

Review Article

## Acute and Long-Term Variations in Variables Related to Redox, Inflammation and Hormonal Status in Male Football Players: A Systematic Review and Recommendations

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### Abstract

**Introduction:** The present study aimed to review the acute and long-term variations in variables related to redox, inflammation and hormonal status in male footballers.

**Materials and methods:** A PRISMA-compliant systematic review was conducted. The entire content of PubMed, Scopus and Science Direct were systematically searched until May 2022. Studies with outcomes including: (1) adult male football players, (2) a redox and/or an inflammatory and/or a hormonal marker after a training period, and (3) variables measured in blood/saliva.

**Results:** Thirty-four studies met the inclusion criteria for the qualitative synthesis. Fourteen studies on redox status, 16 on inflammation/muscle damage and 20 on hormonal variations. Only 4 studies incorporated markers related to all 3 statuses, while 8 studies looked at a combination of 2. Studies around redox homeostasis found several markers to fluctuate with MDA, TBARS, protein carbonyls, GSSG, GPx, CAT, and uric acid increasing immediately after a game. Hormonal markers, such as testosterone in blood, revealed no significant change after training. Some found T to increase post-exercise, and some a decrease. Cortisol increased in both short- and long monitoring periods. Markers associated with inflammation and muscle damage found creatine kinase elevated immediately post-game and over extended periods. LDH, C-RP, and IL-6 were also higher post-match.

**Discussion:** Exposure to short or long-term participation in football training and competitions could significantly affect footballers' redox, inflammation and hormonal status. However, greater consistency across studies is required to ascertain the implications of structured training regimens on measured variables. Selecting the most relevant protocol/conditions and biochemical markers, including the collection time and the type of specimen, must be considered.

**Keywords:** Oxidative stress; Cytokines; Testosterone, Cortisol; Football; Review

**Abbreviations:** RONS - Reactive Oxygen and Nitrogen Species; MDA – Malondialdehyde; TBARS - Thiobarbituric Acid Reactive Substances; LOOH - Lipid Peroxides; PC - Protein Carbonyls; SH-group - Sulfhydryl-Group; GPx - Glutathione Peroxidase; CAT - Catalase; SOD - Superoxide Dismutase; GSH - Reduced Glutathione; GSSG - Oxidized Glutathione; UA

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- Uric Acid; TAC - Total Antioxidant Capacity; TAS - Total Antioxidant Status; T - Testosterone; C - Cortisol; T:C ratio – Testosterone to Cortisol Ratio; CK - Creatine Kinase; LDH - Lactate Dehydrogenase; Mb - Myoglobin; CRP – C-Reactive Protein; IL-6 - Interleukin-6.

## Introduction

High-level football players are continuously exposed to many training sessions and competitions during the training season. This level of exposure poses a potential issue when it comes to player fatigue or sports injuries. The accumulation of training and competition load without adequate recovery can lead to overtraining syndrome or possible injuries because of the tremendous physical, psychological, and loading demands placed on the individual. Recent investigations have found that sports teams or individual athletes that can avoid injuries demonstrate greater success during the competitive season [1-3]. The absence of several training sessions, both because of sports injuries or overtraining can affect the athletes' performance and expected financial benefits.

Furthermore, severe disturbances in professional sports like football can cause the elimination of contracts and sponsorships. Sports performance deterioration can markedly affect athletes from a physical and a mental aspect. To improve, athletes must spend an extended period performing specific activities while dedicating a lot of time following specialised programs to gain benefits. Therefore, there is a great need to monitor athletes and gather vital and insightful information about their physical condition, which can help and guide practitioners in preventing injury. Since a proper recovery approach integrates the 4Rs [4] (refuel, rehydrate, remodelling and recovery), some main parameters to consider are biochemical variables related to athletes' redox, inflammation, muscle damage and hormonal status.

It is well known that sports exercise and participation in events can significantly alter the athletes' redox, hormonal, and inflammation condition [5,6]. By continuously engaging in well-structured training programmes, players are exposed to diversified types of stress that ultimately benefit them to accomplish the desired body adaptations and performance improvements. The oxidative stress generated from exercise can be viewed as signalling to determine adaptations, especially in endurance workouts [7]. Typically, acute alterations in oxidative stress molecules pre-, during, and post-exercise stimulate the body's upregulation and transformations. At the same time, an increment in distinct redox homeostasis biomarkers for an extended duration might indicate the need for adjustments in the provided training prescription [8].

Oxidative stress mechanisms can be strongly correlated with inflammation (acute or systemic) through the activities of neutrophils and macrophages. After an injury, leucocyte subpopulations relocate to the impaired tissue for healing by

discharging Reactive and Nitrogen-Oxygen Species (RONS). Besides, when an athlete's immune status is suppressed, an enhanced presence of pro-inflammatory cytokines occurs [9,10]. Cytokines, such as IL-6, play a central role in controlling inflammation, clearing antigens, and repairing tissue [11].

During a football game, players perform definite movements during their attacking or defending attempts, frequently producing intense muscle contractions that eventually contribute to muscle damage. This transient period of muscle damage is characterized by muscle strength loss and Delayed Onset of Muscle Soreness (DOMS) [12]. Throughout the football training season, alterations in specific hormones, such as testosterone and cortisol, are observed, which are essential in determining the performance adaptations. Free bioavailable testosterone is closely associated to anabolism, cortisol, to catabolic processes, while the ratio of those two hormones can be used as an indicator of overtraining [13].

The effect of training on footballers' performance adaptation and health protection depends, among other factors, on the specificity of applied training load and recovery period, individual's training background and current physical status [14]. Recent recommendations indicate that different types of exercise may provoke varying degrees of metabolic stress and lead to redox, hormonal, and inflammatory responses in adult footballers. However, the related information is not straightforward because studies assessing different biomarkers diversify in testing protocols and sample collection timings. The lack of standardization of methods and procedures has hindered some results and needs further clarification.

The periodical assessment of variables linked to oxidative stress hormones, inflammation, and muscle damage can help coaches, and sports practitioners optimise training load sequences, maximize adaptations, and positively influence football performance. Therefore, the paper aimed to systematically review data documenting acute and long-term changes in variables related to redox status, inflammation, and hormonal responses in adult football players over short- or prolonged training periods.

## Materials and Methods

### Reporting standard

This systematic review conforms to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines [15]. The PRISMA 2020 checklist is presented in Appendix 1, indicating the page numbers where items of information are present in the current manuscript.

### Eligibility criteria

The inclusion criteria were based on the Cochrane guidelines

for conducting systematic reviews [16]. The criteria for inclusion and exclusion were set and agreed upon by all five authors. Following the initial selection process of studies, two authors (EV & SP) independently completed the eligibility assessment in a blinded, standardized way by screening the titles and abstracts. To be considered eligible, the manuscript had to meet the following inclusion criteria:

1. **Population:** Only healthy males and adult participants ( $\leq 18$  years of age). Females were excluded due to the impact of the menstrual cycle (hormonal fluctuations) on football performance parameters, thereby rendering it difficult to interpret findings accurately. Female sex hormones have displayed substantial physiological effects on altering fluid regulation and modifications in thermoregulatory, muscular, and metabolic responses. Maximal endurance performance, injury rates, weight gain, mood profiles, and dysmenorrhea have all been negatively affected in female soccer players during different menstrual cycle stages [17].
2. **“Football” specific:** Only studies related to Association football (soccer) were included, meaning any studies related to Rugby football (e.g., Rugby Union or Rugby League), Gridiron football (e.g., American football or Canadian football), Australian Rules Football (AFL) and Gaelic football were excluded.
3. **Training period:** Short-term training (i.e., a soccer match, a Loughborough test) and/or long-term training periods (i.e., a more extensive training period).
4. **Biomarkers:** Biomarkers associated with redox status, specific hormones, inflammation, and muscle damage measured in saliva or blood were included.
5. **Design:** Non-Randomised Control Trials (NRCTs) and case-control study designs were considered.

### Literature search strategy and information sources

A computerised English-language literature search of the grey literature (EV); Liverpool John Moores University Library (SP); and electronic databases: PubMed (MEDLINE), Scopus and Science Direct were conducted (November 2021 – May 2022) and ended on the 22<sup>nd</sup> of May 2022. A search for relevant content related to differences in biomarkers associated with oxidative stress, inflammation mediators and hormonal variations in male soccer players using the following three search syntaxes using Boolean operators in titles, abstracts, and keywords of indexed documents was conducted:

(“oxidative stress” OR “oxidative damage” OR “redox alterations” OR “redox status”) AND (“adult”) AND (“soccer” OR “football”).

(“CK” OR “inflammation” OR “IL-6” OR “C-RP” OR “LDH” OR “Myoglobin”) AND (“adult”) AND (“soccer” OR “football”).

(“testosterone” OR “cortisol” OR “ratio”) AND (“adult”) AND (“soccer” OR “football”).

Additional search techniques using wildcards, truncation and proximity searching were incorporated to widen the search. Secondary searches consisting of the reference lists of all papers included were screened manually for additional relevant documents as part of the secondary search (CB and CS). In addition, forward reference searching was conducted to explore potential follow-up studies through citations and authors (SP). One author (EV) independently carried out the searches for study selection to minimize potential selection bias. Figure 1 presents the flow of papers through the study selection process using the PRISMA 2020 flow diagram [17].

### Study selection

Where both male and female participants took part in a research study, the article was included if the data from male participants could independently be identified. In instances where the title and abstract did not contain enough detail to indicate whether an article was relevant to the review, the complete article was obtained and read. This process enabled the authors to determine whether the paper met the primary inclusion criteria. In instances where the article's primary purpose was not an investigation related to redox status and/or hormonal variations and/or inflammation markers, the papers were excluded from the review. Letters to the editor, conference abstracts and literature reviews were excluded as these studies were not found to be methodologically-quality-assessable and/or critically appraisable.

### Data extraction

Data extraction was performed by two authors (CB and CS) independently and a data check was following performed by two authors (EV and SP) with the following data extracted from the included studies: The study authors and date.

1. The number of participants, their age, and the level of soccer they “compete/perform” (e.g., professional players from Serie A in Italy, professional players from a 2<sup>nd</sup> Division soccer team in Brazil).
2. The considered variables concerning redox status, hormonal variations, inflammation, and muscle damage.
3. The sampling time and the description of the activity and performance test used (e.g., training phase, the number of assessed time-points, the time of sampling).
4. The effects of the activities or performance tests on the selected variables at different time points.

### Quality assessment

A modified 27-item methodological quality assessment checklist on each included article using the Downs and Black scale was conducted [18]. The checklist consisted of 27 “yes”-or- “no” questions which were scored, totaling

up to a possible 28 points. Item 27: “Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?” to a yes (1-point) or no/unable to determine (0 points) scoring. The questions were categorized under 5 sections: Reporting (10 items; 1-10), External validity (3 items; 11-13), Internal validity study bias (7 items; 14-20), internal validity confounding selection bias (7 items; 21-26) and power (1 item; 27). The quality assessment of the articles was conducted by two reviewers (CB and SP) independently, with disagreement on 26 answers across the 34 manuscripts (2.8 %). The observed differences were resolved by a third reviewer (CS).

### Selected biochemical parameters

The biochemical parameters analysed within the review were divided into three categories:

- 1. Redox homeostasis:** molecules related with lipid peroxidation (Malondialdehyde – MDA; Thiobarbituric Acid Reactive Substances – TBARS; Lipid Hyperoxides - LOOH), Protein Modifications (Protein Carbonyls – PC; SH-group - Sulfhydryl-group), Enzymatic Antioxidants (Glutathione Peroxidase – GPx; Catalase – CAT; Superoxide Dismutase - SOD), Non-enzymatic Antioxidant (reduced glutathione – GSH; Oxidized Glutathione – GSSG; Uric Acid - UA) and total antioxidant capacity (Total Antioxidant Capacity – TAC; Total Antioxidant Status - TAS).
- 2. Hormonal responses:** endocrinological variations in

testosterone, cortisol, and their ratio T: C.

- 3. Muscle damage and inflammation markers:** activity of intracellular enzymes (Creatine Kinase -CK; Lactate Dehydrogenase – LDH; Myoglobin – Mb; C-Reactive Protein - CRP) and Pro- and Anti -inflammatory cytokines (interleukin-6 - IL-6).

## Results

### Search results

The primary database revealed 730 articles identified from databases and 1309 articles via organisation and citation searching. Figure 1 presents the number of articles found in each electronic database or through other methods and a detailed flow chart of the literature search, including all the steps performed. Once all duplicates were removed, 491 manuscripts obtained via databases remained in the reference manager (Mendeley, Elsevier, Amsterdam, The Netherlands). After examining titles, abstracts, and keywords, 61 were sought for retrieval and retained for full-text analysis. When assessing for eligibility, 29 were deemed eligible, and 32 were excluded. A further 34 reports identified via organization’s and citation searching were assessed for eligibility, with an additional 5 articles considered eligible. Therefore, a total of 34 reports were included in the review. The reasons for exclusion can be found in Table 1.

### Study characteristics

The detailed participant characteristics for each study included within the review are shown in Tables 1, 2 and 3. In

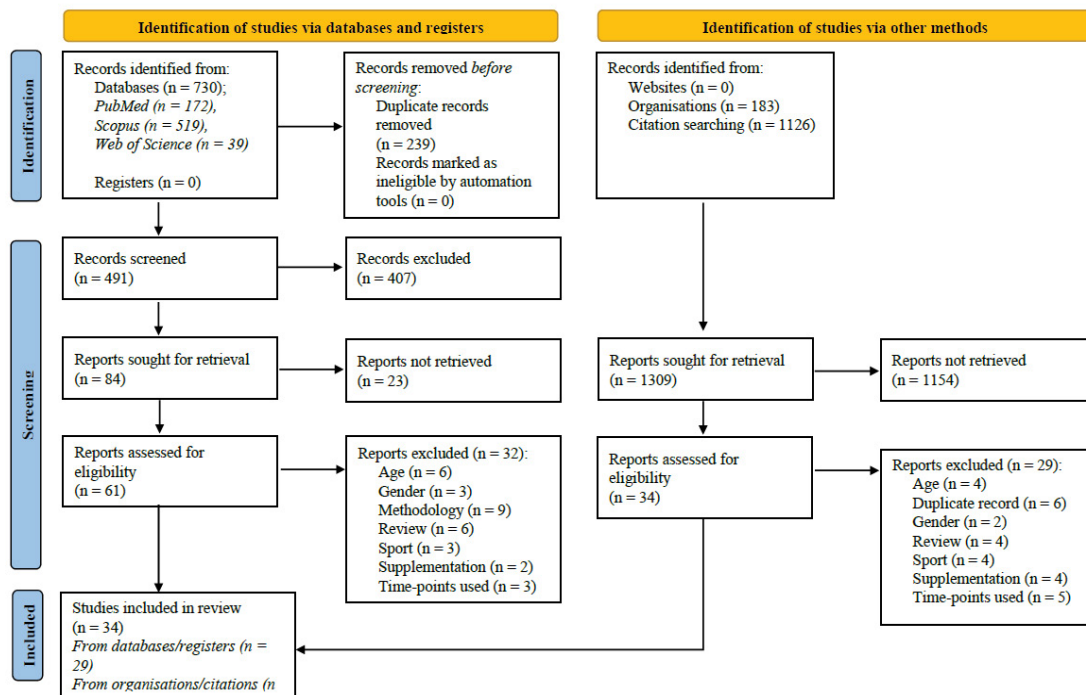


Figure 1: PRISMA 2020 flow diagram of the study selection process [15].



**Table 1:** Summary of all studies on redox status of adult soccer players with an overview of the variables examined the type of activity and sampling times and the main findings in relation to each variable.

Author and Date	Participants	Variables examined	Type of activity and sampling times	Main findings
Ascensão et al. [27]	Sixteen soccer players from Secondary divisions in Portugal, age 21.3 ± 1.1 yrs.	TAS, UA, MDA, SH-groups*	Blood samples pre-match and 30-min, 24-, 48- and 72-h of recovery period in response to a competitive (2 × 45 min) soccer match.	Post 30-min: TAS↑ Post 72-h: UA, MDA↑ SH ↓ (with exception of 72-h)
Becatti et al. [21]	Thirty-six male soccer players from the Italian first division ("Serie A"), age 28 (range 17–35) yrs.	TBARS, PC, GSH/GSSG	Blood samples at 4 time points during the soccer season: T0: just prior to the first team training session (August); T1: at the beginning of the season (September); T2: in the middle of the season (January); T3: at the end of the season (May).	GSH/GSSG, TAC ↓ in T1, ↑ in T2, and ↔ in T3, TBARS, PC ↑ in T1, T2 and T3 UA ↔
Bolner et al. [24]	Thirty-four professional players of a soccer team playing in the Italian A series championship, age 24.9 ± 5.2 yrs.	total (GSSG + GSH), GSH, 8OHdG, 3-NT*	Players were evaluated every 2 months from pre- until end-season (visits V0–V4).	At V2: GSSG+GSH, GSH↑ At V3: GSH↓ At V4: 3-NT↑
da Costa et al. [35]	Ten trained male soccer players from the junior category of an elite Brazilian football association. age 18.3 ± 0.7 yrs.	TPAP, MDA, LOOH*	Blood samples before, during and immediately after a Loughborough Intermittent Shuttle Test.	Immediately after: TPAP↓, MDA↑, LOOH↔
Fatouros et al. [36]	Thirty male soccer players from an U21's division 1 soccer league in Greece and 10 controls, age 20.3 ± 0.3 yrs.	UA, MDA, TAC, GSH, GSSG, GPx, PC, CAT, GSH/GSSG	Blood samples before, immediately post, 24-h, 48-h and 72-h post-match (2 × 45-min match in-house).	Immediately post-match: ↑MDA, PC, CAT Post 24-h and 48-h: UA, MDA, PC, GSSG, TAC, GPx↑; GSH/GSSG ↓ Post 24-h: GSH↓
Rodrigues de Araujo et al. [37]	Thirty-two professional soccer athletes from the Centro de Treinamento de Futebol/Soccer in Rio de Janeiro, Brazil, age 21.2 ± 4.2 yrs.	TBARS, MDA, GSH, GSSG, CAT, SOD, UA	Saliva aliquots were collected at rest and immediately after HIIE protocol.	UA↓ Rest saliva markers did not change
Sopić et al. [38]	Sixteen soccer players of the young selection of football club "Teleoptik", Belgrade, Serbia, age 18.1 ± 0.4 yrs.	O2-, MDA, TAS, TOS, PAB, SH-groups	After a single soccer training and after 45 days of preparation.	After 45 days: ↓TOS, MDA, ↑SH
Mello et al. [39]	Twenty-two professional male soccer players from a second-division soccer team in Brazil, age 26.5 ± 1.9 yrs.	GSH, TAC, TBARS*	Blood samples obtained 5-min pre and post moderate intensity game simulation (2 × 40-min) in pre-season.	Post-game: TAC, TBARS↔ GSH↓
Le Moal et al. [40]	Nineteen professional soccer players from Stade Rennais Football Club in the French League, age 18.3 ± 0.6 yrs.	SOD, GPx, GSH/GSSG	Measures were carried out at 5-time points; T1 (in July), T2 (in September), T3 (in December), T4 (in January) and T5 (in May).	↔ GPX, SOD GSH/GSSG ↑ at T2 and then fluctuated between time points.
Magalhães et al. [28]	Sixteen male soccer players from 2nd and 3rd Portuguese divisions, age 21.3 ± 1.1 yrs.	UA, SH-groups, MDA, TAS*	Blood samples collected pre, 30-min, 24-h, 48-h and 72-h after Loughborough Intermittent Shuttle Test (LIST) and soccer match, separated by 2-weeks.	After both LIST and soccer match: MDA, TAS↑ and SH↓ at 30-min, 24-, 48-, 72-h UA↑ at 30 min
Silva et al. [25]	Seven male soccer players from a team competing in the Portuguese professional soccer league, age range 22-31 yrs.	TAS, UA, SOD, GPx, GR MDA, SH-groups*	Blood samples collected 72-h pre, 24-h, 48-h and 72-h post a 94-min competitive match.	Post 24-h: TAS, GR, SH, SOD, MDA↑; GPx↓ Post 48-h: TAS, SOD, MDA↑ ↔UA across match

Silva et al. [26]	Fourteen male professional soccer from a team competing in the Portuguese professional soccer league, age 25.7 ± 4.6 yrs.	TAS, UA, SOD, GPx, GR MDA, SH-groups*	Blood samples at 4 time points as follows: E1 (preseason), E2 (mid competitive season-Jan), E3 (end competitive season), E4 (end of transition period).	↔ TAS, UA and GPX across season
				E2: MDA, SOD, SH ↑
				E3: MDA, SH, SOD ↑; GR↓
				E4: SOD↓
Souglis et al. [29]	Thirty professional male soccer players divided into playing position: DEF, MID, ATT & thirty healthy volunteers served as controls; 27.0 ± 2.7 yrs.	CAT, GSH, PC, UA*	Seven blood samples collected pre, immediate post, 13-h, 37-h, 61-h, 85-h, and 109-h post-match of an official game (90-min).	Post-game: ↑CAT
				Post 13-h: ↓GSH
				Post 37-h: ↑PC
				Post 61-h: ↑UA
Viana-Gomes et al. [30]	An entire professional football team of the National league in Brazil, age 27.2 ± 5.5 yrs.	In plasma and saliva: TAC, UA, TBARS, PC, GSH, GSSG	Six collections were performed: (1) basal; (2) post-game one (day 2); (3) 48-h post-game-one (day 4); (4) post-game two (day 6); (5) 24-h post-game two (day 7); and (6) 48-h post-game two (day 8).	In plasma: UA↑ at T3, T4, T5
				TBARS ↑ at T6; PC ↑ at T2, T4
				GSH ↑ at T6; GSSG ↑ from T2 to T6
				In saliva: TBARS ↑ at T2, T4

GSH= Glutathione, GSSG= Glutathione disulfide, TBARS= Thiobarbituric acid reactive substances, MDA= Malondialdehyde, PC=Protein carbonyls, SOD= Superoxide dismutase, CAT= Catalase, GPx= Glutathione peroxidase, TAC= Total antioxidant capacity, TAS= Total antioxidant status, TOS= Total oxidant status, SH= Sulfhydryl-groups, UA= Uric acid, 8OHdG= 8-hydroxy-2'-deoxyguanosine, 3-NT= 3-nitrotyrosine, TPAP= Tetrapropylammonium perruthenate, LOOH= Lipid hydroperoxide, GR= Glutathione reductase, O2- = Superoxide anion radical, PAB= Pro-oxidant-antioxidant balance.

\*Study includes more biomarkers.

total 1130 male football athletes were included across the 34 studies, with an average of 33 participants per study, with the lowest number of participants included 5 [19] and the highest number of participants included 467 [20]. The average age of participants was 23.2 ± 2.9 yrs, with a total of 3 studies failing to mention the age or age range of participants [21-23]. The total number of studies looking at redox status was 14 (Table 1), while 20 were on hormonal variations (Table 2) and 16 related to inflammation and/or muscle damage (Table 3).

Four studies assessed biomarkers associated with redox status, specific hormones, inflammation, and muscle damage combined [21, 24-26], 4 other studies assessed biomarkers associated with redox status and inflammation and muscle damage [27-30], and a further 4 studies assessed biomarkers associated with hormonal fluctuations and inflammation and muscle damage [31-34]. All other studies either assess biomarkers associated with redox status or biomarkers associated to hormonal fluctuations or biomarkers associated to inflammation and muscle damage alone.

### Redox homeostasis

#### Lipid and protein peroxidation

The two most frequently used molecules to determine lipid peroxidation were MDA and TBARS (Table 1). Acute levels of MDA displayed an increase following a competitive/simulated soccer match [25-27,35], a training session/performance trial [36-38] or a combination of both [28].

After completing a Loughborough Intermittent Shuttle Test (LIST), MDA values increased immediately after completion of the test, even remaining elevated up to 72-h post a soccer match. When comparing values at different time points throughout a competitive season, MDA values increased during the mid-and the end of a competitive season in soccer, while no differences were established during pre-season or at the end of the transition period. When assessing values after a single training session, values decreased 45 days after [38]. However, no differences were established in MDA levels in saliva following an acute High-Intensity Interval Exercise (HIIE) protocol [37].

Salivary TBARS also fluctuated at different time points throughout the competitive season, with the lowest values observed before the pre-season with higher values at the start, the mid and the end of the season [21]. A study performed by Viana-Gomes et al. [30] found TBARS in saliva to increase immediately after 2 soccer matches performed 4 days apart (day 2 and day 6), while plasma TBARS also showed to increase 48-h post the second match (day 6). Salivary TBARS did not change immediately after an HIIE protocol [37] or in plasma post a simulated match [39].

Protein Carbonyls (PC), as an index of protein carbonylation, showed increases in all examined studies. Two studies found PC values to differ across a competitive season [21] and at different time points after two competitive games spaced 4 days apart [30]. Values were also higher

**Table 2:** Summary of all studies on hormonal variations in adult soccer players with an overview of the variables examined the type of activity and sampling times and the main findings in relation to each variable.

Author and Date	Participants	Variables examined	Type of activity and sampling times	Main findings
Ali et al. [43]	Thirty-six professional, universities, male soccer players split in 3 groups: complex (n=12, age 22.0 ± 2.4 yrs.), contrast (n=12, age 20.8 ± 2.1 yrs.) and control (n=12, age 21.5 ± 1.8 yrs.)	Free T, C	Blood samples pre- and post a 6-week training regimen. Two participating groups performed 4 different exercises, at different intensities.	Both training groups (complex and contrast): ↑T, ↓C
Banfi and Dolci, [13]	Twenty-six in total professional soccer players, Italian first division during a 3-year period, age not stated	C, T	Blood samples collected over two seasons at several pre-season, preparatory and competitive time points.	Between seasons: ↔C, T
Becatti et al. [21]	Thirty-six male soccer players from the Italian first division ("Serie A"), age 28 (range 17–35) yrs.	C*	Blood samples collected at 4 time points during the soccer season: T0: baseline (August); T1: at the beginning of the season (September); T2: in the middle of the season (January); T3: at the end of the season (May).	T2, T3: ↑C
Bolner et al. [24]	Thirty-four professional players of a soccer team playing in the Italian A, age of 24.9 ± 5.2 yrs.	T:C*	Players were evaluated every 2 months from pre- until end-season (visits V0–V4).	↔T:C
Coelho et al. [41]	Ten novices (i.e., healthy students); male, age 22.0 ± 2.8 yrs.	C, T, T:C	Blood/plasma pre and post a collegiate tournament.	↔T, ↓T:C, ↑C
Filaire et al. [47]	Seventeen French players of national level, age 23.7 ± 2.2 yrs.	T, C, T:C	Saliva samples were collected 1 day following the start of season training (T1), before and after a 7-weeks high-intensity training programme (T2 and T3, respectively) and after 4 months (T4). During each collection 3 samples were taken at 8:00 h, 11:30 h and 17:00 h.	T4 vs. T1, T3 vs. T2: ↓T at 11:30 h and 17 :00 h T3 vs. T2: ↓C at 11:30 h T3 vs. T2: ↓T:C at 8:00 h, 11:30 h and 17:00 h
Francavilla et al. [19]	Five elite male soccer players, Italian Serie A, age 25.3 ± 4.9 yrs.	C, T, T:C*	Saliva and blood samples taken at three different time-points: (T1) after the pre-season period (T2) after winter break; (T3) 2 days after the final match of the championship and 19 matches played.	Saliva: T3 vs. T1: ↑T
Handziski et al. [22]	Thirty professional soccer players, (national soccer league), age not stated	C, T, T:C*	Blood samples taken before the start of preparatory phase (I), before the pre-competition phase (II) and post-competition phase (III).	T2 vs. T1: ↑T, T:C, ↓C T3 vs. T2 and T1: ↓T, T:C, ↑C
Ispirlidis et al. [31]	Twenty-four elite male soccer players, age 20.1 ± 0.8 yrs.	C, free T*	Blood samples were collected pre and immediately post, 24-h, 28-h, 72-h, 96-h, 120-h and 144-h post a competitive match.	Immediate post ↑C, Post 0-144-h: ↔T
Lupo et al. [44]	Eighteen semi-professional football players, age range 20-25 yrs.	T, C	Blood samples collected T pre (I), half time (II), immediately post (III), 45-min (IV) and 90-min (V) post-match.	III & IV vs. I: ↓T II- & III vs. I: ↑C
Michailidis [23]	Fifteen players from a soccer club of the first division, age not stated	C, T, T:C	Before (1) and post re-building period (2), mid-season (3), and after ending the competition phase (4).	T4 vs. T3 & T2: ↓T, T3 vs. T2 & T1: ↑C, ↓T:C T4 vs. T3: ↓C

Moreira et al. [48]	Twenty-two high-level Brazilian male athletes divided in team A (n=11) versus team B (n=11); age 23 ± 4 yrs.	C	Saliva samples were obtained from subjects before and after a competitive training soccer match during the competitive season.	Both teams: ↑C Immediately Post
Opaszowski et al. [42]	Twenty players of a 3 <sup>rd</sup> Division soccer club, age 23.6 ± 3.9 yrs.	C, T, T:C*	During performed a running exercise performed twice at the beginning and 1	Post both tests: ↑T Post 2 <sup>nd</sup> test: ↑T:C
Peñailillo et al. [46]	Nine elite male football players of a South American team, age 26 ± 3.5 yrs.	T, C, T:C*	Salivary samples were evaluated before and 10-min post-match.	↔Salivary cortisol ↓Testosterone, T:C by 30.6%, 64.2% respectively after the match.
Romagnoli et al. [32]	Twenty-two young professional male soccer players from an Italian Serie A team, age range 17-20 yrs.	T, C*	Before match and 30-min, 24-h and 48-h after the match, blood samples were drawn.	Post 30-min, 24-h and 48-h: ↔C, T
Saidi et al. [45]	Sixteen elite soccer players from a team playing in the first Tunisian soccer, age range 19-22 yrs.	C, T, T:C	Athletes were evaluated three times (T1, T2, and T3) over 12 weeks.	T3 vs. T2 and T1: ↓T, T:C
Silva et al. [25]	Seven male soccer players Portuguese professional soccer league, age range 22-31 yrs.	C, T, T:C*	Blood samples collected pre (72-h pre), 24-h, 48-h and 72-h and 94-min post competitive match.	Post 24 and 48 h: ↑C, ↓T:C, ↔T
Silva et al. [26]	Fourteen male soccer players from Portuguese professional soccer league, age 25.7 ± 4.6 yrs.	C, T, T:C*	Blood samples collected at 4 time points: E1 (preseason), E2 (mid competitive season- Jan), E3 (end competitive season), E4 (end of transition period).	E2 and E3 vs. E1: ↓T:C E3 vs. E1: ↓C
Souglis et al. [33]	Seventy-two male elite: soccer (n = 18), basketball (n = 18), volleyball (n = 18) and handball (n = 18), & 18 non-athletes served as controls, age 25.6 ± 2.9 yrs.	C*	Blood samples were drawn before, immediately after and 13 and 37-h post-match.	Post-game: ↑C
Thorpe and Sunderland [34]	Seven semi-professional male soccer players, age 25 ± 6 yrs.	Saliva C, T, T:C*	Blood and saliva samples were obtained 1-h before kickoff and immediately post-match.	Post-match: ↑C 78%, ↑T 44%. ↔T:C

T = Testosterone, C = Cortisol, T: C = Testosterone to cortisol ratio. \*Study includes more biomarkers.

**Table 3:** Summary of all studies on inflammation and/or muscle damage markers of adult soccer players with an overview of the variables examined the type of activity and sampling times and the main findings in relation to each variable.

Author and Date	Participants	Variables examined	Type of activity and sampling times	Main findings
Ascensão et al. [27]	Sixteen male second division soccer players, age 21.3 ± 1.1 yrs.	CK*	Blood samples pre, 30-min, 24-h, 48-h and 72-h post competitive match.	Post-match: 30-min, 24-h, 48-h and 72-h: ↑CK
Becatti et al. [21]	Thirty-six male soccer players from the Italian first division ("Serie A"), age 28 (range 17–35) yrs.	CK, Mb, LDH*	Blood samples collected at 4 time points during the soccer season: T0: baseline (August); T1: at the beginning of the season (September); T2: in the middle of the season (January); T3: at the end of the season (May).	CK: ↑T1 LDH: ↓T1, T2, T3 MYO: ↑T1, T3
Bolner et al. [24]	Thirty-four professional players of a soccer team playing in the Italian A, age of 24.9 ± 5.2 yrs.	IL-6, LDH, C-RP*	Players were evaluated every 2 months from pre- until end-season (visits V0–V4).	↔IL-6, LDH In V2: ↑C-RP
Fransson et al. [52]	Twelve male soccer players from Swedish 2 <sup>nd</sup> and 3 <sup>rd</sup> divisions, age 23 ± 1 yrs.	CK, C-RP*	Blood samples taken pre and post a simulated soccer protocol (soccer Copenhagen test 2 x 45 min) at 0-h, 24-h and 48-h. Testing protocol repeated twice.	↑CK at 24-h; return to baseline at 48-h ↔C-RP



Gharahdaghi et al. [51]	Twelve male soccer players from third Iranian divisions, age 21.9 ± 2.2 yrs.	CK, LDH*	Athletes performed one session of exhaustive exercise (Hoff session) twice: pre and post 4 weeks of special soccer training.	After both tests: ↑CK, LDH Pre CK and LDH before 2 <sup>nd</sup> test were lower compared to baseline
Ispiridis et al. [31]	Twenty- four elite male soccer players, age 20.1 ± 0.8 yrs.	CK, C-RP, IL-6*	Blood samples collected pre- and 0-h, 24-h, 28-h, 72-h, 96, 120-h and 144-h post a competitive match	0-h: ↑C-RP, CK, IL-6 24-h: ↑C-RP, CK 48-h: ↑CK 72-96-h: ↑CK
Magalhães et al. [28]	Sixteen male soccer players from the 2 <sup>nd</sup> & 3 <sup>rd</sup> Portuguese divisions, age 21.3 ± 1.1 yrs.	CK, Mb	Blood samples collected pre, 30-min, 24-h, 48-h and 72-h after Loughborough Intermittent Shuttle Test (LIST) and soccer match, separated by 2-weeks.	After LIST and soccer match: ↑CK 30-min, 24-48-72-h; ↑MYO 30 min
Meyer and Meister [20]	Four hundred sixty-seven male soccer players of the 2 highest German leagues, age 24.9 ± 4.4 yrs.	CK, C-RP*	4 samples were collected at baseline (July 2008), in October/ November 2008, in February/March and in April/May 2009 (i.e., T0, T1, T2, T3).	In T2: ↑CK ↔C-RP
Pimenta et al. [50]	Ten professional athletes, age 26 ± 6 yrs.	CK*	Serum concentrations were determined at the beginning and end of two months of pre-season training.	↑CK
Romagnoli et al. [32]	Twenty-two young professional male soccer players from an Italian Serie A team, age range 17-20 yrs.	IL-6, CK*	Before the match and 30 minutes, 24 and 48 hours after the match, blood samples were drawn.	Post 30-min: ↑CK, IL-6. Post 24-h and 48-h: ↑CK
Silva et al. [25]	Seven male soccer players from the Portuguese professional soccer league, age range 22-31 yrs.	CK, Mb, C-RP*	Blood samples pre (72-h pre), 24-h, 48-h and 72-h post 94-min competitive match.	Post 24-h: ↑Mb, C-RP, CK Post 48-h: ↑CK
Silva et al. [26]	Fourteen male soccer players from Portuguese professional soccer league, age 25.7 ± 4.6 yrs.	CK, Mb, C-RP*	Blood samples at 4 time points: E1 (preseason), E2 (mid competitive season-Jan), E3 (end competitive season), E4 (end of transition period).	E2 and E3: ↑CK, C-RP E2: ↑Mb
Souglis et al. [33]	Seventy-two male elite: soccer (n = 18), basketball (n = 18), volleyball (n = 18) and handball (n = 18), & 18 non-athletes served as controls, age 25.6 ± 2.9 yrs.	LDH, CK, C-RP, IL-6*	Blood samples were drawn before, immediately after and 13-h and 37-h post-match.	Post-game: ↑IL-6, Post 13-h: ↑C-RP Post 13 and 37-h: ↑CK, LDH
Souglis et al. [29]	Thirty professional male soccer players divided into playing position: DEF, MID, ATT & thirty healthy volunteers served as controls; 27.0 ± 2.7 yrs.	CK, IL-6, C-RP, LDH*	Seven blood samples collected pre, immediate post, 13-h, 37-h, 61-h, 85-h and 109-h post-match of official game (soccer 90-min game).	Post-game: ↑IL-6, Post 13-h: ↑C-RP, CK, LDH
Thorpe and Sunderland [34]	Seven high-level males (i.e., semi-professional), age 25 ± 6 yrs.	CK, Mb*	Various samples were obtained 1-h before kickoff and immediately post-match.	Post-match: ↑Mb
Viana-Gomes et al. [30]	An entire professional football team in Brazil of which eight players were included, age 27.2 ± 5.5 yrs.	CK*,	Six collections were performed: (1) basal; (2) post-game one (day 2); (3) 48-h post-game-one (day 4); (4) post-game two (day 6); (5) 24-h post-game two (day 7); and (6) 48-h post-game two (day 8).	↑CK from T1 to T6
IL-6= Interleukin-6, C-RP= C-Reactive Protein, CK/CPK= Creatine kinase/Creatine phosphokinase, Mb= myoglobin, LDH= Lactate dehydrogenase *Study includes more biomarkers.				

**Table 4:** Results of the detailed methodological quality assessment scores based on Downs and Black (1998) checklist.

Date	Study Author	Reporting (Items 1-10)										External validity (Items 11-13)			Study bias (Items 14-20)						Confounding selection bias (Items 21-26)						Power (Item 27)	Total Score	Rating									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25				26								
2019	Ali et al. [43]	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22	81
2008	Ascensão et al. [27]	1	1	1	1	2	1	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	17	63
2006	Banfi and Dolci [13]	1	1	0	1	0	1	1	1	0	1	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	11	41	
2017	Becatti et al. [21]	1	1	0	1	1	1	1	0	0	1	1	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	14	52	
2019	Bolner et al. [24]	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	13	48	
2013	Coelho et al. [41]	1	1	1	1	1	1	1	0	0	1	1	0	1	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	15	56		
2011	da Costa et al. [35]	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	13	48	
2010	Fatouros et al. [36]	1	1	1	1	0	1	1	1	0	0	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	15	56		
2001	Filaire et al. [47]	1	1	1	1	2	1	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	16	59	
2018	Francavilla et al. [19]	1	1	1	1	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	17	63	
2018	Fransson et al. [52]	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	17	63	
2013	Gharahdaghi et al. [51]	1	1	1	1	1	1	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	15	56	
2006	Handziski et al. [22]	1	1	0	1	1	1	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	13	48	
2008	Ispiridis et al. [31]	1	1	1	1	1	1	0	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	15	56	
2016	Le Moal et al. [40]	1	1	0	1	1	1	0	1	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	14	52	
1985	Lupo et al. [44]	1	1	1	1	1	1	0	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	16	59		
2010	Magalhães et al. [28]	1	1	1	1	1	1	1	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	19	70		
2017	Mello et al. [39]	1	1	1	1	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	17	63		
2011	Meyer and Meister [20]	1	1	0	1	1	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	1	1	0	0	0	0	0	0	18	67		
2014	Michailidis [23]	1	1	1	1	2	1	1	0	1	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	20	74		
2009	Moreira et al. [48]	1	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	0	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	16	59		
2013	Opaszowski et al. [42]	1	1	1	1	0	1	1	0	0	0	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	16	59		
2015	Peñailillo et al. [46]	1	1	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	1	0	1	0	0	1	0	0	0	0	0	0	0	12	44		
2015	Pimenta et al. [50]	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	18	67		
2018	Rodrigues de Araujo et al. [37]	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	17	63		
2016	Romagnoli et al. [32]	1	1	1	1	0	1	1	0	0	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	18	67		
2020	Saidi et al. [45]	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	18	67		
2013	Silva et al. [25]	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	19	70		

2014	Silva et al. [26]	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	1	1	0	0	0	0	1	1	0	19	70
2014	Sopić et al. [38]	1	1	1	1	0	1	1	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	1	0	0	16	59
2015	Sougliis et al. [33]	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	1	1	1	0	1	1	0	0	0	1	0	0	15	56
2018	Sougliis et al. [29]	1	1	1	1	0	1	1	0	0	0	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0	16	59
2012	Thorpe and Sutherland [34]	1	1	1	1	0	1	1	0	0	1	0	1	1	0	0	1	0	0	1	1	1	1	0	0	1	0	0	15	56
2018	Viana-Gomes et al. [30]	1	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	19	70
<b>Total</b>		<b>34</b>	<b>34</b>	<b>26</b>	<b>34</b>	<b>25</b>	<b>34</b>	<b>30</b>	<b>8</b>	<b>12</b>	<b>18</b>	<b>27</b>	<b>19</b>	<b>19</b>	<b>0</b>	<b>0</b>	<b>33</b>	<b>27</b>	<b>33</b>	<b>29</b>	<b>33</b>	<b>23</b>	<b>14</b>	<b>2</b>	<b>2</b>	<b>21</b>	<b>12</b>	<b>2</b>	<b>16</b>	<b>60</b>
<b>Maximum</b>		<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>68</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>	<b>34</b>			
<b>% of lost points</b>		<b>0</b>	<b>0</b>	<b>24</b>	<b>0</b>	<b>63</b>	<b>0</b>	<b>12</b>	<b>76.5</b>	<b>65</b>	<b>47</b>	<b>21</b>	<b>44</b>	<b>44</b>	<b>100</b>	<b>100</b>	<b>2.9</b>	<b>20.6</b>	<b>2.9</b>	<b>15</b>	<b>2.9</b>	<b>32</b>	<b>59</b>	<b>94.1</b>	<b>94.1</b>	<b>38.2</b>	<b>64.7</b>	<b>94</b>		

immediately, 24-h, 37-h, or 48-h post competitive soccer match, while other time points did not differ [29,35]. The redox status of protein sulfhydryl groups (SH-group related proteins) increased after a competitive match at different time points throughout the season or after 45 days of training [26,38]. However, some studies found SH-group related proteins to decrease following a LIST protocol or 45 days of training [28,38].

### Glutathione, antioxidant enzymes, and total antioxidant capacity

Glutathione (GSH) levels dropped immediately post [39], 13-h post [29], 24-h and 48-h post [35] a soccer game. When two matches were performed four days apart, GSH values increased 48-h after the second competitive game [30]. GSH fluctuated throughout a soccer training season in professional players of the Italian series A, with values increasing after 4 months and then decreasing after 6 months into the training season. However, GSH samples collected after an HIIE protocol did not change [37].

Glutathione Peroxidase (GPx) and glutathione oxidized (GSSG) also showed to vary, with values increased 24-h and 48-h post-match [35]. However, Silva et al. [25] found values of GPx to decrease 24-h post-match, with no differences observed 48-h post. When assessing the GSH/GSSG ratio, it was observed that values increased at the start of the season in September in a group of players competing in the French League [40] and players of the Italian League [21]. Acutely, Fatouros et al. [35] found this ratio to decrease 24-h and 48-h post-match.

Superoxide Dismutase (SOD) increased 24-h after the completion of a soccer match [25], at mid-and end of the competitive season, to decrease at the end of the transition period compared to the pre-season levels [26]. However, SOD did not change in players competing in the French league throughout the season [40]. Catalase (CAT) increased

immediately post-match, but it did not differ from 13-h to 109-h post-match [29,35]. Antioxidant enzymes, SOD & CAT, remained unaltered when measured in saliva [37].

Uric Acid (UA) showed increases in most studies, with higher values directly after the completion of a LIST test [28] and 24-h up until 72-h post-match [27,29,35]. Besides, plasma UA also increased after two competitive matches with a four-day break in-between [30]. On the contrary, UA decreased when measured in saliva after an HIIT protocol [37]. At the same time, it did not fluctuate in a group of Portuguese footballers when measured short- or long-term [25,26]. Total antioxidant capacity and total antioxidant status increased immediately after a LIST assessment [28] and up to 72-h after a soccer match [26,27,35]. Some studies did not find any variations following a simulated soccer [39], a period of 8 days where players had 2 games [30], at different time points during the competitive season [26] or after 45 days of training [38].

### Hormones

#### Testosterone

The measurement of Testosterone (T) in blood did not reveal significant changes in ten of the examined studies either after short- or long periods of monitoring, e.g., two seasons where several samples were collected at different time points during the pre-season, preparatory phase, or in-season [13,19,21,24,33], or pre-and-post a tournament [41] or immediately after a soccer game [25,31,32]. The concentration of T did not also show noteworthy changes from 24-h to 144-h post-game [31].

However, five studies showed T increased immediately post-match [34], following a running exercise performed at the start and end of the 7-week preparation period [42] and before the pre-competition phase when compared to the beginning of preparatory phase [22]. In Ali et al. [43] and Francavilla et al. [19], concentration was high as well. Still,

we must mention that the free testosterone was analysed in the first study, and the second study also contained saliva samples.

Further, four studies found T to decrease; immediately and 45 min post-game [44], after 12-weeks of training [45], post the re-building period, and mid-season [23] and post-competition phase compared to preparatory and pre-competition stages [22]. When T was analysed in saliva, two studies displayed low levels: 10-mins post-match [46] and after 4-months of training into the season at 11:30 h and 17:00 h [47].

### Cortisol

Cortisol (C) values in most studies showed an increase at mid-season [23], at the end of the season (May) [21] and two days after the final match of a 19-game Championship [14]. Acutely, C increased directly after an official soccer match [25,31,33] or training match [48,49] and immediately after a collegiate tournament [41]. Levels of C were also high during halftime, immediately after and 45-min after a match [44]. Though, C levels dropped following a 6-week training regimen [43], before the pre-competition phase [22], and at the end of the competitive season when compared to the start of the season [26]. However, C concentration did not change significantly over two training seasons when it was tested at several different time points [13], after a running exercise performed twice, at the beginning and the end of the seventh-week preparatory period [42], over 12 weeks of training [45], and from 90-min, 24-h, to 48-h after a match [32].

### Testosterone to cortisol ratio

In six studies, the T:C ratio significantly decreased immediately and up to 72-h post-game [25,46] post a collegiate tournament [41], at mid-season [23], after 12-weeks of training [45] and at the end of the competitive season [26]. In two studies, the T:C ratio increased post-competition phase [22] immediately and at the end of the seventh week of the preparatory period [42]. Two further studies did not find fluctuations in the T:C ratio immediately post-match [34] or when molecules were evaluated every two months throughout a season [24].

### Inflammation and muscle damage related variables

Creatine Kinase (CK) is the most frequently explored biomarker, with 15 studies analysing CK and mentioning increases (Table 3). Levels of CK increased immediately post-match until 96-h post-match. Increases in CK were also observed over more extended periods of training: as at the beginning of the season and one month of training [21], after 2 months [50], following 9 months [20], during the mid and end of a competitive season [26], over 8 days when during 2 games played 4 days apart [30], and after an exhaustive exercise (Hoff session) performed pre and post 4 weeks of special soccer training [51].

The concentration of Lactate Dehydrogenase (LDH) was elevated in three studies, such as after a post-Hoff test performed pre and post 4 weeks of training [51], and 13-h to 37-h post-match [29,33]. During more extended periods, LDH levels gradually dropped during the season compared to baseline levels [21], although one study found no differences throughout the season [24]. Myoglobin (Mb) significantly raised up to 20-min post-game [28,34], during mid-season [26] and at the end of the season [21].

Similarly, C-Reactive Protein (C-RP) increased in most considered studies, with higher values mentioned immediately post-match up to 24-h post-match [25,31,33]. C-RP was also higher over more extended training periods, such as after 2-months in-season [24] and during mid-season [26]. However, it did not fluctuate after a simulated soccer protocol [52] or at different time points throughout a competitive soccer season [20]. Interleukin-6 (IL-6) values increased immediately post-match [29,31,33], 30-min post-match [32]. However, IL-6 values remained unchanged throughout the season when measured every 2 months [24].

### Methodological quality control and publication bias

Based on a modified 27-item Downs and Black [18] checklist, the results of the methodological quality assessment of the included studies ranged from 13 to 22, with an average of 16 or 60 % over the 34 included studies. Reporting (10 items; items 1-10) showed 4 items to be fully met by all studies (Items 1, 2, 4 and 6), with no studies meeting all the item criteria for reporting. External validity (3 items; items 11-13) displayed all three items to be met by 9 studies, while no items were met by all studies. Internal validity study bias (7 items; items 14-20) reported no items (items 16-20) to be fully met. Items 14 and 15 were met by no studies. Confounding selection bias (6 items; items 21-26) also reported no studies to meet all the item criteria. Power (Item 27) was met by only 2 studies [37,42]. Detailed methodological quality assessment scores can be found in Table 4.

## Discussion

### Redox Homeostasis related variables

Lipid peroxidation denotes the reaction of reactive species with different types of lipids producing several by-products. Most investigations focus on responses on cell membranes despite the existence of more types of lipids placed in the cytosol [53]. The reaction of oxygen with unsaturated lipids produces a wide assortment of oxidation products, with the primary product being lipid hydroperoxides (LOOH). One study assessed LOOH variations before, during and after a LIST, but without significant changes [36]. Most of the studies have used blood samples compared to saliva ones. Within this review, lipid peroxidation-associated indicators (MDA, TBARS) of examined studies increased acutely,



such as after a soccer game or performance test, and over longer periods, such as following a more extended period throughout the training season [25-28,35,36,38]. The use of saliva is a straightforward, non-invasive process that can be applied frequently; however, the use of this specimen to evaluate redox status markers requires more examinations to get a better understanding. Currently, there is a lack of data on the biological fluctuation of the same biomarker in differing fluids [6].

Protein modifications have meaningful physiological consequences because they directly influence the function of many proteins and may lead to an indirect transformation of biomolecules [54]. Protein oxidation has been mostly measured through protein carbonyls [55]. In the present review, protein carbonyls were found raised after all types of engaging stimuli. Two studies found SH-group related proteins to be decreased following a HIIT protocol or 45 days of training [38]. However, it is crucial to be mentioned that sampling time plays a significant role in results. Indicatively, a research study reveals that the best sample time-point for PC assessment is 4 hours post-exercise for non-muscle damage exercises [49].

Many studies incorporated the measure of non-enzymatic low molecular weight fragments such as GSH, UA and the ratio of GSH/GSSG. Immediately and 24-h post soccer game, GSH dropped, but it fluctuated during the training cycle. The GSH/GSSG ratio increased during extended periods, implying adaptations of antioxidant enzymes [21,40], while acutely, after a game, it was dropped [35]. UA levels increased in plasma samples immediately and up to 72-h post-game [27-29,30,35].

All enzymatic antioxidants showed a tendency to increase mostly acutely. SOD increased in most examined acutely and chronically studies [25,26]. However, in the French league, SOD did not change when players were monitored for 10 months [40]. CAT and GPx increased post-game [29,35] until 48-h post-match [45]. One study by Silva et al. [25] observed GPx levels to decrease post-match.

TAC and TAS increased immediately post-match [25,27,28,45] and remained high up to 72-h post [28]. Though, these markers did not change during a simulated game [39] or an extensive training period [26,38]. The measurement of those variables in saliva samples needs further exploration since, as in most cases, no changes were detected.

These findings further highlight that the sampling time is pivotal for detecting fluctuations in specific molecules as the time-to-peak differs. Besides more detailed information about the applied training programme during lengthy periods is required. There is no “best” or “most accurate” time-point for assessing all markers at once. The literature refers, for example, that the best sampling time for CAT is immediately

after completing an aerobic exercise and for TBARS 1-h post. Molecules like TAC, GSH, and GSSG require a 2-h post sampling time while PC 4-h post. [49]. Studies investigating variables related to redox homeostasis should possibly prioritize the biomarkers of interest and arrange proper timing.

## Hormones

Testosterone is a commonly used biomarker within soccer but yields inconsistent results. In this review, nine studies found T levels in the blood to reveal no significant changes. Studies observed variations in testosterone when the free or bioavailable portion was assessed, where decreases were noted immediately and 45-min post-game [44]. Longer-term observations also established free T levels to decrease after 12 weeks of training [45], during the post-re-building period and mid-season measures [23], and during the post-competition period compared to post-evaluation [22]. When T levels were assessed in saliva, two studies did yield significant decreases 10 min post-match [46] and after 4 months of soccer training [47].

One key point for consideration when the analysis of T is required is that the total T is typically measured in blood samples unless this has been accurately specified. In some cases, when the free or bioavailable T is measured, mainly in saliva samples, outcomes can reflect the effect of training. Free or bioavailable is the amount of this hormone that is not bound to albumin or Sex Hormone-Binding Protein (SHBP) and thus is available for use (anabolic processes, etc.). Cortisol is a catabolic hormone secreted by the adrenal cortex and consists of one of the primary physical and physiological stress markers. It has been established that exercises requiring 60% or more of an individual's maximal oxygen consumption (VO<sub>2</sub>max) can result in physical stress that causes increased cortisol secretion [56]. Besides, among the vital functions of cortisol are the quick spurts of energy and the maintenance of blood glucose levels.

Cortisol acts on the skeletal muscle and adipose tissue to increase the mobilisation of amino acids and lipids and stimulate gluconeogenesis. Most studies found C levels raised in soccer, irrespective of whether these were measured in the blood [21,23,31,33,41,44,48] or saliva samples [34,47]. Differences were observed acutely [31,33,34,41,48] and after more extended examinations [21,23].

When viewing the ratio of T to C, it is crucial to notice the distinction between “T to C” and “free T to C” ratios since they compare different testosterone portions. The ratio of these two hormones may illustrate the balance between training and recovery desired to maintain the body in an “optimal” state to reach muscle mass and strength increments. In five studies, the ratio was significantly lower after a scholarly tournament [41], up to 72 h post-game [25], in mid-season [23], after 12 weeks of training [45], and at the end of the competitive

season [26]. Corresponding to some researchers, a drop in the ratio of around 30% between assessments potentially represents an overtraining state [57]. Recently, the free T:C ratio has been recommended as a more sensitive marker to evaluate overreaching overtraining in athletes [13].

### Inflammation and muscle damage variables

Variations in CK activity can be used to assess muscle tolerance, skeletal muscle micro-injury and athletes' adaptation. Movement patterns in football require individuals to change direction regularly and involve a stop-and-start nature, leading to a severe eccentric biomechanical strain on the working muscles [20,58]. The return of CK to baseline may consist of athletes' recovery index. CK levels rose acutely in all investigations immediately post-game, with values increasing as much as 96-h post [25,27-29,31-33,51,52]. Values of CK also increased after applying a considerably extended period of training [20,21,30,50].

Levels of LDH were elevated after performing a Hoff session, pre- and post-two months of training [51], immediately, 13-h post [29], and 37-h post an official match [33]. Like CK, LDH increases significantly during exercise, and the level and intensity of training influence the response to exercise. Values of LDH have risen markedly in untrained individuals, and its efflux from the tissue into circulation after strenuous exercise continues until the ninth day after the end of the effort [59]. Monitoring metabolites like CK and LDH can enhance our understanding of muscle response to exercise and adaptation to physical work [60].

Other markers, such as Mb and IL-6, also showed variations in football players. Mb is a protein that supports the store of oxygen in myocytes, and its concentration is associated with mitochondrial enzyme activity and the capillary supply [61]. Levels of Mb increased significantly in several examined studies, such as immediately post until 20-min post-match [28,34], in mid-season [26] and at the end of the season [21].

In the present systematic review, IL-6 was raised post-match [29,31-33]. Based on work conducted by Helsten [62], IL-6 has shown to peak up to 90-min post-exercise and can remain elevated for 4 days. IL-6 has pro- and anti-inflammatory roles and might significantly affect metabolic and musculoskeletal adaptation to exercise. Besides, in skeletal muscle, IL-6 enhances both glucose uptake and fat oxidation [63].

C-RP is an acute phase inflammation protein that transiently increases after medium to high-intensity exercise. Long-term, systematic training is linked to lower values of both pro-inflammatory cytokines and acute-phase proteins in the blood [64]; this point might explain why some studies did not observe changes over longer time periods. C-RP was elevated

in most studies, such as immediately, 13-h [29,33] and 24 h post-game [25,31]. Also, it raised after 4 months of training (V2) when athletes were monitored every 2 months [24], and at the mid-period of the competitive season (Jan) and the end-period of the competitive season [26]. However, it did not significantly change after a simulated soccer protocol [52], or long term when 4 samples were collected from July 2008 till May 2009 [20].

### Conclusion

The results showed that exposure to short or long-term participation in football training and competitions could significantly affect players' redox, inflammation and hormonal status. However, greater consistency across studies is required to ascertain the implications of structured training regimens on examined variables. Depending on the research or the purpose of the athlete's evaluation, selecting the most relevant testing protocol/condition and markers, including the collection time and type of specimen, must be considered. The measurement of redox status-related variables in saliva requires more examination regarding the variability of those molecules in diverse specimens. To be accurately identified, many molecules need different sampling times; thus, studies exploring specific biomarkers should prioritize the collection time close to the variables of interest.

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