasileira de Nutrição Esportiva* [Internet]. 2019;13(77):17-6. [citado 2021 Jan 15]. Available from: <http://www.rbne.com.br/index.php/rbne/article/view/1215>.
4. Ministério da cidadania [Internet]. AFEs, Desenvolvimento humano e esporte de alto rendimento. [citado 2021 Jan 15]. Available from: <http://www.esporte.gov.br/snear/departamentos.jsp>.
5. Coqueiro AY, Mata Godois AM, Raizel R, Tirapegui J. Creatina como antioxidante em estados metabólicos envolvendo estresse oxidativo. *Revista Brasileira de Prescrição e Fisiologia do Exercício* [Internet] 2017;11(64):128-37. [citado 2021 Jan 15]. Available from: <http://www.rbpfex.com.br/index.php/rbpfex/article/view/1090>
6. Steinbacher P, Eckl P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules* 2015;5(2):356-77. doi: 10.3390/biom5020356
7. Chielle EO, Maziero JS. Efeito do exercício físico intenso nas concentrações sérica, salivar e urinária de marcadores de lesão músculo esquelética. *Evidência - Ciência e Biotecnol* 2018;18(1):41-58. doi:

10.18593/eba.v18i1.16666

8. Fikri S, Pradhila F, Dirganta D. Biomechanical analysis with optimal combination by using foot and distance when the futsal player passing the ball against the accuracy of the target. *Br J Sports Med*. 2016;50(22):e4.13-e4. doi: 10.1136/bjsports-2016-096952.20
9. Pereira-Neto E, Alves RC, Souza Júnior TP, Brandão LHA, Silva-Grigoletto ME, Almeida MB. Efeito agudo do treinamento de força com restrição de fluxo sanguíneo sobre demanda metabólica de lactato em jovens futebolistas. *Rev Bras Fisiol Exerc* 2019;18(3):136. doi: 10.33233/rbfe.v18i3.3239
10. Tavana B, Nourian R, Memari AH, Noormohammadpour P, Kordi R. O-33 Futsal and injuries among recreational sport participants: a prospective field study. *Br J Sports Med* 2016;50(1):A19.1-A19. doi: 10.1136/bjsports-2016-097120.33
11. Silva TT. Questões éticas na prática da medicina do esporte na contemporaneidade. *Rev Bioética* 2019;27(1):62-6. doi: 10.1590/1983-80422019271287
12. Bezerra JA, Farias NO, Melo SVA, Silva RPM, Castro ACM, Martins FSB, et al. Respostas de indicadores fisiológicos a um jogo de futebol. *Rev Bras Med do Esporte* 2016;22(3):200-5. doi: 10.1590/1517-869220162203137068
13. Neto OPM, Carvalho AS, Nicolau LS, Costa AC, Dias GPS. Análise da proteinúria após exercício físico intenso. *Rev Bras Anal Clin* 2017;49(3):256-62. doi: 10.21877/2448-3877.201700564
14. Castro KR, Dantas MP, Teixeira R V, Santos GC, Neto PA, Ururahy MG, et al. Relação de magnésio sérico e capacidade de sprints repetidos em crianças. *Rev Motricidade* 2019;15(1)23-8.
15. Bueno C. Competições de alto risco. *Ciência e Cultura* 2017;69(2):22-4. doi: 10.21800/2317-66602017000200009
16. Marques GC, Ferre-Douza V. Análise do perfil hematológico de praticantes de atividade física para hipertrofia muscular, usuários ou não de suplementos e esteróides anabolizantes. *FOCO: Caderno de Estudos e Pesquisa [Internet]* 2017;13:54-70. [citado 2021 Jan 15]. Available from: <http://www.revista-foco.inf.br/index.php/FocoFimi/article/view/497>
17. Duclos M, Tabarin A. Exercise and the hypothalamo-pituitary-adrenal axis. *Front Horm Res* 2016;47:12-26. doi: 10.1159/000445149
18. Lima FD, Oliveira RJ, Albuquerque RC, Correia ALM, Dantas RAE, Mota MR. Respostas hematólogicas agudas ao teste incremental máximo em esteira. *Motricidade* 2016;12(3):39-44. doi: 10.6063/motricidade.6327.
19. Dias R, Baganha RJ, Cieslak F, Krinski K, Camarço NF, Verlengia R, et al. Parâmetros imunológicos e infecções do trato respiratório superior em atletas de esportes coletivos. *Rev Bras Med do Esporte* 2017;23(1):66-72. doi: 10.1590/1517-869220172301149299
20. Morales JV, Guimarães J, Barros E. Proteinúria: avaliação clínica e laboratorial. *Clin Biomed Res [Internet]* 2006;16(3):95-101. [citado 2021 Jan 15]. Available from: <https://seer.ufrgs.br/hcpa/article/view/99823>
21. Wolyniec W, Ratkowski W, Kasprowicz K, Malgorzewickz S, Aleksandrowickz E, Witek K, et al. Changes in electrolytes and uric acid excretion during and after a 100 km run. *J Biol Regul Homeost Agents [Internet]* 2018;5:1205-10. [citado 2021 Jan 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/30334414/>
22. Ndrepepa G. Uric acid and cardiovascular disease. *Clinica Chim Acta* 2018;484:150-63. doi: 10.1016/j.cca.2018.05.046
23. Silva ASR, Santhiago V, Papoti M, Gobatto CA. Comportamento das concentrações séricas e urinárias de creatinina e uréia ao longo de uma periodização desenvolvida em futebolistas profissionais: Relações com a taxa de filtração glomerular. *Rev Bras Med Esporte* 2006;12(6):327-32. doi: 10.1590/S1517-86922006000600006
24. Paula A, Ferreira S, Picolli T, Bordin A, Rech A, Poeta J, et al. Baixos níveis de atividade física estão associados a prejuízos no perfil lipídico e aumento do percentual de gordura de indivíduos idosos. *Rev Bras Ciênc Mov* 2015;23(3):135-42. doi: 10.18511/0103-1716/rbcm.v23n3p135-142
25. Ghafouri K, Cooney J, Bedford DK, Wilson J, Caslake MJ, Gill JMR. Moderate exercise increases affinity of large very low-density lipoproteins for hydrolysis by lipoprotein lipase. *J Clin Endocrinol Metab* 2015;100(6):2205-13. doi: 10.1210/jc.2015-1196
26. Córdova A, Mielgo-Ayuso J, Fernandez-Lázaro D, Roche E, Caballero-García A. Impact of magnesium supplementation in muscle damage of professional cyclists competing in a stage race. *Nutrients* 2019;11(8):1927. doi: 10.3390/nu11081927

27. Zhang Y, Xun P, Wang R, Mao L, He K. Can magnesium enhance exercise performance? *Nutrients* 2017;9(9):946. doi: 10.3390/%2Fnu9090946
28. Barranco T, Tvarijonaviciute A, Tecles F, Carrillo JM, Sánchez-Resalt C, Jimenez-Reyes P, *et al.* Changes in creatine kinase, lactate dehydrogenase and aspartate aminotransferase in saliva samples after an intense exercise: A pilot study. *J Sports Med Phys Fitness* 2018;85(6):910-6. doi: 10.23736/s0022-4707.17.07214-0
29. Callegari GA, Novaes JS, Neto GR, Dias I, Garrido ND, Dani C. Creatine kinase and lactate dehydrogenase responses after different resistance and aerobic exercise protocols. *J Hum Kinet* 2017;58(1):65-72. doi: 10.1515/%2Fhukin-2017-0071
30. Isik O, Yildirim I, Ersoz Y, Koca HB, Dogan I, Ulutas E. Monitoring of pre-competition dehydration-induced skeletal muscle damage and inflammation levels among elite wrestlers. *J Back Musculoskeletal Rehabil* 2018;31(3):533-540. doi: 10.3233/bmr-170955
31. Ferreira BE, Morel EA, Tsalikis J, Costa CH, Mullher PDTG, Pontes ERJC. Alterações agudas induzidas por competição de ciclismo em biomarcadores enzimáticos e imunológicos. *Revista Brasileira Prescrição e Fisiologia do Exercício [Internet]* 2015;9(52):175-80. [citado 2021 Jan 15]. Available from: <http://www.rbpfex.com.br/index.php/rbpfex/article/view/752>
32. Krefta B, Vecchi C, Pinezi FG, Mezzomo TR. Monitoramento de eletrólitos, de marcadores de danos musculares e função renal em atletas de futebol de elite. *Revista Brasileira de Nutrição Esportiva [Internet]* 2018;11(68):1042-9. [citado 2021 Jan 15]. Available from: <http://www.rbne.com.br/index.php/rbne/article/view/960>
33. Filippella M, Altieri B, Falchetti A, Cosso R, Cena H, Musso C, *et al.* Bone metabolism, bone mass and structural integrity profile in professional male football players. *J Sports Med Phys Fitness* 2020;60(6):912-8. doi: 10.23736/S0022-4707.20.09913-2
34. Cardiovascular Physiology Concepts [Internet]. *Laminar Flow*; 2018. [citado 2021 Jan 15]. Available from: [www.cvphysiology.com/Hemodynamics/H006](http://www.cvphysiology.com/Hemodynamics/H006)