2024, Retos, 59, 435-443

Biomarker response in professional football athletes after matches: a systematic review
Respuesta de biomarcadores en deportistas de fútbol profesionales después de los partidos: una revisión sistemática

*Leandro de Lima e Silva, ** Douglas Rodrigues dos Santos, *Yuri Rolim Lopes Silva, ***Luciano Alonso, **Juliano Spineti, *Giullio César Pereira Salustiano Mallen da Silva, *Rodrigo Gomes de Souza Vale, *Rodolfo de Alkmim Moreira Nunes
*Universidade do Estado do Rio de Janeiro - UERJ (Brasil), **Fluminense Football Club - FFC (Brasil), ***Universidade Federal do Rio de Janeiro - UFRJ (Brasil)

Abstract. Purpose: The objective of this systematic review was to analyze studies that investigated serum biochemical markers used to measure internal load in football matches. Method: The review followed the recommendations proposed for PRISMA systematic reviews. The bibliographic review was carried out until May 2023. Searches were carried out in the SPORTDiscus, Scopus, MedLine (via PubMed), Lilacs (via VHL) and Web of Science databases to find studies that analyze biomarkers after football matches with athletes male professionals, where the following data were extracted: author/year, sample size, competitive level, sample characteristics, exposure, analyzed biomarkers and results. The methodological quality was assessed by the Critical Assessment Skills Program (CASP) Tool. Results: Fifteen articles were included in the review out of 1,402 identified in the search. These articles were published between 2008 and 2022 and involved a total sample of 287 football athletes at regional, national, and international levels. Regarding biochemical analyses, 50 different biomarkers were studied, among which creatine kinase was the biomarker most used in studies, along with lactate dehydrogenase and aspartate aminotransferase. Conclusion: The results indicate that football games impact several biomarkers, including those related to muscle damage, hematological response, hormonal variations, immune function, and oxidative stress. Creatine kinase was the most used biomarker due to its greater association with the load imposed by the game in assess muscle damage.
Keywords: soccer; biological markers; load markers; player monitoring; inflammation

Resumen. Objetivo: El objetivo de esta revisión sistemática fue analizar estudios que investigaron los marcadores bioquímicos séricos utilizados para medir la carga interna en partidos de fútbol. Método: La revisión siguió las recomendaciones propuestas para las revisiones sistemáticas PRISMA. La revisión bibliográfica se realizó hasta mayo de 2023. Se realizaron búsquedas en las bases de datos SPORTDiscus, Scopus, MedLine (vía PubMed), Lilacs (vía VHL) y Web of Science para encontrar estudios que analicen biomarcadores después de partidos de fútbol con deportistas masculinos profesionales, donde se extrajeron los siguientes datos: autor/año, tamaño de la muestra, nivel competitivo, características de la muestra, exposición, biomarcadores analizados y resultados. La calidad metodológica fue evaluada por Critical Herramienta del Programa de Evaluación de Habilidades (CASP). Resultados: Se incluyeron en la revisión quince artículos de un total de 1.402 identificados en la búsqueda. Estos artículos fueron publicados entre 2008 y 2022 e involucraron una muestra total de 287 deportistas de fútbol a nivel regional, nacional e internacional. En cuanto a los análisis bioquímicos, se estudiaron 50 biomarcadores diferentes, entre los que la creatina quinasa fue el biomarcador más utilizado en los estudios, junto con la lactato deshidrogenasa y la aspartato aminotransferasa. Conclusión: Los resultados indican que los juegos de fútbol impactan varios biomarcadores, incluidos aquellos relacionados con el daño muscular, la respuesta hematológica, las variaciones hormonales, la función inmune y el estrés oxidativo. La creatina quinasa fue el biomarcador más utilizado por su mayor asociación con la carga que impone el juego para evaluar el daño muscular.
Palabras clave: fútbol; marcadores biológicos; marcadores de carga; seguimiento de jugadores; inflamación

Fecha recepción: 24-05-24. Fecha de aceptación: 19-07-24
Leandro de Lima e Silva
l.limaesilva@gmail.com

Introduction

Football is a widely practiced sport worldwide and is characterized by intense intermittent physical activities with high aerobic and anaerobic demands (Martínez-Cabrera et al., 2020). To optimize performance and minimize the risk of injuries, it's important to monitor athletes' internal load (Springham et al., 2020). In this regard, monitoring the demanded load in this sport is relevant as modern elite professional athletes experience an increasingly congested sports calendar combined with increased training and competition loads (Pillay et al., 2022; Schwellnus et al., 2016).

It's a reality in sports that training load, combined with an "overloaded" or "congested" competition schedule, exposes athletes to difficulty in adapting optimally to the overall load. This can result in decreased performance and is associated with an increased risk of injury (Howle et al., 2020; Ekstrand et al., 2011). Monitoring internal load in football is not an easy task since the association between external and internal loads is not straightforward. The evaluation of internal load can be defined as the physiological response of the body to a given training stimulus and can be influenced by various factors such as intensity, duration, and type of exercise (Alves et al., 2022; Contreras et al., 2023). Additionally, internal load can be affected by external factors such as environment and nutrition (Póvoas et al., 2022; McGlory et al., 2017).

Serum biochemical markers are molecules present in the blood that can be used as indicators of the body's physiological state (Hernández-Cruz et al., 2022; Anđelković et al., 2015). Measuring these markers can provide valuable information about the body's response to training and physical stress (Kozioł et al., 2020). In football, biochemical markers have been used to monitor muscular fatigue, muscular damage, inflammation, and oxidative stress (De Carvalho et al., 2021; Akubat et al., 2018). According to McLaren et al. (2018), monitoring internal load can help identify the optimal timing for high-intensity training, minimizing the risk of injuries and excessive fatigue. According to Quintas et al. (2020), there is a significant association between external load and the metabolic profile analyzed through urine, with changes in biochemical pathways associated with long-term adaptation to training.

Several biochemical markers have been used in football, among which creatine creatine kinase (CK), myoglobin (Mb), lactate dehydrogenase (LDH), C-reactive protein (CRP), and malondialdehyde (MDA) stand out (Coppalle et al., 2019). The evaluation of internal load in football has been carried out by analyzing biochemical markers before and after training sessions and competitions. Furthermore, the evaluation can be performed at different times during the training and competition period, allowing for the analysis of athletes' adaptation process and the identification of moments of higher injury risk (Selmi et al., 2022; Djaoui et al., 2017). Some studies have shown that measuring biochemical markers can be useful in preventing injuries in football players. For example, a study conducted by Bengtsson et al. (2013) demonstrated that CK concentration was associated with the risk of muscle injuries in professional football players.

However, it's important to note that evaluating internal load through biochemical markers should not be done in isolation. Other methods, such as subjective assessment of training load, monitoring of heart rate, and the use of physical activity monitoring devices, should be used in conjunction to provide a more comprehensive assessment of internal load (Teixeira et al., 2021; O'Sullivan et al., 2018; Bittencourt et al., 2016).

In this context, the objective of this systematic review is to analyze studies that have investigated biochemical markers used to measure internal load in field football matches, to identify the main trends and contributions in this area.

Materials and methods

Procedures

This systematic review was conducted following the recommendations proposed for systematic reviews and meta-analyses by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). It was submitted and registered in PROSPERO with the registration number CRD42024527354. A search strategy was performed in the following databases: SPORTDiscus, Scopus, MedLine (via PubMed), Lilacs (via BVS), and Web of Science, with no date filters, and the last search was conducted on May 9, 2024.

For the search, descriptors were used according to DeCS and MESH. It's worth noting that descriptors that had no affinity or possibility of relation with the research focus and objective were discarded. The following keywords were used: football, soccer, training load, workload, internal training load, internal load, match, game, play, Biomarker, Biological Marker, Biologic Marker, Immune Marker, Serum Marker, Biochemical Marker, and Laboratory Marker. Boolean operators "OR" between synonyms and "AND" between terms were used to construct the search phrase, structured as follows: (("football"[Title/Abstract] OR "soccer"[Title/Abstract]) AND ("training load"[Title/Abstract] OR "workload"[Title/Abstract] OR "internal training load"[Title/Abstract] OR "internal load"[Title/Abstract] OR "match*"[Title/Abstract] OR "game*"[Title/Abstract] OR "play*"[Title/Abstract])) AND ("Biomarker*"[Title/Abstract] OR "Biological Marker*"[Title/Abstract] OR "Biologic Marker*"[Title/Abstract] OR "Immune Marker*"[Title/Abstract] OR "Serum Marker*"[Title/Abstract] OR "Biochemical Marker*"[Title/Abstract] OR "Laboratory Marker*" OR[Title/Abstract]), using a title and abstract search filter in all databases.

After the search, the found documents were sent to an online library called EndNote shared among three researchers, and duplicates were removed. Then, the same three researchers conducted title and abstract screening to assess inclusion criteria. After this analysis, the remaining documents underwent full-text reading. Subsequently, the documents that met the inclusion criteria were included in the review.

The inclusion criteria were adopted according to the PECO strategy: participants (P) studies conducted with male professional football athletes; exposure (E) one or more football matches; control (C) not applicable; outcomes (O) quantified some type of biochemical marker as a response. The exclusion criteria adopted were a) review articles, theses, or dissertations, and articles in the submission phase; b) articles not written in English, Spanish, or Portuguese.

Methodological Quality

The methodological quality was assessed using the Critical Appraisal Skills Programme (CASP) tool (CASP, 2018), available at: http://www.casp-uk.net/casp-tools-checklists. The CASP tool consists of 12 questions to be answered with "yes," "no," or "can't tell." A score of 10 to 12 "yes" responses will be considered high quality, seven to nine moderate quality, and zero to 6 low quality, as recommended by Smith et al. (2016). To ensure greater rigor in the assessment, the tool was designed to consider some possibilities of bias that may be present in a scientific article within the context of the research. Thus, two researchers familiar with the study topic conducted the assessment of factors that could affect the quality of the articles, and a third evaluator was consulted in case of disagreement during the assessment.

Data Extraction

From the included studies, the following data were extracted: number of participants, level of competitiveness, and sample characteristics (age, body mass, height, and % body fat) when available, exposure, type of biomarkers analyzed, and findings/results.

2024, Retos, 59, 435-443

Results

A total of 1.402 studies were found in the databases. After using the eligibility criteria, 15 studies were included in the systematic review (Figure 1).

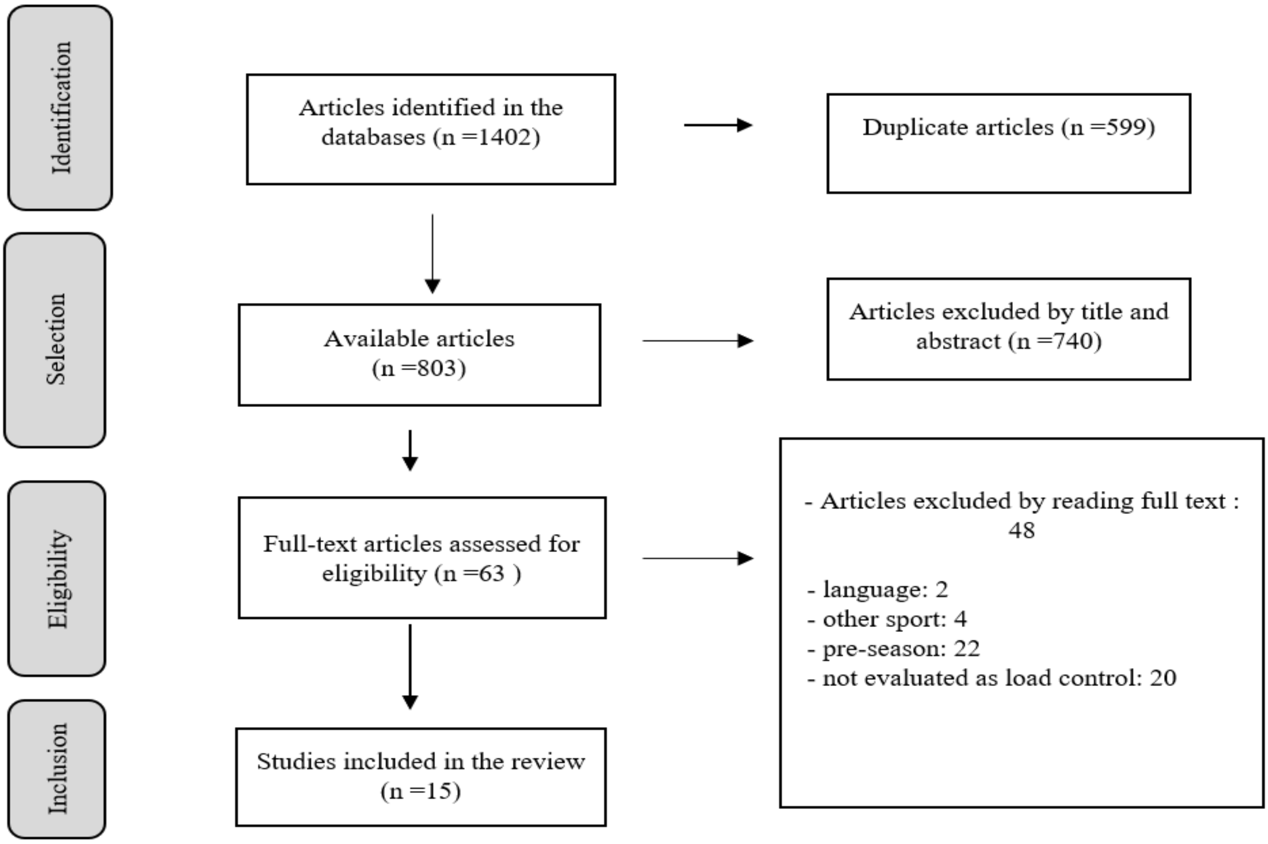

Figure 1. Flow diagram of the PRISMA study selection procedure

Table 1.
Data extraction from included studies.

Author/year	Sample	Exhibition	Biomarkers	Results
Ascensão et al. 2008	17 athletes; 21.3±1.1 years; 70.7±6.3 kg; NN	A competitive football match (2 × 45 min) where blood samples were collected before and 30 minutes, 24 hours, 48 hours, and 72 hours after the match. does not inform the time of day of blood collection	CK, Mb, MDA, -SH, TAS, AU	Mb: significantly higher at 30 minutes compared to pre-match. CK: significantly higher at 30 minutes, 24 hours, 48 hours, and 72 hours compared to pre-match. MDA: significantly higher at 30 minutes, 24 hours, 48 hours, and 72 hours compared to pre-match. -SH: significantly lower at 30 minutes, 24 hours, and 48 hours compared to pre-match. TAS: significantly higher at 30 minutes compared to pre-match. AU: significantly higher at 30 minutes, 24 hours, 48 hours, and 72 hours compared to pre-match.
Bezerra et al. 2014	GC= 15 athletes GE= 15 athletes; NR	GE = a 90-minute match (2x45min). GC = participated only in the measurements. Analyses occurred pre-game and 24 hours post-game. does not inform the time of day of blood collection	CK, CK Heart Fraction (CKMB), LDH e GGT	GE significantly increased at 24 hours post-match for CK, CKMB, and LDH compared to pre-match. GC showed significantly smaller changes for CKMB at 24 hours post-analysis compared to pre-match.
Bezerra et al. 2016	42 athletes; 25,7±4,6 years; 75,8±6,36kg; NR	A 90-minute match (2x45min). Analyses before the game, after, and at 24 hours, 48 hours, and 72 hours. does not inform the time of day of blood collection	CK, LDH, AST, cortisol, testosterone, CRP, HGB, HCT, Erythrocytes, MCV, MCH, MCHC, Leukocytes, Neutrophils, Eosinophils, Monocytes, and Lymphocytes	Erythrocytes, HGB, and HCT at 24- and 48-hours post-match. The values of leukocytes, neutrophils, and monocytes significantly increased after the match. The values of eosinophils and lymphocytes decreased after the match. Peaks of elevation were observed after the matches for LDH and at 24 hours for CK and AST. The levels of CRP increased after the match, with peak concentration at 24 hours. Cortisol concentrations signifi-

				cantly increased after the matches, whereas testosterone levels decreased post-match.
Colombini et al. 2014	19 athletes; 26,5±3,7 years; NN	A 90-minute match (2x45min). Analyses before and immediately after the game. blood samples were collected at 8:00 hours in the morning	HGB, HCT, VCM, MCH, CHCM, RBC, ammonia, UA, urea, and creatinine	Higher post-match values for RBC, HCT, and HGB than pre-match. Increase in post-match concentrations of ammonia and creatinine compared to pre-match. A reduction in the urea/creatinine ratio was observed.
Duarte et al. 2022	11 athletes; 29,26±4,52 years, 178,95±6,42 cm, 77,32±7,24 kg; NI	3 matches of 90 minutes each (2x45min) with a 3-day interval between them. Analyses before and after, 24 hours, 48 hours, and 72 hours after each game. does not inform the time of day of blood collection	PCR	The levels of CRP increased at 24 hours and 48 hours post-each game compared to pre-match, peaking at 24 hours post-match in each game.
Fatouros et al., 2010	30 athletes 20,3±0,3 years,75,4±3,1 kg, 177±1,3 cm GE: 20 athletes GC:10 athletes; NN	GE: A 90-minute match (2x45min) GC: Did not engage in any activity, only participated in measurements. Analyses before and after, 24 hours, 48 hours, and 72 hours blood samples were collected between 8:00 and 12:00 hours in the morning	CK, AU, leukocytes, MDA, PC, GSH, GSSG, TAC, catalase, GPX	For GE, leukocytes increased compared to GC after and 24 hours post-match. AU increased for GE compared to GC after, 24 hours, and 48 hours post-match. CK increased in GE after, 24 hours, 48 hours, and 72 hours post-match, reaching its peak 48 hours post-match compared to GC. PC increased for GE after, 24 hours, 48 hours, and 72 hours post-match compared to GC. MDA increased for GE compared to GC after, 24 hours, and 48 hours post-match. GSH decreased for GE only at 24 hours post-match. On the other hand, GSSG increased for GE at 24 hours and 48 hours. Post-match, TAC increased at 24 hours and 48 hours post-match for GE compared to GC. Catalase increased immediately after the match for GE compared to GC. GPX increased at 24 hours and 48 hours for GE compared to GC.
García-Romero-Pérez et al. 2021	29 athletes; 27,59±3,83 years; 1,83±0,05 m; 80,16±7,45 kg; NI	Matches of 90 minutes each (2x45min). Multiple Matches Group (MMG) with an interval between matches < 4 days and Single Match Group (SMG) with an interval between matches. Analysis before and after the matches. blood samples were collected between 9:00 and 10:00 hours in the morning	CK	No significant difference was found for CK values when comparing MMG and SMG. Players who completed more than 60 minutes in the previous game had significantly increased CK levels pre-match in MMG compared to SMG.
Haller et al. 2019	22 athletes; 24,2±2,9 years; NI	Matches of 90 minutes each (2x45min) over 4 months. Pre-match analyses (BL-1), one day post-match (BL+1), and two or more days post-match (BL+2/3). does not inform the time of day of blood collection	Circulating cell-free DNA (cfDNA)	The concentrations of cfDNA significantly increased at BL+1 compared to BL-1. cfDNA concentrations during weeks with multiple matches were significantly higher compared to weeks with single matches.
Ispirlidis et al. 2008	24 athletes; 20,1 ±0,8 years; 1,78± 0,08 m; 75,2±6,8 kg GE:14 GC:10	GE: Engaged in a 90-minute football match (2x45min). GC: Did not engage in any activity, only participated in measurements. Analysis before and after, 24h, 48h, 72h, 96h, 120h, 144h post-match. blood samples were collected in the morning of the game-day	leukocytes, CK, LDH, CRP, cortisol, testosterone, cytokines (IL-6) and (IL-1b), TBARS, PC, and AU	Leukocytes and PCR increased after and at 24 hours post-match in GE compared to GC. CK and LDH increased after, peaking at 48 hours post-match, but LDH decreased from 96 hours and CK only at 120 hours post-match compared to GC. AU increased at 24 hours and remained elevated until 96 hours post-match. PC increased at 24 hours, peaked at 48 hours, and remained elevated until 96 hours post-match. TBARS increased after the match and remained elevated until 48 hours. Cortisol and IL-6 increased only after the match.
Jamurtas et al., 2015	GC= 8 athletes; 22,2±1,3 years; 75,7±4,4kg GE= 10 athletes; 22,8±1,8 years; 76,3±5,2; NN	GE: A 90-minute match (2x45min). GC: Only trained and participated in stretching. Analyses occurred pre-match and at 2h, 12h, 36h, and 60h post-match. blood samples were collected between 7:00 and 8:00 hours in the morning	CK, RBC, HGB, HCT, VCM, MCH, CHCM, RDW CV, RDW-SD, iron, total iron binding capacity (TIBC), transferrin saturation (TS).	GE obtained significantly higher CK levels at 2h, 12h, and 36h post-match compared to pre-match. Compared to GC, it was statistically higher at 2h, 12h, 36h, and 60h post-match. GE had significantly lower iron levels at 2h post-match compared to pre-match and GC. TIBC was statistically higher for GE at 36h and 60h post-match compared to pre-match and GC.
Peñailillo et al. 2015	9 athletes; 26±3,5 years, 177±3,7 cm e 74±6,1 kg; NI	Matches of 90 minutes each (2x45min). Analysis pre-match and 10 minutes post-match. blood samples were collected	salivary concentrations of cortisol, testosterone, and immunoglobulin A (IgA)	There was no significant change in cortisol between pre and post-match. Testosterone, IgA, and testosterone/cortisol significantly decreased post-match compared to pre-match.

		between 18:30 and 20:30 hours of the night		
Russell et al. 2015	14 athletes; NI	4 football matches of 90 minutes each (2x45min), held between November and January. Analysis pre-match and post-match, at 24 hours and 48 hours. blood samples were collected in the morning of the game-day	CK	There was a significant increase in CK at 24 hours and 48 hours post-match compared to pre-match in all matches. No difference in CK was observed between the matches.
Silva et al. 2013	7 athletes; 22-31 years; 172-191 cm;71-95 kg; 7-10,7 %G; NI	A 90-minute football match (2x45min). Analyses were conducted pre-match and at 24 hours, 48 hours, and 72 hours post-match. blood samples were collected 18:00 hours of the night	Testosterone, cortisol, CK, -SH, SOD, GPX, reductase, Mb, CRP, AU, MDA and TAC	Cortisol, CK, SOD, MDA, and TAC significantly increased at 24 hours and 48 hours post-match compared to pre-match. The testosterone/cortisol ratio significantly decreased at 24 hours and 48 hours post-match compared to pre-match. Mb, PCR, GPX, and -SH significantly increased at 24 hours post-match compared to pre-match.
Thorpe et al. 2012	7 athletes; 25±6 years; 75,3±4,6 kg; 179±6 cm; NR	A 90-minute football match (2x45min). Analyses were conducted pre-match and post-match. Salive and blood samples were collected 14:00 hours of the night	Testosterone, cortisol, CK, Mb, immunoglobulin A G and M (IgA), (IgG) and (IgM)	CK, Mb, cortisol, and testosterone increased post-match compared to pre-match.
Viana-Gomes et al. 2018	8 athletes; 27,2±5,5 years; NN	Two consecutive football matches of 90 minutes each (2x45min) with a break of 4 days in between. Analyses were conducted pre-match, post-first game (PG1), 48h after PG1, post-second game (PG2), 24h after PG2, and 48h after PG2. does not inform the time of day of blood collection	CK, AST, ALT, GGT, LDH, TAC, AU, TBARS, PC, GSH e GSSG	LDH and PC increased in PG1 and PG2 compared to pre-match. CK and GSSG significantly increased above pre-match levels at all analyzed time points. AST, ALT, and GSH increased at 48hPG2 compared to pre-match. GGT increased in PG2, 24hPG2, and 48hPG2 compared to pre-match. AU increased at 48hPG1, PG2, and 24hPG2 in plasma and decreased at 48hPG2 in saliva compared to pre-match. TBARS increased at 48hPG2 in plasma and increased in PG1 and PG2 compared to pre-match in saliva.

Legend: CK: creatine kinase; Mb: myoglobin; MDA: malondialdehyde; -SH: sulfhydryl groups; AU: uric acid; RBC: red blood cell count; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW CV: red cell distribution width-CV; RDW-SD: red blood cell distribution width; LDH: lactate dehydrogenase; GGT: gamma-glutamyl transferase; AST: aspartate aminotransferase; ALT: alanine transaminase; PCR: C-reactive protein; PC: carboxyl proteins; GSH: reduced glutathione; GSSG: oxidized glutathione; TAC: total antioxidant capacity; GPX: glutathione peroxidase; TBARS: thiobarbituric acid reactive substances; SOD: superoxide dismutase; GC: control group; Experimental group; NI: international level; NN: national level; NR: regional level.

The included studies had a publication timeframe between 2008 and 2022, with a total sample of 287 football athletes at regional, national, and international levels. Among these studies, 4 had control groups, totaling 43 athletes. Regarding biochemical analyses, 50 different biomarkers were analyzed.

Table 2.
Methodological quality

CASP Checklist	1	2	3	4	5 (a)	5 (b)	6 (a)	6 (b)	7	8	9	10	11	12	Escore
Ascensão et al., 2008	S	NI	S	S	S	S	S	S	S	NI	S	S	S	S	10
Bezerra et al., 2014	S	NI	NI	NI	NI	NI	NI	NI	S	NI	S	S	S	S	6
Bezerra et al., 2016	S	NI	NI	S	NI	NI	S	S	S	NI	S	S	S	S	8
Colombini et al., 2014	S	NI	S	S	S	S	S	S	S	NI	S	S	S	S	10
Duarte et al., 2022	S	NI	NI	S	NI	NI	NI	NI	S	NI	S	S	S	S	7
Fatouros et al., 2010	S	NI	S	S	S	S	S	S	S	NI	S	S	S	S	10
García-Romero-Perés et al., 2021	S	NI	NI	S	NI	NI	S	S	S	S	S	S	S	S	9
Haller et al., 2019	S	NI	NI	S	NI	NI	S	S	S	NI	S	S	S	S	8
Ispirlidis et al., 2008	S	NI	S	S	S	S	NI	NI	S	NI	S	S	S	S	9
Jamurtas et al., 2015	S	S	S	S	S	S	S	S	S	NI	S	S	S	S	11
Peñailillo et al., 2015	S	NI	NI	S	NI	NI	NI	NI	S	S	S	S	S	S	8
Russel et al., 2015	S	NI	NI	S	NI	NI	S	S	S	S	S	S	S	S	9
Silva et al., 2013	S	NI	S	S	S	S	S	S	S	NI	S	S	S	S	10
Thorpe et al., 2012	S	NI	S	S	S	S	S	S	S	NI	S	S	S	S	10
Viana-Gomes et al., 2018	S	NI	NI	S	NI	NI	S	S	S	NI	S	S	S	S	8

Legend: 1: The study addressed a focused question; 2: The cohort was recruited acceptably; 3: Exposure was measured accurately to minimize bias; 4: The result was measured accurately to minimize bias; 5: (a) The authors identified all important information, confounding factors; (b) They have taken confounding factors into account in the design and/or analysis; 6: (a) Monitoring of the subjects was complete enough; (b) Follow-up of the subjects was long enough; 7= What are the results of this study; 8: How accurate are the results; 9: You believe in the results; 10: The results can be applied to the local population; 11: The results of this study are adequate with other evidence (discussion); 12: What are the implications of this study for practice. S: yes; N: no; NPD: can't say; NI: not informed

The methodological quality assessed by the CASP tool was high for six studies. Eight studies showed moderate quality, and only one study exhibited low quality.

Discussion

This review aimed to analyze the effect of soccer matches on different biomarkers and identify which biomarkers are most used in the context of professional soccer. In this study, the analysis of 50 different biomarkers was observed, and categorized into five groups: enzymatic, hematological, hormonal, immunological, and oxidative stress-related biomarkers.

Enzymatic markers were the most used, with Peñailillo et al. (2015) being the only exception. Among the analyzed markers, those related to muscle damage such as CK=11, LDH=4, Mb=3, and aspartate aminotransferase (AST)=2 were the most studied. CK was the most utilized biomarker, showing a characteristic increase immediately after the match (Thorpe et al., 2012), peaking between 24 and 48 hours (Bezerra et al., 2016; Fatouros et al., 2010) and potentially remaining at elevated concentrations up to 72 hours post-match (Ascensão et al., 2008). This aligns with previous studies analyzing CK concentrations in other sports such as Rugby (McLellan et al., 2010), suggesting that the physical demands and muscle damage induced by soccer and rugby are similar.

LDH exhibited an increase immediately after the match (Viana-Gomes et al., 2018; Bezerra et al., 2016; Ispirlidis et al., 2008) and at 24 hours post-match (Bezerra et al., 2014), with its peak occurring at 48 hours post-match in the study by Ispirlidis et al. (2008). However, LDH concentration in the blood can vary due to various factors, including fitness level, age, and individual metabolic adaptations (Brancaccio et al., 2008). Mb was evaluated in three studies, with increases observed at different time points after the match: immediately after the match in the study by Thorpe et al. (2012), at 30 minutes in the study by Ascensão et al. (2008), and at 24 hours in the study by Silva et al. (2013). AST showed different responses in the two studies that evaluated it, with Bezerra et al. (2016) showing an increase immediately after the match and remaining elevated up to 48 hours post-match, while in the study by Viana-Gomes et al. (2018), which analyzed biochemical markers from two matches, AST only increased after 48 hours of the second match. Therefore, it can be understood that a soccer match can generate sufficient mechanical load to induce muscle damage, as identified by different biomarkers.

Other enzymatic biomarkers were also analyzed, such as PCR, which is a marker related to the inflammatory process and is a protein produced by the liver in response to inflammatory processes in different parts of the body, including skeletal muscle. Increased levels of PCR indicate the presence of acute or chronic inflammatory processes (Jatene et al., 2019). Four studies analyzed this marker, all showing an increase in this biomarker at 24 hours post-match (Duarte et al., 2022; Bezerra et al., 2016; Silva et al., 2013; Ispirlidis et al., 2008). This response is consistent with findings of muscle damage, which consequently induces an inflammatory process to repair these structures.

Another frequently used biomarker was Uric Acid (AU), a metabolic biomarker whose increase due to exercise is caused by renal clearance inhibition, leading to an elevation in plasma AU concentration (Suzuki et al., 2006). In this review, AU increased after the match and remained elevated up to 96 hours post-match (Fatouros et al., 2010; Ascensão et al., 2008; Viana-Gomes et al., 2018). However, in the studies by Colombini et al. (2014) and Silva et al. (2013), no significant differences were observed between pre-match and post-match concentrations. This can be explained by the fact that AU is one of the principal antioxidants in blood plasma, being taken up from the plasma into the muscles post-exercise (Hellsten et al., 2001).

Only testosterone and cortisol were analyzed as hormonal biomarkers in five studies, always analyzed together. In four studies, cortisol increased after the match (Thorpe et al., 2012; Silva et al., 2013; Bezerra et al., 2016; Ispirlidis et al., 2008). However, testosterone decreased immediately after the match but returned to baseline levels within 24 hours or showed no significant difference between pre- and post-match levels (Peñailillo et al., 2015; Silva et al., 2013; Bezerra et al., 2016; Ispirlidis et al., 2008), with only Thorpe et al. (2012) showing an increase in testosterone post-match. These results suggest a catabolic state immediately after the soccer match, as confirmed by the reduced testosterone: cortisol ratio (Thorpe et al., 2012; Silva et al., 2013; Bezerra et al., 2016; Ispirlidis et al., 2008; Peñailillo et al., 2015).

Oxidative stress is associated with the production of reactive oxygen species during physical exercise, which can contribute to cellular damage. For oxidative stress-related biological markers, two studies analyzed reduced glutathione (GSH) and oxidized glutathione (GSSG), showing different results for GSH concentration, where Viana-Gomes et al., (2018) found an increase up to 48 hours post-match and Fatouros et al., (2010) found a decrease 24 hours post-match. However, these same two studies showed similar results for GSSG, with an increase of 48 hours post-match. This discrepancy can be explained by the fact that Viana-Gomes et al. (2018) analyzed these markers in two matches while Fatouros et al. (2010) only analyzed one. Viana-Gomes et al. (2018) also analyzed TBARS, which increased immediately after the first and second matches and 48 hours after the second match. In the study by Ispirlidis et al. (2008), it increased up to 48 hours post-match. Glutathione peroxidase (GPX) was analyzed in two studies and increased up to 48 hours (Silva et al., 2013; Fatouros et al., 2010). MDA increased up to 48 hours post-match in the three studies that analyzed it (Silva et al., 2013; Fatouros et al., 2010; Ascensão et al., 2008). These results show that one or more soccer matches produce oxidative stress capable of being detected up to 48 hours post-match, even with the increase in antioxidant capacity up to 48 hours post-match (Silva et al., 2013; Fatouros et al., 2010). The alterations in these biomarkers after soccer matches reflect the impact of physical exertion on antioxidant response and oxidative damage.

Some studies analyzed changes in hematological markers after soccer matches, where there was no difference between pre-match and post-match for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Bezerra et al., 2016; Jamurtas et al., 2015; Colombini et al., 2014). These findings may be explained by the lack of restriction and control of fluid replacement during the match. However, increases in red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT) were identified post-match up to 48 hours, representing a decrease in plasma volume (Bezerra et al., 2016; Jamurtas et al., 2015; Colombini et al., 2014). Another finding was the decrease in plasma iron concentration 2 hours post-match (Jamurtas et al., 2015), which may be related to decreased aerobic performance in athletes (Hinton et al., 2014; Schumacher et al., 2002).

Immunological markers were also studied, with leukocytes standing out and being analyzed in 3 studies, showing an increase at 24 hours (Bezerra et al., 2016; Fatouros et al., 2010; Ispirlidis et al., 2008) and 48 hours post-match (Bezerra et al., 2016). Leukocytes are blood cells that naturally respond to infection processes in defense of the body, which in the context of sports responds according to the intensity, effort, and duration of exercises (Ostojic et al., 2009). Immunoglobulin (Ig) was analyzed in 2 studies, showing a decrease immediately after the match in IgA (Peñailillo et al., 2015) and no changes in IgA, IgG, and IgM (Thorpe et al., 2012), indicating a possible temporary suppression of the immune system. These changes can increase athletes' susceptibility to infections, highlighting the importance of considering strategies to minimize this negative effect.

The methodological quality of the included studies was assessed using the CASP tool. Most studies showed moderate to high methodological quality, indicating a robust approach to conducting the research. However, it is essential to consider the limitations and possible biases present in each study when interpreting, as the results obtained in each study can be influenced by various factors, including the athletes' training level, the intensity of the matches, the position on the field, and nutrition, among others. Additionally, the choice of biomarkers and analysis methods used may vary between studies and influence the analysis, making direct comparisons challenging.

The present study has some limitations regarding the review being carried out only with professional male athletes and with studies that analyzed biomarkers after matches and without analyzing training, however this review presents strengths including methodological approaches for discovery and analysis of diverse data, which allowed a more comprehensive understanding of the impacts of football games on various biomarkers.

Conclusion

The present systematic review examined studies investigating the effects of soccer matches on biomarkers in professional athletes. The results indicate that soccer matches have an impact on various biomarkers, including those related to muscle damage, hematological response, hormonal variations, immune function, and oxidative stress.

Considering these results, soccer matches produce a physiological effect that can be assessed by various systems of the body, making it possible to determine the resulting impact. However, concerning the muscle damage caused by internal load, CK is the most reliable marker for evaluation.

Recommendations for future research include standardizing collection and analysis protocols, considering variables such as training level, position on the field, and long-term assessment of the impacts of soccer matches on athletes' health.

Acknowledgements

This research did not receive external funding.

Disclosure statement

Absence of any conflict of interest.

Author’s contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by LLS. YRLS, GCPSMS, RGSV and RAMN. The first draft of the manuscript was written by YRLS, LLS, DRS, JS, LAVS. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

Akubat, I., Barrett, S., Sagarra, M. L., & Abt, G. (2018). The validity of external: internal training load ratios in rested and fatigued soccer players. *Sports*, *6*(2), 44.

Anđelković, M., Baralić, I., Đorđević, B., Stevuljević, J. K., Radivojević, N., Dikić, N., ... & Stojković, M. (2015). Hematological and biochemical parameters in elite soccer players during a competitive half season. *Journal of Medical Biochemistry*, *34*(4), 460.

Ascensão, A., Rebelo, A., Oliveira, E., Marques, F., Pereira, L., & Magalhães, J. (2008). Biochemical impact of a soccer match—analysis of oxidative stress and muscle damage markers throughout recovery. *Clinical biochemistry*, *41*(10-11), 841-851.

Bengtsson, H., Ekstrand, J., & Hägglund, M. (2013). Muscle injury rates in professional football increase with fixture congestion: an 11-year follow-up of the UEFA Champions League injury study. *British journal of sports medicine*, *47*(12), 743-747.

Bezerra, J. A., Silva, R. P. M., Jácome, J. G., Costa, J. M. M., Melo, S. V. A., & dos Santos, J. A. R. (2014). Respostas de biomarcadores musculares a uma partida de futebol. *RBFF-Revista Brasileira de Futsal e Futebol*, *6*(19).

Bezerra, J. D. A., Farias, N. D. O., Melo, S. V. A., Silva, R. P. M., Castro, A. C. M. D., Martins, F. S. B., & Santos, J. A. R. D. (2016). Respostas de indicadores fisiológicos a um jogo de futebol. *Revista Brasileira de Medicina do Esporte*, *22*, 200-205.

Bittencourt, N. F., Meeuwisse, W. H., Mendonça, L. D., Nettel-Aguirre, A., Ocarino, J. M., & Fonseca, S. T. (2016). Complex systems approach for sports injuries: moving from risk factor identification to injury pattern recognition—narrative review and new concept. *British journal of sports medicine*, *50*(21), 1309-1314.

Brancaccio, P., Maffulli, N., Buonauro, R., & Limongelli, F. M. (2008). Serum enzyme monitoring in sports medicine. *Clinics in sports medicine*, *27*(1), 1-18.

CASP Checklists - Critical Appraisal Skills Programme. (s.d.). CASP - Critical Appraisal Skills Programme. http://www.casp-uk.net/casp-tools-checklists

Colombini, A., Machado, M., Lombardi, G., Lanteri, P., & Banfi, G. (2014). Modifications of biochemical parameters related to protein metabolism and renal function in male soccer players after a match. *J Sports Med Phys Fitness*, *54*(5), 658-64.

Contreras, M. M., Garcia, A. I. P., Jiménez, A. R., Torres, R. P. H., & Guevara, I. A. C. (2023). Aplicaciones de la Máxima Oxidación de Grasas y FATmax en la evaluación del rendimiento deportivo en atletas-de resistencia: una revisión narrativa. *Retos: nuevas tendencias en educación física, deporte y recreación*, (47), 806-813.

Coppalle, S., Rave, G., Ben Abderrahman, A., Ali, A., Salhi, I., Zouita, S., ... & Zouhal, H. (2019). Relationship of pre-season training load with in-season biochemical markers, injuries and performance in professional soccer players. *Frontiers in physiology*, *10*, 426414.

de Carvalho, G., Girasol, C. E., Gonçalves, L. G. C., Guirro, E. C. O., & Guirro, R. R. D. J. (2021). Correlation between skin temperature in the lower limbs and biochemical marker, performance data, and clinical recovery scales. *Plos one*, *16*(3), e0248653.

Djaoui, L., Haddad, M., Chamari, K., & Dellal, A. (2017). Monitoring training load and fatigue in soccer players with physiological markers. *Physiology & behavior*, *181*, 86-94.

Duarte, W., Júnior, J. L. R., Paula, L. V., Chagas, M. H., Andrade, A. G., Veneroso, C. E., ... & Pimenta, E. M. (2022). C-Reactive Protein and Skin Temperature of the lower limbs of Brazilian elite soccer players like load markers following three consecutive games. *Journal of Thermal Biology*, *105*, 103188.

Ekstrand, J., Hägglund, M., & Waldén, M. (2011). Injury incidence and injury patterns in professional football: the UEFA injury study. *British journal of sports medicine*, *45*(7), 553-558.

Fatouros, I. G., Chatzinikolaou, A., Douroudos, I. I., Nikolaidis, M. G., Kyparos, A., Margonis, K., ... & Jamurtas, A. Z. (2010). Time-course of changes in oxidative stress and antioxidant status responses following a soccer game. *The Journal of Strength & Conditioning Research*, *24*(12), 3278-3286.

García-Romero-Pérez, Á., Ordonez, F. J., Reyes-Gil, F., Rodríguez-López, E. S., & Oliva-Pascual-Vaca, Á. (2021). Muscle damage biomarkers in congestion weeks in English premier league soccer players: A prospective study for two consecutive seasons. *International Journal of Environmental Research and Public Health*, *18*(15), 7960.

Haller, N., Ehlert, T., Schmidt, S., Ochmann, D., Sterzing, B., Grus, F., & Simon, P. (2019). Circulating, cell-free DNA for monitoring player load in professional football. *International journal of sports physiology and performance*, *14*(6), 718-726.

Hellsten, Y., Svensson, M., Sjödin, B., Smith, S., Christensen, A., Richter, E. A., & Bangsbo, J. (2001). Allantoin formation and urate and glutathione exchange in human muscle during submaximal exercise. *Free Radical Biology and Medicine*, *31*(11), 1313-1322.

Hernández-Cruz, G., Estrada-Meneses, E. F., Ramos-Jiménez, A., Rangel-Colmenero, B. R., Reynoso-Sánchez, L. F., Miranda-Mendoza, J., & Quezada-Chacón, J. T. (2022). Relación entre el tipo de ejercicio físico y la fatiga cuantificada mediante VFC, CK y el lactato en sangre (Relationship between physical exercise type and fatigue quantified through HRV, CK, and blood lactate). *Retos*, *44*, 176–182.

Hinton, P. S. (2014). Iron and the endurance athlete. *Applied Physiology, Nutrition, and Metabolism*, *39*(9), 1012-1018.

Howle, K., Waterson, A., & Duffield, R. (2020). Injury incidence and workloads during congested schedules in football. *International journal of sports medicine*, *41*(02), 75-81.

Ispirlidis, I., Fatouros, I. G., Jamurtas, A. Z., Nikolaidis, M. G., Michailidis, I., Douroudos, I., ... & Taxildaris, K. (2008). Time-course of changes in inflammatory and performance responses following a soccer game. *Clinical journal of sport medicine*, *18*(5), 423-431.

Jamurtas, A. Z., Douroudos, I. I., Deli, C. K., Draganidis, D., Chatzinikolaou, A., Mohr, M., ... & Fatouros, I. G. (2015). Iron status markers are only transiently affected by a football game. *Journal of sports sciences*, *33*(20), 2088-2099.

Jatene, P., Dos Santos, G. S., & Portella, D. L. (2019). C-reactive protein serum levels as an internal load indicator of sprints in competitive football matches. *International Journal of Sports Medicine*, *40*(12), 762-767.

Kozioł, K., Zebrowski, J., Betlej, G., Bator, E., Czarny, W., Bajorek, W., ... & Kwiatkowska, A. (2020). Changes in γH2AX and H4K16ac levels are involved in the biochemical response to a competitive soccer match in adolescent players. *Scientific reports*, *10*(1), 14481.

Lima-Alves, A., Claudino, J., Boullosa, D., Couto, C. R., Teixeira-Coelho, F., & Pimenta, E. M. (2022). The relationship between internal and external loads as a tool to monitor physical fitness status of team sport athletes: a systematic review. *Biology of Sport*, *39*(3), 629-638.

Martínez-Cabrera, F. I., Núñez-Sánchez, F. J., Muñoz-López, A., & de Hoyo, M. (2020). Aceleraciones de alta intensidad en el fútbol.¿ Por qué es importante el método de evaluación?(High-intensity acceleration in soccer. Why is the evaluation method important?). *Retos*, *39*, 750-754.

McGlory, C., Devries, M. C., & Phillips, S. M. (2017). Skeletal muscle and resistance exercise training; the role of protein synthesis in recovery and remodeling. *Journal of applied physiology*, *122*(3), 541-548.

McLaren, S. J., Macpherson, T. W., Coutts, A. J., Hurst, C., Spears, I. R., & Weston, M. (2018). The relationships between internal and external measures of training load and intensity in team sports: a meta-analysis. *Sports medicine*, *48*, 641-658.

McLellan, C. P., Lovell, D. I., & Gass, G. C. (2010). Creatine kinase and endocrine responses of elite players pre, during, and post rugby league match play. *The Journal of Strength & Conditioning Research*, *24*(11), 2908-2919.

O'Sullivan, K., O'Sullivan, P. B., & Gabbett, T. J. (2018). Pain and fatigue in sport: are they so different?. *British Journal of Sports Medicine*, *52*(9), 555-556.

Ostojic, S. M., & Ahmetovic, Z. (2009). Indicators of iron status in elite soccer players during the sports season. *International journal of laboratory hematology*, *31*(4), 447-452.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., ... & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj*, *372*.

Peñailillo, L., Maya, L., Niño, G., Torres, H., & Zbinden-Foncea, H. (2015). Salivary hormones and IgA about physical performance in football. *Journal of sports sciences*, *33*(20), 2080-2087.

Pillay, L., Burgess, D., van Rensburg, D. J., Kerkhoffs, G. M., & Gouttebarge, V. (2022). The congested International Match Calendar in football: views of 1055 professional male players. *BMC Sports Science, Medicine and Rehabilitation*, *14*(1), 200.

Póvoas, S., Ascensão, A., Magalhães, J., Silva, P., Wiig, H., Raastad, T., ... & Andersson, H. (2022). Technical match actions and plasma stress markers in elite female football players during an official FIFA Tournament. *Scandinavian Journal of Medicine & Science in Sports*, *32*, 127-139.

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological reviews*, *88*(4), 1243-1276.

Quintas, G., Reche, X., Sanjuan-Herráez, J. D., Martínez, H., Herrero, M., Valle, X., ... & Rodas, G. (2020). Urine metabolomic analysis for monitoring internal load in professional football players. *Metabolomics*, *16*, 1-11.

Russell, M., Northeast, J., Atkinson, G., Shearer, D. A., Sparkes, W., Cook, C. J., & Kilduff, L. P. (2015). Between-match variability of peak power output and creatine kinase responses to soccer match-play. *The Journal of Strength & Conditioning Research*, *29*(8), 2079-2085.

Schumacher, Y. O., Schmid, A., Grathwohl, D., Bültermann, D. I. R. K., & Berg, A. (2002). Hematological indices and iron status in athletes of various sports and performances. *Medicine and science in sports and exercise*, *34*(5), 869-875.

Schwellnus, M., Soligard, T., Alonso, J. M., Bahr, R., Clarsen, B., Dijkstra, H. P., ... & Engebretsen, L. (2016). How much is too much?(Part 2) International Olympic Committee consensus statement on load in sport and risk of illness. *British journal of sports medicine*, *50*(17), 1043-1052.

Selmi, O., Ouergui, I., Levitt, D. E., Marzouki, H., Knechtle, B., Nikolaidis, P. T., & Bouassida, A. (2022). Training, psychometric status, biological markers and neuromuscular fatigue in soccer. *Biology of sport*, *39*(2), 319-327.

Silva, J. R., Ascensão, A., Marques, F., Seabra, A., Rebelo, A., & Magalhães, J. (2013). Neuromuscular function, hormonal and redox status and muscle damage of professional soccer players after a high-level competitive match. *European journal of applied physiology*, *113*, 2193-2201.

Smith, T. O., Davies, L., De Medici, A., Hakim, A., Haddad, F., & Macgregor, A. (2016). Prevalence and profile of musculoskeletal injuries in ballet dancers: A systematic review and meta-analysis. *Physical Therapy in Sport*, *19*, 50-56.

Springham, M., Williams, S., Waldron, M., Strudwick, A. J., Mclellan, C., & Newton, R. U. (2020). Prior workload has moderate effects on high-intensity match performance in elite-level professional football players when controlling for situational and contextual variables. *Journal of Sports Sciences*, *38*(20), 2279-2290.

Suzuki, K., Peake, J., Nosaka, K., Okutsu, M., Abbiss, C. R., Surriano, R., ... & Laursen, P. B. (2006). Changes in markers of muscle damage, inflammation and HSP70 after an Ironman Triathlon race. *European journal of applied physiology*, *98*, 525-534.

Teixeira, J. E., Forte, P., Ferraz, R., Leal, M., Ribeiro, J., Silva, A. J., ... & Monteiro, A. M. (2021). Monitoring accumulated training and match load in football: A systematic review. *International Journal of Environmental Research and Public Health*, *18*(8), 3906.

Thorpe, R., & Sunderland, C. (2012). Muscle damage, endocrine, and immune marker response to a soccer match. *The Journal of Strength & Conditioning Research*, *26*(10), 2783-2790.

Viana-Gomes, D., Rosa, F. L. L., Mello, R., Paz, G. A., Miranda, H., & Salerno, V. P. (2018). Oxidative stress, muscle and liver cell damage in professional soccer players during a 2-game week schedule. *Science & Sports*, *33*(5), e221-e228.

Datos de los/as autores/as y traductor/a:

Leandro de Lima e Silva	l.limaesilva@gmail.com	Autor/a
Douglas Rodrigues dos Santos	rsantosdouglas@icloud.com	Autor/a
Yuri Rolim Lopes Silva	professoryurirolim@gmail.com	Autor/a
Luciano Alonso Valente dos Santos	alonso.lvs@gmail.com	Autor/a
Juliano Spineti	juliano.spineti@hotmail.com	Autor/a
Giullio César Pereira Salustiano Mallen	giulliocesar.gc@hotmail.com	Autor/a
Rodrigo Gomes de Souza Vale	rodrigogsvale@gmail.com	Autor/a
Rodolfo de Alkmim Moreira Nunes	rodolfoalkmim@gmail.com	Autor/a
Eduardo Borba Neves	neveseb@gmail.com	Traductor/a