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Thèse de doctorat
Discipline : Sciences et Techniques des Activités Physiques

Réponses biologiques et psychologiques chez les footballeurs élites: Effets de la congestion des matchs

Présentée par Karim SAIDI

Soutenue le 7 décembre 2020

Jury

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UNIVERSITE RENNES 2

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SOUS LE SCEAU DE L'UNIVERSITÉ BRETAGNE LOIRE

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DIRECTEUR DE THÈSE : Abderraouf BEN ABDERRAHMAN

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1 INTRODUCTION GENERALE

Le football est l'un des sports les plus populaires de la planète. Ce sport est considéré comme une activité multifactorielle. En effet, La performance en football a plusieurs composantes. Elle est psychologique : résistance au stress, capacité de concentration, gestion de l'échec…
Elle est tactique, à travers les comportements collectifs offensifs et défensifs.
Elle est aussi technique dans les gestes individuels, avec ou sans ballon.
Elle est enfin physique, du fait de la nature et de la répétition des efforts.
Dans le football, ces quatre aspects sont indispensables pour garantir la performance (Bangsbo, 1994). L'analyse temporelle dans le football d'élite montre des changements de l'activité physique du footballeur toutes les quatre à cinq secondes pendant un match de quatre-vingt-dix minutes (Mohr et al., 2003). En raison des exigences physiologiques propres au football, les programmes d'entraînement doivent être spécifiquement adaptés et doivent aborder plusieurs qualités physiques telles que la capacité aérobie, la force, la puissance, la vitesse et les changements de direction. L'objectif des entraîneurs et des préparateurs physiques serait de trouver le stimulus d'entraînement optimal permettant de maximaliser et d'optimiser la performance, tout en minimisant les risques liés à l'entraînement (fatigue, blessure, surentraînement). Certains auteurs ont parlé d'équilibre dans la gestion de la charge d'entraînement, entre le travail et la récupération (Impellizzeri et al., 2004 ; Gaudino et al., 2015). Or, pour obtenir ce point d'équilibre, il faut pouvoir quantifier les charges liées à l'entraînement et au match, à travers un processus qui est individualisé tant de manière objective (indicateurs physiologiques) que subjective (questionnaires) (Bartlett et al., 2017). Cette évaluation va permettre de mieux comprendre la fatigue des joueurs engendrée par la pratique de football (Halson, 2014).
Pendant et après un entrainement et/ou un match de football, les différentes systèmes physiologiques du corps sont mises à contribution entraînant un changement des différents marqueurs biologiques (paramètres hématologiques, hormonaux, des processus inflammatoires, et des dommages musculaires...) qui peuvent finalement avoir une incidence sur la performance physique (Romagoli et al., 2016 ; Coppalle et al., 2019; Sparkes et al., 2020). Bien comprendre les effets aigus et à long terme de l'entrainement et de compétition de football sur la performance physique et les marqueurs biologiques est indispensable à la gestion de la charge d'entrainement et au processus de préparation à la performance de haut niveau.

Dans le football contemporain, les équipes de football peuvent jouer entre 50 et 80 matchs pendant la période compétitive d'une saison footballistique et parfois elles sont obligées de

jouer régulièrement des matchs successifs avec seulement deux à trois jours de récupération (Ekstrand et al., 2004 ; Dellal et al., 2015; Arruda et al., 2015). La période durant laquelle les joueurs jouent plusieurs matchs rapprochés est appelée « période congestive ».

Des études récentes ont examiné les effets d'une période compétitive congestive sur les profils d'activité physique chez des footballeurs élites. En effet, Mohr et al. (2016) ont évalué la distance totale parcourue pendant les matchs chez des footballeurs élites jouant trois matchs en 9 jours. Ces auteurs ont observé une diminution de 7 à 14% de la distance parcourue à haute intensité dans le deuxième match par rapport au premier (joué trois jours avant) et au troisième (joué quatre jours après) matchs. De plus, Pnemedo Jamardo et al. (2017) ont observé que la distance totale parcourue par des footballeurs allemands pendant les matchs est moins importante lorsque la période de récupération entre les matchs est moins de 4 jours par rapport à celle observée lorsque la période de récupération est supérieure ou égale à cinq jours. Bien que ces études aient montré que le niveau d'activité physique et la capacité d'effectuer des courses pendant les matchs peuvent être affectés en raison de la congestion des matchs, très peu de données existent concernant les indicateurs des performances physiques chez des footballeurs élites pendant cette période. Ces connaissances semblent très importantes car elles permettraient de fournir des informations quant aux effets négatifs de la période compétitive congestive sur les différentes qualités physiques déterminantes de la performance physique en football (par exemple, endurance, répétitions des sprints, puissance musculaire…).

De même, les effets de la congestion des matchs sur les marqueurs biologiques et psychologiques chez les footballeurs élites sont encore relativement peu clairs. Les résultats des études précédentes suggèrent que des matchs successifs et des périodes d'entraînements intenses peuvent affecter les réponses psychophysiologiques chez les footballeurs elites. En effet, Moreira et al. (2016) ont montré une diminution de la concentration de testostérone mesurée après une période compétitive congestive (sept matchs joués pendant sept jours). De plus, Freitas et al. (2014) ont démontré qu'une période d'entraînement et de compétition intensive pourrait entraîner une augmentation de la concentration de cortisol salivaire, et une diminution de la tolérance au stress chez des footballeurs élites. Ainsi, le niveau de stress physique et psychologique engendré par les périodes d'entrainement et de compétition intense pourrait influencer l'homéostasie corporelle et conduirait à des variations critiques des paramètres biologiques qui pourraient à leur tour affecter la performance physique (Reinke et al., 2009). Connaitre les effets d'une période congestive sur la performance physique, les parameters biologiques et pschychologiques représente un enjeu determinant dans la gestion

d’une équipe engagée dans plusieurs compétitions. De plus, examiner l’interaction entre ses differents paramètres nous permettra de mettre en évidence la pertinence de certains indicateurs objectifs et subjectifs pour quantifier l'équilibre entre la charge d'entraînement et la charge compétitive avec la tolérance du footballeur.

Objectif principal de cette thèse

L’objectif principale de cette thèse est de mieux comprendre l'impact du processus d'entraînement et de compétition intense (période congestive) sur les réponses biologiques (hématologiques, hormonales, inflammatoires), psychologiques et physiques chez des footballeurs élites. Ainsi, dans le cadre de cette thèse, nous avons effectué une revue systématique ayant pour objectifs de résumer et de discuter les effets aigus et à long terme de l’entrainement et de compétition de football sur les paramètres hématologiques, hormonaux, inflammatoires, les marqueurs de dommages musculaires et la performance physique des footballeurs. Par la suite nous avons entrepris des travaux originaux ayant pour objectifs de :

1) Etudier la variation du volume plasmatique, des marqueurs hématologiques, de la charge d’entrainement et de la performance physique après six semaines d’entrainement et de compétition intense (période congestive).

2) Etudier les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs hormonaux, psychologiques et la performance physique.

3) Etudier les effets de la période compétitive de la saison (non congestive vs.congestive) sur les marqueurs inflammatoires, les dommages musculaires, les états de bien être (sommeil, fatigue, stress et douleurs musculaires) et la performance physique chez des footballeurs élites.

Ce travail de thèse nous permettra ainsi d’identifier les marqueurs et les indicateurs les plus pertinents qui aideraient à la gestion de la charge d'entrainement et à la prévention d’un état de fatigue ou de surentrainement durant la période compétitive congestive. Ceci semble, à nos yeux, très important surtout avec l’augmentation importante de la congestion des matchs de football durant les différents championnats à cause de la pandémie du COVID-19 (exemple du Final 8 de la Champions League au Portugal en août 2020).

2 REVUE DE LITTERATURE

Hématologie, hormones, inflammation et dommages musculaires chez les footballeurs : une revue systématique ayant des implications pour l'exercice

Hematology, hormones, inflammation and muscle damage in soccer players: A systematic review with implications for exercise

(Soumis à Sports Medicine le 10/10/2020)

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Résumé

Contexte: La connaissance des effets aigus (exemple., match de football ou séance d'entraînement) et à long terme de l'entraînement et de compétition de football sur les paramètres hématologiques, hormonaux, inflammatoires, les marqueurs de dommages musculaires et la performance physique peuvent aider à mieux concevoir les programmes d'entrainement et de conditionnement physique pour le développement de la performance et la prévention des blessures chez le footballeur.

Objectifs: L'objectif de cette revue systématique était de résumer et de discuter les effets aigus (exemple, match de football ou séance d'entrainement) et à long terme de l'entrainement et de compétition de football sur les marqueurs hématologiques, hormonaux, inflammatoires, les marqueurs de dommages musculaires et la performance physique chez des footballeurs de différents niveaux d'expertise. Le deuxième objectif était d'étudier les associations entre certains marqueurs biologiques et la performance physique.

Matériaux et méthodes: Une recherche systématique dans la littérature a été menée dans quatre bases de données électroniques (PubMed, ISI Web of Knowledge, Web of Science, et SPORTDiscus) depuis sa création jusqu' à août 2020. La clé suivante et les termes MeSH ont été inclus et combinés en utilisant les opérateurs "AND", "OR", "NOT": ((soccer OR football) AND ("soccer training*" OR "football training*" OR "small sided soccer game*" OR "soccer match*") AND ("hematological response*" OR "haematological variation*" OR "hematological parameter*" OR "plasma volume variation*" OR "blood parameter*") AND ("hormonal adaptation*" OR "biochemical marker*" OR "cortisol change*" OR "testosterone change*" OR "androgen level*" OR endocrine response*) AND (inflammation OR "inflammatory marker*" OR "cytokines marker*" OR "muscle damage")). En outre, La liste des références et les citations (Google Scholar) des études identifiées ont été explorées afin de détecter d'autres articles pertinents. La recherche a identifié initialement deux milles deux cent soixante articles. Après avoir examiné les titres, les résumés et les textes intégraux, quarante-huit études ont été sélectionnées pour examiner les effets aigus (un match et/ou une séance d'entrainement) et à long terme de l'entraînement (> quatre semaines) et de compétition de football sur les paramètres hématologiques, hormonaux, inflammatoires, les marqueurs de dommages musculaires et la performance physique chez des footballeurs.

Résultats: Onze études ont examiné les effets aigus du match de football. Huit études ont examiné les effets aigus d'une séance d'entrainement de football. La durée de la séance d'entrainement est entre 60 et 120 minutes. Vingt neuf études ont examiné les effets à long terme de l'entrainement et/ou de compétition de football avec des durées d'entraînement allant de quatre à dix-huit semaines dans dix-sept études, environ un an dans six études et deux ans dans une étude. Parmi les quarante-huit études inclues dans la revue, trente-sept études portaient sur des joueurs de football professionnels/élites, cinq incluaient des joueurs de football semi-professionnels et six études impliquaient des jeunes joueurs de football.

Les matchs de football ont montré une augmentation (faible à modérée) des paramètres hormonaux (exemple, cortisol (C), intervalle de la taille d'effet; ES= 0,3 - 0,8), une augmentation des paramètres inflammatoires (exemple, interleukine 6 (IL-6), ES= 0,4 - 3,9), une augmentation des marqueurs de dommages musculaires (par exemple, créatine kinase (CK); ES= 0,5 - 4,8), et une diminution des paramètres de la performance physique (par exemple, saut de contre-mouvement jump (CMJ), ES= -0,3 à -1,4). Aucune relation significative n'a été trouvée entre les marqueurs hématologiques, hormonaux, inflammatoires et de dommage musculaire avec les paramètres de performance physique après un match de football.

Les séances d'entrainement de football ont entraîné une légère diminution des marqueurs hématologiques telles que l'hémoglobine (Hb), (ES= -0,2), une légère augmentation des paramètres hormonaux tels que la testostérone totale (T), (ES= 0,32 - 0,34) et une augmentation modérée des marqueurs de dommages musculaires comme la CK (ES= 0,54). De plus, une légère diminution de la performance a été constatée pour les mesures des paramètres de performance physique telles que la hauteur enregistrée dans le test de CMJ (ES= -0,3). Une corrélation positive a été trouvé entre Δ% T et Δ% CMJ (r= 0,90 ; p= 0,001). De plus, une corrélation négative entre Δ% CK et Δ% CMJ (r= -0,99; p= 0,0001) a été observé.

Les effets à long terme de l'entraînement et de compétition ont révélé une altération (augmentations ou diminutions) des marqueurs hématologiques, hormonaux, inflammatoires, de dommages musculaires et de la performance physique. Deux études ont montré une augmentation modérée des marqueurs hématologiques tels que l'Hb, (ES= 0,7 à 0,8). Des changements spécifiques des paramètres hormonaux ont été démontrés; une augmentation de T (ES= 0,2 à 0,8) et une diminution du C (ES= -0,2 à -2,4). Enfin, on a trouvé une augmentation des marqueurs de dommages musculaires tels que la CK (ES= 0,6 à 9,9) et des paramètres de la performance physique tels que la hauteur enregistrée en CMJ (ES= 0,28 à

1,66) et en squat jump (SJ). Après une longue période d'entraînement et/ou de compétition de football, des corrélations positives significatives ont été trouvées entre Δ% de la testostérone libre (FT) et Δ% SJ (r= 0,98; p= 0,01) et entre Δ% du ratio testosterone : cortisol (T/C) avec Δ% SJ (r= 0,90; p= 0,03).

Conclusions: Les résultats de cette revue systématique suggèrent que les paramètres hématologiques, hormonaux, inflammatoires et les marqueurs de dommages musculaires ont été altérés après un match de football et/ou une séance d'entrainement. Les changements des concentrations de T et de CK étaient en relation avec le changement de la performance en CMJ après une séance d'entraînement de football. De plus, nous avons observé que l'entraînement à long terme induit une augmentation ou une diminution des paramètres hématologiques, hormonaux, inflammatoires, de dommages musculaires et de la performance physique. Ces fluctuations peuvent être expliquées par plusieurs facteurs (charge d'entrainement, durée d'entrainement, facteur psychologique, état d'humeur). Les changements des paramètres hormonaux (T libre et T/C) étaient en relation avec le changement de la performance en SJ. Les hormones anabolisantes et les marqueurs de dommages musculaires peuvent être utilisés comme des outils utiles pour identifier les modifications aigues et à long terme des paramètres de la performance physique. Des études supplémentaires sont nécessaires pour mieux examiner les corrélations entre les paramètres biologiques, les paramètres psychologiques, et la charge d'entrainement.

Points clés

- Les paramètres hématologiques, hormonaux, inflammatoires et les marqueurs de dommages musculaires ont été altérés après un match de football et/ou une séance d'entrainement.
- Les changements des concentrations de T et de CK après une séance d'entrainement de football étaient en relation aux changements de la performance en CMJ.
- Les changements des paramètres hormonaux (FT et T/C) après une longue période d'entraînement et de compétition de football étaient en relation aux changements de la performance en SJ.
- Les hormones anabolisantes et les marqueurs de dommages musculaires peuvent être utilisés comme des outils utiles pour identifier l'altération des paramètres de performances physiques après un match de football, une séance d'entrainement, et un entrainement à long terme.

- Les entraîneurs peuvent utiliser l'interaction entre les marqueurs biologiques et les paramètres de performance physique pour mieux gérer la charge de travail et surveiller la fatigue pendant l'entrainement et la compétition.

Abstract

Background: Knowledge on the acute (e.g., soccer match or training session) and long-term effects of soccer training and competition on hematological, hormonal, inflammatory, muscle damage markers, and physical performance may help to better design strength and conditioning programs for performance development and injury prevention of the individual player and the team.

Objectives: The aim of this systematic review was to summarize and discuss evidence on the acute and long-term effects of soccer training and competition on selected hematological, hormonal, inflammatory, muscle damage markers and physical performance in soccer players. A second goal was to investigate associations between selected physiological and performance markers.

Materials and Methods: A systematic literature search was conducted in four electronic databases (PubMed, ISI Web of Knowledge, Web of Science, SPORTDiscus) from inception until August 2020. The following key and MeSHterms were included and combined using the operators "AND", "OR", "NOT": ((soccer OR football) AND ("soccer training*" OR "football training*" OR "small sided soccer game*" OR"soccer match*") AND ("hematological response*" OR "haematological variation*" OR "hematological parameter*" OR "plasma volume variation*" OR "blood parameter*") AND ("hormonal adaptation*" OR "biochemical marker*" OR "cortisol change*" OR "testosterone change*" OR "androgen level*" OR endocrine response*) AND (inflammation OR "inflammatory marker*" OR "cytokines marker*" OR "muscle damage")). In addition, the reference lists and citations (Google Scholar) of the identified studies were explored in order to detect further relevant research papers.The search syntax initially identified 2,260 records. After screening for titles, abstracts, and full-texts, 48 studies remained that examined the acuteand long-term effects of soccer training (> 4weeks) and/or matches on hematological, hormonal inflammatory, muscle damage, and performance markers in soccer players.

Results: Eleven studies examined the effects of soccer matches and eight studies the effects of soccer training sessions. The duration of single soccer training sessions lasted between 60-120 minutes.Twenty-eight studies investigated the effects of long-term soccer training with training durations lasting between 4-18 weeks in 17 studies, around one year in six studies and two years in one study. Among the total 48 included studies, 37 studies investigated professional/elite soccer players, five included semi-professional soccer players, and six studies involved young soccer players.

Soccer matches resulted in small-to-moderate increases in hormonal parameters (e.g., cortisol (C), ES= 0.3 to 0.8), small to very large increases in inflammatory parameters (e.g., interleukine6 (IL-6), ES=0.4 to 3.9), small to very large increases in muscle damage markers (e.g., creatine kinase (CK), ES=0.5 to 4.8), and small to large impairments in physical performances (e.g., countermovement jump (CMJ), ES= -0.3 to -1.4). No significant relationships were found between hematological, hormonal, inflammatory, and muscle damage markers with physical fitness after an acute soccer match.

Single soccer training sessions resulted in small decrements in hematological markers such as hemoglobin (Hb) (ES=-0.2), small increases in hormonal parameters such as total testosterone (T), (ES=0.32 to 0.34) and increases in muscle damage markers like CK (ES=0.54). In addition, small performance decrements were found for measures of physical performance such as CMJ height (ES=-0.3). Significant positive correlations were found between Δ% T and Δ% CMJ height (r=0.90; p=0.001) and significant negative correlations between Δ% CK and Δ% CMJ (r=-0.99; p=0.0001).

Effects of long-term soccer training revealed parameter-specific increases or decreases in hematological, hormonal, inflammatory, muscle damage markers, and physical performance. Two studies showed a moderate increase in hematological markers such as Hb (ES=0.7 to 0.8). Parameter-specific changes were noted for hormonal markers in the form of increases for T (ES=0.2 to 0.8) and decreases for C (ES=-0.2 to -2.4). Finally, moderate to very large increases were found for muscle damage markers such as CK (ES=0.6 to 9.9) and physical performance such as CMJ height (ES=0.28 to 1.66) and squat jump (SJ) height. After long-term periods of soccer training, significant positive correlations were found between Δ% free testosterone (FT) and Δ% SJ height (r=0.98; p=0.01) and between Δ% testosterone: cortisol ratio (T/C) with Δ% SJ (r=0.90; p=0.03).

Conclusions: Findings suggest that hematological, hormonal, inflammatory, and muscle damage biomarkers were altered after acute soccer match play and training session. The observed changes in T and CK concentrations were related to changes in CMJ performanceafter acute training session. Additionally, we observed that the long-term soccer training induced increases/decreases in hematological, hormonal, inflammatory, muscle damage markers, and physical performance. These fluctuations can be explained by different factors (training load, duration of training, psychological factors, mood state). The observed changes in hormonal parameters (T and T/C) were related to SJ performance changes. Anabolic hormones and muscle damage markers can possibly be used as a useful tool to identify physical performance alteration after soccer session or matches and long-term soccer training.

Key points

- The hematological parameters, hormonal, inflammatory, and muscle damage markers were altered after acute soccer match play and training.
- The changes in T and CK concentrations after single soccer sessions were related to changes in CMJ height performance.
- The changes in hormonal parameters (FT and T/C) after long-term training were related to SJ performance.
- Anabolic hormones and muscle damage markers can be used as a useful tool to identify physical performance alterations after soccer sessions or matches and long-term soccer training.
- Coaches may use the interplay between biomarker alterations, physical performance changes to better manage workload and monitor fatigue during long-term soccer training.

1. Introduction

Soccer has been characterized as a high intensity sport that combines intermittent work periods of anaerobic and aerobic physical actions [1]. During the competitive soccer season, player's bodies are continuously subjected to physical and/or mental stress from both, training and competition [1]. As a consequence of the soccer-specific physiological demands, training programs should address physical qualities such as aerobic capacity, strength, power, linear and change-of-direction speed. Besides a high level of training volume, soccer players are often exposed to more than one competitive match per week [2].This level of physical stress may influence body homeostasis and could eventually reduce immune function [3], and lead to critical changes in hematological [4], hormonal[5], and inflammatory factors [6] which may ultimately affect physical performance [4]. Consequently, it is important to regularly monitor fluctuations in these different physiological systems due to soccer matches, single training sessions, and long-term training to prevent injuries, illnesses, and overtraining [7, 8, 9, 10, 11, 12]. Recent studies showed an alteration in hematological [13, 14], hormonal [15, 16], inflammatory, and muscle damage markers after single soccer matches and training sessions [7, 17]. Several long-term soccer training studies demonstrated that biological markers changed over the course of a soccer season according to variations in training intensity and volume [18, 19]. While the use of biological markers for long-term monitoring of exercise stress is widespread, the relationship between hematological, hormonal, inflammatory, and muscle damage markers with physical performance standards after acute and long-term training and competition remain equivocal. Some authors have also observed that high testosterone levels are positively correlated with strength and performance levels in elite soccer players [10]. Another study observed that the decrease in testosterone concentration after long-term training and competition was associated with physical performance declines [20]. With regards to hematological markers, several studies have indicated that improved performance in soccer is associated with a significant increase in hematocrit and hemoglobin concentrations at rest [21] but, elsewhere results have shown the opposite effect [4].

To the authors' knowledge, only three systematic reviews have focused on the physiological effects of soccer matches [22, 23, 24]. The first review reported outcomes on the effects of soccer matches on steroid hormones and psychological parameters [22]. The second systematic review described the development of fatigue during soccer matches and its recovery time using physiological, neuromuscular, technical, biochemical, and perceptual

responses [23]. The third article focused on the acute and residual changes in post-match muscle damage, neuromuscular and perceptual responses [24]. However, no systematic reviews exist which examine the effects of a single soccer training session exposure and long-term soccer training on biological markers in soccer players. Consequently, it appears timely to conduct such a review on the effects of soccer matches, single soccer training sessions, and long-term soccer training on hematological, hormonal, inflammatory, muscle damage markers, and physical performance in elite soccer players.

Accordingly, the objective of this systematic review was to summarize recent evidence on the effects of a soccer match and a single soccer session exposure as well as the long-term effects of soccer training on selected hematological, hormonal, inflammatory, and muscle damage markers as well as physical performance in soccer players. Moreover, we tried to describe associations between biological markers and physical performance changes after acute and long-term soccer training. We hypothesized that hematological, hormonal, inflammatory, and muscular damage markers and physical performance may be altered after acute (e.g., soccer match or training session) and long-term soccer training.

2.2 Methods

2.2.1 Eligibility criteria

The research questions were defined by the PICOS-model in accordance with the Preferred Reporting Item for Systematic Reviews and Meta-Analysis (PRISMA) statement [25]. Studies were included in the current systematic review if they were in accordance with the following criteria **(Table 1):** (1) articles published in peer-reviewed journals; (2) included male and female soccer players of different expertise level (national or/and international); (3) participants aged > 12 years including youth and senior soccer players; (4) analyzed biological markers such as hematology (e.g., plasma volume variations [PVV], hematocrit [Ht], hemoglobin [Hb], mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], and mean hemoglobin concentration [MCH]), hormones (e.g., testosterone [T], cortisol [C], testosterone : cortisol ratio [T/C]), inflammation (e.g., interleukin 6 [IL-6], C-reactive protein [CRP], tumor necrosis factor [TNF-α]), and muscle damage (e.g., creatine kinase [CK], lactate desydrogenase [LDH]) after soccer training programs (> 4 weeks) and/or soccer game and/or training session; (5) full article text availability in English; (6) reported soccer training design (e.g., lack of information on volume, frequency, and/or training methodology) and information about soccer match and

training session (duration and/or intensity); (7); reported information about the significant effect of soccer training or soccer match on biological markers (i.e., effect size [ES Cohen's d]) or reported alterations in percentage [Δ%] or mean and standard deviations. Since the scope of this review article is large in terms of outcome measures (e.g., hematological, hormonal, inflammatory and muscle damage markers), systematic review approach and not a meta-analysis was performed. Our rationale being that the large number of outcome parameters would have produced high heterogeneity within the data.

Table 1 Inclusion criteria according to the PICOS approach.

Criteria	Inclusion	Exclusion
1	Article published in peer-reviewed journals	Books, reviews, case studies, opinion pieces, non-peer-reviewed
2	Soccer players from different levels of practice	Non-soccer players, amateur or recreational soccer player
3	Participants > 12 years old, described as young and, or senior soccer players	Participants < 12 years old, or described as child athletes
4	Analyzing biological markers; hematology (e.g., PVV, Hb, Ht, MCV, MCH,and MCHC), hormones (e.g., C, T), inflammation (e.g., IL-6, CRP, TNF-α) and muscle damages (e.g.,CK, LDH) after soccer training programs > 4 weeks and/or soccer game and/or training session	Soccer training programs < 4 weeks. Training or soccer match was coupled with environment manipulation, nutritional and use of ergogenic aids.
5	Full text available in English	Cannot access full text in English
6	Reported soccer training (e.g., lack of information on volume, frequency, and/or training methodology), and information about soccer match and training session (duration and/or intensity).	Did not meet the minimum requirements regarding soccer training design, duration or/and intensity of soccer match and training session.
7	Reported information about the significant effect of soccer training or soccer match on biological markers (effect size [ES] or alteration percentage [Δ%] or mean and standard deviation**.**	Did not report information and measure about the effect of soccer training or soccer match on biological markers.

PVV plasma volume variations**, Hb** hemoglobin, **Ht** hematocrit**, MCV** mean corpuscular volume**, MCHC** mean corpuscular hemoglobin concentration**, MCH** mean hemoglobin concentration**, IL-6**interleukine 6**, TNF-α** tumor necrosis factor**, CRP** C-reactive protein**, CK**creatine kinase**, LDH** lactate dehydrogenase

2.2.2 Literature search strategy

Literature searches were conducted in four electronic databases including PubMed, ISI Web of Knowledge, Web of Science and SPORTDiscus from inception until August 2020. The following key terms (and synonyms searched for by the MeSH database) were included and combined using the operators "AND", "OR", "NOT": ((soccer OR football) AND ("soccer training*" OR "football training*" OR "small sided soccer games") AND ("haematological responses" OR "haematological variations" OR "haematological markers" OR "plasma volume variations" OR "blood parameters") AND ("hormonal adaptation" OR "biochemical markers" OR "cortisol changes*" OR "testosterone changes*" OR "androgen levels" OR endocrine response) AND ("inflammation" OR "inflammatory markers" OR "cytokines markers" "muscle damage")). In addition, the reference lists and citations (Google Scholar) of the identified studies were explored in order to detect further relevant research papers.

2.2.3 Study selection

The final screening was done by two investigators (KS and ABA) based on the relevance of the inclusion and exclusion criteria and the identified items for assessing hormones, hematology, inflammation, and muscle damage markers in male and female soccer players. If the citation showed any potential relevance, it was screened at the abstract level. When abstracts indicated potential inclusion, full-text articles were reviewed. A third-party consensus meeting was held with a third author (HZ) if the two reviewers were not able to reach agreement on inclusion of an article.

2.2.4 Quality assessment

The methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) scale (http://www.pedro.fhs.usyd.edu.au), which has been shown to have good reliability and validity [26]. The PEDro scale has 11 possible points and examines external validity (criterion 1) and internal validity (criteria 2–9) of controlled trials and whether there is sufficient statistical information for interpreting results (criteria 10–11). A cut-off point of 6 on the PEDro scale was used to indicate high-quality studies, as this has been reported to be sufficient to determine the methodological quality level in previous studies [26]. Two independent researchers (KS and ABA) assessed the quality of the studies, if any unambiguity arose, a third researcher (HZ) was contacted and a unanimous decision was achieved.

2.2.5 Data Analysis

2.2.5.1 Independent variables

Each study was read and coded by two independent investigators (KS and ABA). All relevant information from the different studies was extracted.

Players' characteristics

Players' gender, age, and expertise level were taken into account. Four distinct levels of expertise were observed: young, elite, semi-professional, and professional (national and/or international level).

2.2.5.2 Dependent variables

The dependent variables extracted from the selected studies were objective outcomes being hematological, hormonal, and inflammatory, and muscle damage parameters, physiological and physical parameters.

Hematological alterations

Hematological responses to acute (soccer match or training session) and long-term training were analyzed by recording alterations in Plasma volume (PV), Ht, Hb, MCV, MCHC, and MCH.

Hormonal markers

Hormones secreted by the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis can be used as indices of physical stress to evaluate the balance between anabolic and catabolic responses (C, T and T/C ratio).

Inflammatory markers

To detect inflammation, the acute phase protein (IL-6, CRP), and pro-inflammatory cytokines responses (TNF-α) are frequently used.

Muscle damage markers

To investigate muscle damage after soccer training sessions or matches, the activity of intracellular enzymes (CK and LDH) represents common measures.

SAIDI, Karim. Réponses biologiques et psychologiques chez les footballeurs élites : effets de la congestion des matchs - 2020

Physiological markers

The classical physiological markers used in soccer are anaerobic thresholds, maximal oxygen consumption (VO_{2max}), and blood lactate**.**

Physical performance markers

The following physical performance markers are usually used by the staff in soccer teams:
(a) Vertical jump (VJ) performance, which includes countermovement jump height (CMJ) and squat jump (SJ) height.
(b) Sprint time during runs with change-of-direction (COD) is usually recorded (e.g., T-tests).
(c) Linear sprint measures (SL, time) for a given distance. Best single sprint performances obtained during repeated-sprint ability tests were also included in this category (e.g., fastest time after 20 meters sprints or 30 meters sprints)
(d) Repeated shuttle sprint ability (RSSA) was used to assess players' ability to cope with the intermittent demands of soccer. This test includes rapid CoDs, which represents sport-specific movement patterns in soccer. Mean time ($RSSA_{mean}$) obtained during 6 × 40 maximal sprints interspersed with 20 s of passive recuperation.
(e) Intermittent-running endurance capacity. The capacity to perform high intensity intermittent endurance exercise (total distance covered) was recorded during the Yo–Yo intermittent recovery level 1 (YYIR1).

2.2.5.3 Statistics

To evaluate the magnitude of the effects, percent changes (Δ%) were calculated for each study outcome using the following equation:

$$\mathbf{\Delta\% = (Mpost - Mpre)/Mpre \times 100}$$

Where M_{post} represents the mean value after (soccer match or training session or long-term training) and M_{pre} the baseline mean value. Effect sizes (ES) were computed as Cohen's d to present standardized effects of acute (soccer match or training session) and long-term training on the respective outcome variables [27]. The following equation was used to compute Cohen's d:

$$\mathbf{ES = Cohen's\ d = ((M1 - M2)/SDpooled)}$$

Where M1 represents the mean at post soccer match, training session and long-term soccer training and M2 is the mean at baseline.

Values for ES were defined as trivial (< 0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0), and very large > 2 [28]. All data analysis was processed using Sigma Stat 3.5 software (Systat, Inc, USA). Mean percentages of change at different time points extracted from all studies were presented as mean (Range: minimum and maximum) for all parameters. The effect sizes and percentage changes were analyzed in studies where sufficient data was available. A significant difference was indicated when the 95% confidence interval (CI) of the ES did not overlap with zero. In addition, relations between Δ% of physical performance markers and Δ% of biological markers were assessed where sufficient data was available using Pearson's product-moment correlation coefficients (r). The magnitude of correlation coefficients was considered as trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($0.9 < r < 1$), and perfect ($r = 1$), [29]. Statistical significance for all analyses was set at $p < 0.05$.

2.3. Results

2.3.1. Selection of the studies

The initial search yielded 2,260 potentially eligible articles (**Figure 1**). After removing duplicates and application of the inclusion and exclusion criteria, 48 articles were included for analysis in this systematic review. **Table 2** illustrates the characteristics of 19 acute studies. These19 acute studies involved 255 male and 115 female participants from ten different countries (United Kingdom, Brazil, Spain, Turkey, Poland, Portugal, Croatia, Italy, Norway, and Greece). Eight studies examined the effects of soccer specific training sessions. On average, duration of single sessions lasted between 1-2 hours. Eleven studies examined the effects of soccer matches (eight official matches and three non-official matches). Within the long-term studies, 29 studies involved 655 male and 25 female soccer players from 14 countries (Brazil, Tunisia, Denmark, Serbia, Poland, Portugal, France, Spain, Greece, Italy, United States, India, Germany, and Iran). Among the 29 long-term studies **(Table 3**), 28 studies investigated male subjects and one study examined females. The training frequency was at least three sessions per week [30]. In several studies, the training duration was between 4 to 18 weeks. One study reported duration of 1 year [31] and another study examined a 2 years intervention period [10].

Among the included studies, 24 studies investigated professional soccer players, 5 included semi-professional soccer players, 9 studies involved young soccer players, and 13 studies

investigated elite soccer players. The mean average of methodological quality of the include articles was 7.2 considered as a high quality and only four studies were considered as low-quality studies (Mean 5 in the Pedro scale-score), [17, 31, 32, 33].

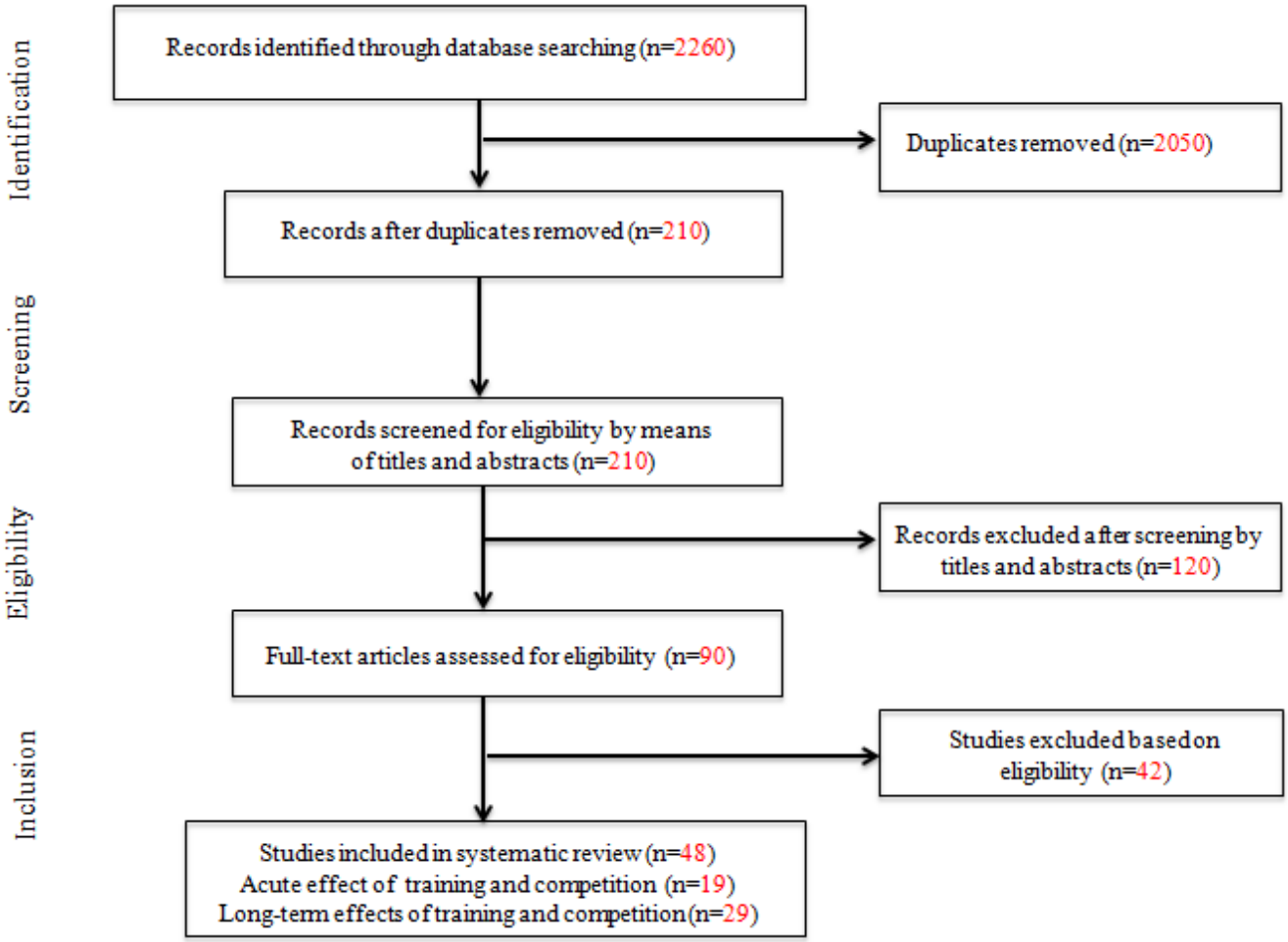


Figure 1. Selection process for research articles (n = 48) included in this systematic review Adapted version of the recommendations in the PRISMA (Preferred Reporting Items for systematic Reviews and Meta-Analyses) statement [25].

Table 2 Characteristics of the studies that examined the acute effects of soccer matches and training sessions on hematological, hormonal, inflammatory parameters and muscle damage markers.

Study Acute training sessions	PEDro scale	Year	Country	Sex/status	Sample Size	Age, years (mean ± SD or range)	Training characteristics	Duration
Sparkers et al. [42]	8	2020	United Kingdom	Male Semi- professional soccer players	12	21.2	Single training session duration: 59 min, SSG: (24 × 29 m): 7 × 6 min, 2 min rec. Player load: 452 ± 59 A.U. Double training session: same SSG + 60 min of strength training (4 × 4 min, 85% of 1RM, 4 min rec). Player load: 452 ± 59 A.U.	-
Sparkes et al. [15]	8	2018	United Kingdom	Male Professional soccer players	16	21 ± 2	Single training session duration: 59 min, SSG: (24 × 29 m): 7 × 6 min, 2 min rec. Player load: 483 ± 38 A.U.	-
Chmura et al. [44]	9	2019	Poland	Male Young soccer players	18	17.2 ± 0.88	SSG: Pitch size (10-15 m), First form: 6 × 30 sec (1 × 1), 2 min rec. Second form: 6 × 45 sec (1 × 1), 3 min rec.	-
Mascarin et al. [43]	7	2018		Female Professional soccer	13	18.8 ± 0.8	SSG: 73 min. pitch size 120 m^2: 16 × 4 min, 3 min rec.	-

				players			HR_{mean}: 94.67 ± 0.87%	
Avloniti et al. [45]	8	2007	Greece	Female Elite soccer players	10	23.8 ± 3.5	Soccer specific training: 2 h, 75% HR_{max}, HR_{mean}: 146 ± 6 bpm	-
Mello et al. [32]	5	2017	Brazil	Male Professional soccer players	22	26.5 ± 1.9	Training session duration: 120 min Simulated game, 75% HR_{max}	-
Karakoc et al. [14]	6	2005	Turkey	Male Professional soccer players	10	18.9-23	Soccer specific training sessions: 90 min	-
Moreira et al. [40] **Acute soccer matches**	6	2009	Brazil	Male Professional soccer players	22	23 ± 4	Competitive training Simulated game: 2 × 35 min, 10 min rec. RPE = 16.6 ± 1.9 Load: 1328 ± 152 A.U	-
Romagnoli et al. [35]	6	2015	Italia	Male Professional soccer players	22	17-20	Soccer match duration: 90 min (non-official), 85% HR_{max}	-
Souglis et al. [17]	5	2015	Greek	Male Elite soccer players	18	25.6 ± 2.9	Soccer match duration: 90 min (official match), 75% HR_{max}	-
Silva et al. [41]	7	2013	Portugal	Male Professional soccer players	7	22-31	Soccer match duration: 94 min (official match), 85% HR_{max}	-

Thorpe et al. [16]	7	2012	United Kingdom	Male Semi-professional soccer players	7	25 ± 6	Soccer match duration: 90 min (official match) HR_{mean}: 165 ± 10 bpm	-
Souglis et al. [56]	9	2015	Greece	Male and Female Elite soccer players	22 male 21 female	23.1 ± 3.0 22.9 ± 2.4	Soccer match duration: 90 min (official match) Male: 86.9 ± 4.3% HR_{max} Female: 85.6 ± 2.3% HR_{max}	-
Devrnja et al. [55]	8	2018	Croatia	Male Elite soccer players	43	15-18	Soccer match duration: 90 min (non official match) Total distance covered 6432 ± 552 m	-
Fothergill et al. [38]	7	2017	United Kingdom	Male Elite soccer players	18	17.5 ± 0.6	Soccer match duration: 90 min (official match)	-
Jamurtas et al. [34]	9	2015	Greece	Male Elite soccer players	18	21-24	Soccer match duration: 90 min (official match) 85.4 ± 4% HR_{max}	-
Anderson et al. [7]	8	2010	Norway	Female Elite soccer players	10	23 ± 4	Two Soccer matches: 2 × 90 min (official match). First match: 68.6% HR_{max}. Second match: 88.4% HR_{max}.	-
Oliveira et al. [39]	7	2009	Portugal	Female Professional soccer players	33	24.2 ± 4.8	Soccer match duration: 90 min (official match)	-

Gravina et al. [13]	7	2011	Spain	Female Elite soccer players	28	18-30	Soccer match duration: 90 min (non official match)	-

W week, **HIIT** high intensity interval training, **h** hour, **min**: minute, **SSG** small sided games, **HR** heart rate, **HR_{mean}** mean heart rate, **HR_{max}** maximal heart rate, **A.U** arbitrary units, **rec** recuperation, **m** meters, **bpm** beats per minute, **sec** second

Table 3 Characteristics of the studies that examined the long-term effects of soccer training on hematological, hormonal, inflammatory parameters and muscle damage markers.

Study	PEDro scale	Year	Country	Sex/status	Sample Size	Age, years (mean± SD or range)	Training characteristics	Duration
Saidi et al. [4]	7	2019	Tunisia	Male Elite soccer players	18	20.1 ± 0.4	Soccer specific, technical, tactical, and strength training. Endurance training: 90-100% HR_{max}. 4-5 sessions/W- 9-11 h	6 Ws
Requena et al. [37]	7	2017	Spain	Male Professional soccer players	19	19-30	5 trainings sessions/W W1 to W5: 30-40 min of aerobic training (60-70% HR_{max}) W6 and W7: 8 min aerobic training (40-50% HR_{max}) + strength training (3-5 sets × 6-8 repetitions (60-70% of 1 RM)	7 Ws
Perroni et al. [50]	6	2019	Italy	Male Young soccer players	45	14 ± 0.4	Soccer training 6 sessions/W1-W5 - 120 min 3 sessions/W5-W8 – 120 min	8 Ws

Opaszowski et al. [51]	7	2012	Poland	Male Semi-professional soccer players	20	23.6 ± 3.9	Coordination, strength, speed, aerobic, technical and tactical training. 5 sessions/W- duration: 70 min	7 Ws
Kamran et al. [30]	8	2018	India	Male Professional soccer players	36	-	Strength training (complex and contrast). 3 sessions/W	6 Ws
Coppalle et al. [9]	7	2019	France	Male Professional soccer Players	26 24	26.2 ± 5.1 25.9 ± 5.2	Soccer training 6-8 sessions/W – 11 h/W	2 × 6 Ws
Pimenta et al. [60]	6	2015	Brazil	Male Professional soccer players	23	26 ± 6	Soccer training 5-6 sessions/W - 96-132 min	7 Ws
Gharahdaghi et al. [58]	7	2013	Iran	Male Semi-professional soccer Players	12	21.8 ± 2.2	HIIT, and strength training. 3-4 sessions/W	4 Ws
Reinke et al. [57]	6	2009	Germany	Male Professional soccer Players	10	20-36	First 4 weeks: aerobic training <1 hour per day Next 5 weeks: HIIT, soccer specific 2-3 sessions/day	9 Ws
Saidi et al. [20]	7	2020	Tunisia	Male Professional soccer players	16	19-22	Soccer specific, technical, tactical, and strength training, endurance training (90-100% HR_{max}). 4-5 sessions/W - 9-11 h	12 Ws
Andelkovic et al. [6]	7	2015	Serbia	Male Young soccer players	19	17.6 ± 0.1	Soccer field and weight training 7-8 sessions/W - 1.5-2 h	12 Ws

Silva et al. [21]	8	2008	Brazil	Male Professional soccer players	12	19-28	Soccer specific, technical, tactical, and strength training, endurance training (70-80% and 80-90% HR_{max} respectively for the first and the last six weeks of training). 5 sessions/W - 14.6-16.33 h	12 Ws
da Silva et al. [18]	7	2011	Brazil	Male Professional soccer players	18	23.8 ± 3.3	Soccer specific, technical, tactical, and strength training 4-5 sessions/W, 14.7-16.3 h.	12 Ws
Silva et al. [61]	7	2008	Brazil	Male Professional soccer Players	15	23.4 ± 2.5	Soccer specific, technical, tactical, and strength training. 5 sessions/W - 14.6-16.33 h	12 Ws
Kramer et al. [52]	6	2004	United States	Male Elite soccer players	25	18-20	Speed, agility, endurance and strength training. 10 h/W	11 Ws
Gorostiaga et al. [33]	5	2004	Spain	Male Young soccer players	21	16-18.5	Strength training. 6 sessions/W - 25-30 min	11 Ws
Walker et al. [36]	7	2019	United states	Female Professional soccer players	25	20 ± 1.1	Soccer training First training block: 18 sessions/4Ws Second training block: 15 sessions /4Ws Third training block: 13 sessions/4Ws Fourth training block: 11 sessions/4Ws	16 Ws
Aquino et al. [59]	7	2016	Brazil	Male Young soccer players	15	15.4 ± 0.2	Soccer training 4 sessions/W - 14.2-15.7-17.1 h Average RPE: 5.1-6.1-6.3	22 Ws

Heisterberg et al. [8]	8	2013	Denmark	Male Professional soccer players	24	26.3 ± 1.1	Aerobic training, soccer specific, resistance, strength training 5-8 sessions/W- 1.5-2 h (10-16 h)	18 Ws
Silva et al. [19]	7	2014	Portugal	Male Professional soccer players	14	25.7 ± 4.6	aerobic and strength training 5-6 sessions/W	35.5 Ws
Moreira et al. [54]	7	2013	Brazil	Male Young soccer players	45	12-13	Soccer training 4 sessions/W- 6-8 h	21 Ws
Filaire et al. [46]	9	2001	France	Male Professional soccer players	17	23.7 ± 2.2	Endurance, physical and technical training: 20 h/W Strength training: 12 h/W	32 Ws
Michailidis et al. [48]	7	2014	Greek	Male Professional soccer players	15		Aerobic training speed, strength, technical, tactical training 6 sessions/W	57 Ws
Hammami et al. [10]	7	2017	Tunisia	Male Young soccer players	20	14.5 ± 0.5	Soccer specific, technical and tactical training 4 sessions/W- 2 h	2 years
Koundourakis et al. [53]	8	2014	Greece	Male Professional soccer players	67	25.5 ± 1.1	SSG, technical, tactical, speed, agility, endurance, and strength training. 5 sessions/W- 75-95 min	42 Ws
Muscella et al. [5]	7	2019	Italy	Male Elite Soccer players	29	16.51 ± 0.7	Simulated game, technical and tactical training 4 sessions/W- 100 min	36 Ws
Andrzejewski et al. [47]	6	2020	Poland	Male professional soccer players	18	18.1 ± 1.3	Soccer training 5 sessions/W- 80 min	24 Ws

Arruda et al. [31]	5	2015	Brazil	Male Young soccer players	39	14-17	SSG, HIIT, repeated sprint, and strength training. 5–8 sessions/W- 16-17 h/W	1 year

W week, **Ws** weeks, **HIIT** high intensity interval training, **h** hour, **min** minute, **SSG** small sided games, **RPE** rating of perceived exertion.

Table 4 Physiotherapy evidence database (PEDro) score of some long-term studies.

	PEDro items											
Study	**Eligibility criteria**	**Random Allocation**	**Concealed allocation**	**Group Homogeneity**	**Blinded subjects**	**Blinded therapists**	**Blinded assessor**	**Drop out 15%**	**Intention to treat analyses**	**Between group comparison**	**Point estimates and variability**	**Score**
Silva et al. [21]	+	+	+	+	-	-	+	+	-	+	+	8
Heisterberg et al. [8]	+	+	+	+	-	-	+	-	+	+	+	8
Gorostiga et al. [33]	+	+	-	-	-	-	-	+	-	+	+	5
Andelkovic et al. [6]	+	+	+	+	-	-	-	+	-	+	+	7
Silva et al. [19]	+	+	+	+	-	-	-	+	-	+	+	7
da silvaet al. [18]	+	+	+	+	-	-	-	+	-	+	+	7
Filaire et al. [46]	+	+	+	+	-	-	+	+	+	+	+	9

Michailidis et al. [48]	+	+	+	+	-	-	-	+	-	+	+	7
Hammami et al. [10]	+	+	+	+	-	-	-	+	+	+	+	8
Koundourakis et al. [53]	+	+	+	+	-	+	-	+	-	+	+	8
Muscella et al. [5]	+	+	+	-	-	-	-	+	+	+	+	7
Walker et al. [36]	+	+	+	+	-	-	-	+	-	+	+	7
Andrzejewski et al. [47]	+	+	+	+	-	-	-	+	-	+	+	7
Reinke et al. [57]	+	+	+	+	-	-	-	+	-	+	+	7
Silva et al. [51]	+	+	+	+	-	-	-	+	-	+	+	7
Arruda et al. [31]	+	+	-	-	-	-	-	-	+	+	+	5
Requena et al. [37]	+	+	+	+	-	-	-	+	-	+	+	7
Aquino et al. [59]	+	+	+	+	-	-	-	+	-	+	+	7
Kraemer et al. [52]	+	+	-	-	-	-	-	+	+	+	+	6

2.3.2 Effects of acute (*e.g.*, soccer match or training session) and long-term soccer training on hematological parameters

2.3.2.1 Acute and residual effects of soccer matches and single training sessions on hematological parameters

Table 5 illustrates the acute and residual effects of soccer matches and single training sessions on different hematological variations.

Effects of soccer matches

The duration of soccer matches was the same for all studies (90 min). In addition, the intensity of soccer match average was between 68 to 88% of maximal heart rate (HR_{max}) and the environmental conditions were different between studies.

Only three studies were identified that examined the acute effects of soccer matches on hematological parameters [13, 34, 35]. The first two studies showed no significant variations in the analyzed hematological parameters (PV, Ht, Hb, MCV, MCHC, and MCH) pre-and post-match [13, 34]. The third study showed a significant increase in PV immediately (+7.9%), 24 h (+9%) and 48 h (+7.2%) post-match [35]. Along this increase, performance in vertical jump (e.g., CMJ) decreased immediately (Δ%= -3.2; ES= -0.3), 24 h (Δ%= -2.6%; ES= -0.24) and 48 h after soccer match (Δ%= -3%; ES= -0.29).

Effects of single training session

As to the training session, the type of training was categorized as small sided games (SSG), soccer specific training, strength training, and simulated games. The duration of single training sessions was different between studies and ranged between 60 to 120 minutes. For some studies, intensity of training session was between 75 to 95% of HR_{max}; and the player load was between 452 to 1328 Arbitrary Units (AU).

One study examined hematological parameters pre- and post-soccer specific training sessions. It showed a decrease in Hb (-2.5%) and MCV (-3.5%) levels two minutes after the training session [14]. Thus, soccer training session resulted a small decrement in Hb (one study, ES= -0.2) and MCV (one study, ES=-0.3). In addition, a metabolic alteration was shown after training session (e.g., Lactate: Δ%= 148%; ES= 1.8).

2.3.2.2 Effects of long-term soccer training on hematological parameters

For the long term-of training, three distinct levels of training status were considered with number of training hours/sessions per week as units. Player training status was classified as

"Low" (3-4 h/two training sessions per week) "Moderate" (between 5 and 7 h/three to four training session) and "High" (more than 8 h/five training sessions) **(Table 6).**

Six studies investigated the long-term effects (e.g., soccer training) on hematological markers [4, 6, 8, 21, 36, 37]. Three studies evaluated plasma volume variations; the first study demonstrated a large decrease (Δ%= -12%; ES= -1.2) after 12 weeks training periods [21]. The second study observed a moderate increase (Δ%= 6.1%; ES= 0.7) after six weeks intense training periods [4], and the last study observed an increase (Δ%= 7%) ninety days after training periods [6]. The six studies examined Ht variations, when three studies demonstrated a moderate to large decrease for Ht level (Δ%= -3.1 to -6.3%; ES= -0.9 to -1.3) [4, 6, 36] and one study observed an increase (Δ%= 2.9%; ES= 0.6) at the end of competitive periods [37]. Two studies observed an insignificant change in Ht concentration [8, 21]. Five studies investigated Hb variations, three studies observed a moderate to large decrease for Hb level after training period (Δ%= -3.1 to -4.6%; ES= -0.8 to -2) [4, 6, 8]. Two studies observed a moderate increase in Hb level (Δ%= 3.5 to 5.4%; ES= 0.7 to 0.8) after training periods [31, 37]. Five studies investigated MCV variations after training periods. Three studies observed a decrease for MCV levels (Δ%= -3.7 to -1.5%; ES= -2.2 to -0.3) [6, 21, 37] after training periods. One study observed an increase at the end of season period (Δ%= 2.5; ES=1) [8], and one study observed an insignificant change in MCV level after six-week intensive training [4]. Four studies examined MCHC variations after long-term of soccer training. Two studies observed insignificant changes in MCHC markers [4, 6]. One study observed a very large increase (Δ%= 5; ES= 2.7) for MCHC level after 12 weeks training periods [21]. Two studies observed a decrease for MCHC after training periods (Δ%= -3.8 to -1.1; ES= -8 to -0.4) [8, 37]. Five studies evaluated MCH variations after different training periods. Two studies observed a decrease in MCH levels (Δ%= -2.6 to -1.6%; ES=-1 to -0.4) after different training periods [8, 37], and two studies observed insignificant changes for MCH parameters after different training periods [4, 6]. Parallel to the decrease in Hb and/or Ht after periods of training or/and competitions, several studies showed a decrease in physical fitness parameters (e.g., YYIR1: Δ%= -34.9%, $RSSA_{mean}$: Δ%= 2.6, $RSSA_{best}$: Δ%= 3.8%, VJ: Δ%= -2.5 to -3%, and $VO2_{max}$: Δ%= 2.2 to 6.7%), [4,8, 36]. Another study demonstrated an increase in aerobic performance increased (e.g., Anaerobic threshold: Δ%= 8.1%) when the Hb concentration increased [21].

Table 5 Effects of acute soccer matches and training sessions in hematological parameters

Study	**Participants (number/sex/ age)**	**Intervention/Times of evaluations**	**Hematological parameters: plasma volume variations (PVV), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean hemoglobin concentration (MHC).**						
Acute soccer matches			**PVV (%)**	**Ht (%)**	**Hb(g/dl)**	**MCV (fl)**	**MCHC (pg)**	**MCH (pg)**	**Physiological markers and physical fitness**
Gravina et al. [13]	28/F/18-30	(A) 24 h Pre-M (B) +0 h (C) +18 h		(A) 39.7 ± 1.9 (B) NS (C) NS	(A) 13.1 ± 0.8 (B) NS (C) NS	(A) 88.4 ± 4.5 (B) NS (C) NS	(A) 32.9±0.9 (B) 32.9± 0.9 (C) 32.7± 0.8	(A) 29.1±1.9 (B) 29.1± 1.9 (C) 28.9± 1.9	
Jamurtas et al. [34]	18/M/21-24	(A) Pre-M (B) +2 h (C) +12 h (D)+36 h (E)+60 h	A= B= C= D= E	(A) 45 (B) NS (C) NS (D) NS (E) NS	(A)14.8 (B) NS (C) NS (D) NS (E) NS	(A) 84 (B) NS (C) NS (D) NS (E) NS	(A) 30 (B) NS (C)NS (D) NS (E) NS	(A) 28 (B) NS (C) NS (D) NS (E) NS	
Romagnoli et al. [35]	22/M/17-20	(A) Pre-M (B) +0 h (C) +24 h (D) +48 h	(A) , A < B, C, D* (B) ↑7.9%* (C) ↑9%* (D) ↑7.2%*						**CMJ (cm)** (A) 49.6 ± 5.1 (B) ↓3.2%* (C) ↓2.6%* (D) ↓3%* ES (A-B)= -0.3 ES (A-C)= -0.24

									ES (A-D)= -0.29
Acute training sessions									
Karakoc et al. [14]	10/M/18.9-23	(A)Pre- STS-90min (B)+2 min			(A) 154±8.9 (B) ↓2.5%* ES= -0.2	(A) 85.7±3.49 (B) ↓3%* ES= -0.3			**[Lactate]** (A) 1.45± 0.96 (B) ↑148% ES (A-B)= 1.8

STS soccer training session, **M** match, **min** minute,**h** hour, **NS** no significant difference,**ES** effect size,* significant difference, **CMJ** countermovement jump.

Table 6 Effects of long term soccer training in hematological parameters

Study	Participants (number/sex/ age)	Intervention/ Times of evaluations	Hematological parameters: plasma volume variations (PVV), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean hemoglobin concentration (MHC).						
			PVV (%)	**Ht (%)**	**Hb (g/dl)**	**MCV (fl)**	**MCHC (pg)**	**MCH (pg)**	**Physiological markers and physical fitness**
Silva et al. [21]	12/M/19-28	(A) W0 (B) W12	(A-B) ↓12%* ES = -1.2	(A) 40.68 ± 2.54 (B) NS ES (A-B) = 0.07	(A)14.16 ± 0.97 (B) ↑5.4%* ES (A-B) = 0.8	(A) 87.53 ± 2.7 (B) ↓3.7%* ES (A-B)= -1.2	(A) 34.74 ± 0.61 (B) ↑5* ES (A-B) = 2.7		**Anaerobic threshold** (A) to (B) ↑8.12%*
Saidi et al. [4]	18/M/20	(A) W0 (B) +6Ws of training	(A-B) ↑6.1 ± 8.7%* ES = 0.7	(A) 43.9 ± 2.7 (B) ↓3.1%* ES (A-B) = -0.6	(A) 14.9 ± 1.04 (B) ↓4.6 %* ES = -0.8	(A) 83.1 ± 4.5 (B) NS	(A) 28.05 ± 1.7 (B) NS	(A) 33.9 ± 0.7 (B) NS	**YYIR1** ↓34.9%*, ES (A-B) = - 2.5 **$RSSA_{mean}$** ↑2.6%* ES (A-B) = 0.7 **$RSSA_{best}$** ↑3.8%* ES (A-B) = 0.7 **SJ** ↓2.5%* ES (A-B) = -0.26
Requena et al. [37]	19/M/19-30	(A)Mid-season (B) Off season		(A) 43.08 ± 2.18 (B) ↑2.9%* ES (A-B) = 0.6	(A) 14.85 ± 0.94 (B) ↑3.5%* ES (A-B) = 0.7	(A) 89.98 ± 3.51 (B) ↓1.5%* ES (A-B) = -0.3	(A) 34.45 ± 0.93 (B) ↓1.1%* ES (A-B) = -0.4	(A) 31 ± 1.69 (B) ↓ 2.6%* ES (A-B)= -0.4	**MAS** ↓4.5%* ES (A-B) = 0.8 **VJ** ↓17.5* ES (A-B) = -0.2

Andelkovic et al. [6]	19/M/17.62	(A) Baseline (B) +90 days	A-B ↑7.5%*	(A) 0.482 ± 0.01 (B) ↓4.1* ES (A-B) = -1.3	(A) 157 ± 3.9 (B) ↓3.1* ES(A-B)= -1.6	(A) 88.64 ± 0.89 (B) ↓2% ES(A-B)= -2.2	(A) 326 ± 2.0 (B) NS	(A) 28 ± 0.3 (B) NS	
Heisterberg et al. [8]	24/M/26.3	(A) Mid-december (B) Late may		(A) 0.46 ± 0.01 (B) NS	(A) 9.5 ± 0.1 (B) ↓2.1%* ES (A-B) = -2	(A) 86 ± 1 (B) ↑2.3* ES (A-B) = 1	(A) 20.7±0.1 (B) ↓3.8* ES (A-B) = -8	(A) 1.77 ± 0.03 (B) ↓1.6 ES (A-B) = -1	**$VO2_{max}$** ↓2.26* ES (A-B) = -0.95
Walkers et al. [36]	25/F/20	(A) Pre-season (B) +16 Ws		(A) 43.2 ± 2.7 (B) ↓6.3%* ES (A-B) = -0.9					**$VO2_{max}$** ↓6.7% ES (A-B) = -0.83 **VJ** ↓3% ES (A-B) = -0.28

W week,**Ws** weeks, **NS** no significant difference, **ES** effect size, *significant difference, **YYIR1** yo-yo intermittent recovery test level 1. **RSSA** repeated shuttle sprint ability, **SJ** squat jump, **VJ** vertical Jump**, $VO2_{max}$** maximal oxygen consumption, **MAS** maximal aerobic speed

2.3.3 Effects of acute (*e.g.*, soccer match or training session) and long-term soccer trainingon hormonal markers.

2.3.3.1 Acute and residuals effects of soccer match and single training session on hormonal markers

Table 7 depicted the twelve studies that investigated the effects of acute soccer match and training session on selected hormonal parameters.

Soccer match effect

Seven studies investigated hormonal markers before and after the match [16, 17, 35, 38, 39, 40, 41]. Four studies showed an increase of C immediately after the match (Δ%= 0.3 to 0.8%; ES= 16.9 to 59.4) [17, 38, 40, 41]. One study demonstrated an increase in C concentration 24 h (Δ%= 25.9%; ES= 4.6), 48 h (Δ%= 59.4%; ES= 4.9), and 72 h after the match (Δ%= 3.5; ES= 0.62) [41]. One study showed a large decrease in C level 24 h (Δ%= -36%; ES= -1.6), and 48 h after the match (Δ%= -32%; ES= -1.5) [35]. One study observed an insignificant change for the C level before and after soccer match [16]. Four studies investigated T variation after a soccer match [16, 38, 39, 41]. One study observed an increased (Δ%= 44%; ES= 0.64) for T level immediately after soccer match [16]. One study showed an increase for T level thirty minutes after a soccer match in winner group (+2.7) and a decrease (-2.19) of T level in loser group [39]. Two studies demonstrated insignificant changes in T [38, 41]. Additionally, a single study showed a moderate decrease in free testosterone (FT) immediately (Δ%= -25.9; ES= -0.8), 24 h (Δ%=-25.9%; ES= -0.8) and 48 h (Δ%= -30%; ES= -1.2) after a soccer match [35]. Two studies investigated variations of T/C ratio after a soccer match [16, 41]. The first study demonstrated a significant decrease 24 h (Δ%= -12.4%; ES= -3.4), 48 h (Δ%= -14%; ES= -4) and 72 h (Δ%= -8; ES=-1.4). The second study showed insignificant change in T/C ratio. One study showed an insignificant change in free testosterone: cortisol ratio (FT/C) after soccer game [35].

In parallel to the decrease in FT, we showed a decrease in vertical jump performance (e.g., CMJ) immediately (Δ%= -3.2%; ES= -0.3), 24 h (Δ%= -2.6%; ES= -0.24) and 48 h (Δ%= -3%; ES= -0.29) after the match [35]. In addition, we showed that the increase in C and the decrease in T level 24 hours after the match were associated to the decrease in CMJ performance (Δ%= -7%; ES= -1.4) [41]. The RPE score increased significantly (Δ%= 16 to 86%; ES= 0.3 to 0.8) when the C concentration increased after an acute match [40].

Single training session effect

Five studies investigated hormonal variations pre and post training session [15, 42, 43, 44, 45]. Two studies showed a decrease in C concentration immediately after training sessions [15, 42]. One study demonstrated a significant decrease in C level 4 h after the training session (Δ%= -52.7%) [45]. Two studies showed a significant decrease in C level 24 h after training session (Δ%= -8.9 to -21.3%; ES= -0.5 to -0.4) [15, 43]. One study demonstrated a significant increase for the C immediately (ES= 0.8-1.2), 15 min (ES= 0.7-0.92), and 30 min (ES= 0.2-1) after two forms of SSG [44]. One study showed a significant decrease in C level 48 h (Δ%= 8.9%; ES= -0.4) after training session [43]. Three studies investigated T variation after training session [15, 42, 44]. Two studies showed a small increase immediately after training session (Δ%= 11.2 to 11.2%; ES= 0.32 to 0.34) [15, 44], and one study showed an insignificant change in T level immediately after the training session [42]. Two studies analyzed FT variations pre-and post-soccer specific training sessions [43, 44]. The first study observed a significant decrease 24 h (Δ%= -41%; ES=-1.4) and 48 h (Δ%= -9%; ES= -0.3) post-training session [43]. The second study showed a small increase immediately (ES= 0.3 to 0.39) after the training session followed by a small decrease 30 min (ES= -0.3 to -0.4) after training session with two different forms of small sided soccer games [44]. Two studies investigated T/C changes to training session [42, 44]. The first study showed an increase for T/C ratio immediately (+138.3 ±336.8) and 24 h (+53.6 ± 93.9) after a single training session (SSG). In addition, it also observed a significant increase immediately (+74 ±190.2) followed by a significant decrease (-43± 69.5), 24 h after a double training session (small sided games plus strength training sessions) [42].The second study showed a moderate decrease for T/C 15 min (ES= -0.9 to -1.2) and 30 min (ES= -0.80 to -0.85) after training session based on small sided soccer games [44]. Two studies investigated FT/C variations after training session [43, 44]. The first study showed an insignificant change in FT/C ratio immediately, 24 h, 48 h and 72 h after a soccer training session [43]. The second study showed a significant decrease for FT/C, 15 min (ES= -1.1to -0.7) and 30 min (ES= -1.4 to -0.6) after training session based on small sided soccer games [44].

Along with the increase in C and the decreases in T and T/C ratio immediately after an acute training session, it was found that the CMJ performance decreased (Δ%= -59 %; ES= -0.6) and the lactate concentration increased (Δ%= 100%; ES= 1.6) [15].

2.3.3.2 Effect of long-term soccer training on hormonal markers

Nineteen studies examined the long-term effects of soccer training on selected hormones parameters **(Table 8)**. Sixteen studies analyzed C variations. Five studies observed a small to moderate increase on C level after different period of training (ES= 0.4 to 0.9) [18, 36, 46, 47, 48]. Seven studies observed a small to very large decrease of C after different periods and types of training (ES= -0.2 to -2.4) [5, 10, 19, 30, 33, 49, 50,51]. Three studies observed an insignificant change for C level after training periods [20, 37, 52]. In addition, one study investigated free cortisol (FC) variations [36]. They observed a very large increase (Δ%= 88.2%; ES= 3.5) after 16 weeks of training [36]. Sixteen studies examined effects of long–term soccer training on T changes. Seven studies showed a small to large decrease of T level during different soccer training periods (ES=-0.2 to -4.2) [5, 18, 20, 31, 46, 48, 50]. Five studies observed an increase of T throughout soccer training periods (ES= 0.2 to 0.8) [10, 33, 37, 52, 53]. Four studies observed no significant changes of T during the different phases of training periods [19, 47, 51, 54]. In addition, four studies examined FT responses to the long-term periods of training. Two of which showed a significant increase in FT after training periods (ES=0.2 to 0.7) [30, 53]. While the other two studies observed an insignificant change of FT in response to different periods of soccer training [33, 47]. Twelve studies investigated the effects of long-term training periods on T/C values. Six studies observed a small to very large decrease of T/C ratio after training periods (ES= -0.2 to -3.8) [18, 19, 20, 46, 47, 48]. Five studies demonstrated a small to large increase of T/C value after different periods of training (ES=0.44 to 1.6) [10, 19, 33, 50, 51]. One study showed an insignificant change in T/C ratio [5]. One study evaluated FT/C ratio variations during 6-month training and competitive periods; we showed a small decrease (ES= -0.5) at the end of competitive periods in professional soccer players [47].

Parallel to the decrease in C, or/and to the increase in T and T/C ratio, Most of studies showed an increase in physical performance parameters (e.g., $VO2_{max}$: Δ%= 4 to 12%, CMJ: Δ%= 4.6 to 15.2%, SJ: Δ%= 6.5 to 11.7%, YYIR1: Δ%= 33%, T-test: Δ%= -2.2 to -4.8%, 20m sprint: Δ%= -0.9 to 7.1%) after periods of training or/and competitions [5, 10, 19, 30, 33, 50, 53]. In addition, another study showed a decrease in physical fitness ($VO2_{max}$: Δ%= -6.7%, VJ: Δ%= -3%) when the C and the HR increased after training periods [36]. The decreases in T concentrations were associated to physical performance decreases (e.g., YYIR1: Δ%= -34.94%; $RSSA_{mean}$: Δ%= 3.64%; $RSSA_{best}$: Δ%= 0.9%; SJ: Δ%= -3.38%) [20].

Table 6 Effects of long term soccer training in hematological parameters

Study	Participants (number/sex/ age)	Intervention/ Times of evaluations	Hematological parameters: plasma volume variations (PVV), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean hemoglobin concentration (MHC).						
			PVV (%)	**Ht (%)**	**Hb (g/dl)**	**MCV (fl)**	**MCHC (pg)**	**MCH (pg)**	**Physiological markers and physical fitness**
Silva et al. [21]	12/M/19-28	(A) W0 (B) W12	(A-B) ↓12%* ES = -1.2	(A) 40.68 ± 2.54 (B) NS ES (A-B) = 0.07	(A)14.16 ± 0.97 (B) ↑5.4%* ES (A-B) = 0.8	(A) 87.53 ± 2.7 (B) ↓3.7%* ES (A-B)= -1.2	(A) 34.74 ± 0.61 (B) ↑5* ES (A-B) = 2.7		**Anaerobic threshold** (A) to (B) ↑8.12%*
Saidi et al. [4]	18/M/20	(A) W0 (B) +6Ws of training	(A-B) ↑6.1 ± 8.7%* ES = 0.7	(A) 43.9 ± 2.7 (B) ↓3.1%* ES (A-B) = -0.6	(A) 14.9 ± 1.04 (B) ↓4.6 %* ES = -0.8	(A) 83.1 ± 4.5 (B) NS	(A) 28.05 ± 1.7 (B) NS	(A) 33.9 ± 0.7 (B) NS	**YYIR1** ↓34.9%*, ES (A-B) = - 2.5 **$RSSA_{mean}$** ↑2.6%* ES (A-B) = 0.7 **$RSSA_{best}$** ↑3.8%* ES (A-B) = 0.7 **SJ** ↓2.5%* ES (A-B) = -0.26
Requena et al. [37]	19/M/19-30	(A)Mid-season (B) Off season		(A) 43.08 ± 2.18 (B) ↑2.9%* ES (A-B) = 0.6	(A) 14.85 ± 0.94 (B) ↑3.5%* ES (A-B) = 0.7	(A) 89.98 ± 3.51 (B) ↓1.5%* ES (A-B) = -0.3	(A) 34.45 ± 0.93 (B) ↓1.1%* ES (A-B) = -0.4	(A) 31 ± 1.69 (B) ↓ 2.6%* ES (A-B)= -0.4	**MAS** ↓4.5%* ES (A-B) = 0.8 **VJ** ↓17.5* ES (A-B) = -0.2

Andelkovic et al. [6]	19/M/17.62	(A) Baseline (B) +90 days	A-B ↑7.5%*	(A) 0.482 ± 0.01 (B) ↓4.1* ES (A-B) = -1.3	(A) 157 ± 3.9 (B) ↓3.1* ES(A-B)= -1.6	(A) 88.64 ± 0.89 (B) ↓2% ES(A-B)= -2.2	(A) 326 ± 2.0 (B) NS	(A) 28 ± 0.3 (B) NS	
Heisterberg et al. [8]	24/M/26.3	(A) Mid-december (B): Late may		(A) 0.46 ± 0.01 (B) NS	(A) 9.5 ± 0.1 (B) ↓2.1%* ES (A-B) = -2	(A) 86 ± 1 (B) ↑2.3* ES (A-B) = 1	(A) 20.7±0.1 (B) ↓3.8* ES (A-B) = -8	(A) 1.77 ± 0.03 (B) ↓1.6 ES (A-B) = -1	**$VO2_{max}$** ↓2.26* ES (A-B) = -0.95
Walkers et al. [36]	25/F/20	(A) Pre-season (B) +16 Ws		(A) 43.2 ± 2.7 (B) ↓6.3%* ES (A-B) = -0.9					**$VO2_{max}$** ↓6.7% ES (A-B) = -0.83 **VJ** ↓3% ES (A-B) = -0.28

W week,**Ws** weeks,**NS** no significant difference, **ES** effect size, *significant difference, **YYIR1** yo-yo intermittent recovery test level 1. **RSSA** repeated shuttle sprint ability, **SJ** squat jump, **VJ** vertical Jump**, $VO2_{max}$** maximal oxygen consumption, **MAS** maximal aerobic speed

Table 7 Effects of acute soccer matches and training sessions on hormonal markers.

Study	**Participants (number/sex/ age)**	**Interventions/Times of evaluations**	**Hormonal parameters: cortisol (C), testosterone (T), and testosterone: cortiol ratio (T/C)**			
Acute soccer matches			**C (ng/ml) or (nmol/l) or ɲg/dl**	**T (ng/ml) or (nmol/l) or (ɲg/dl)**	**T/C ratio**	**Physiological markers and physical fitness**
Fothergill et al. [38]	18/M/17.5	(A) Pre-G (B) Post-G (a) Home (b) Away CM Saliva	(A) 9.06 (B) ↑56.2%* ES (A-B) = 0.5 (a) 13.32, a> b* (b) 9.99	A= B (a) 140.31, a=b (b) 125.13		
Romagnoli et al. [35]	22/M/17-20	(A) Pre-M (B) +30 min (C) +24 h (D) +48 h CM: serum	(A) 219.2± 51.6, A> C, D* (B) NS (C) ↓36%* (D) ↓32%* ES (A-C)= -1.6 ES (A-D) = -1.5	**FT** (A) 6.5 ±1.5, A> B, C, D* (B) ↓25.9%* (C) ↓25%* (D) ↓30%* ES (A-B)= -0.8 ES (A-C) = -0.8 ES (A-D)= -1.2	**FT/C** (A) 0.77± 0.20 , (B) NS (C)NS (D) NS	**CMJ (cm)** (A) 49.6 ± 5.1 (B) ↓3.2%* (C) ↓2.6%* (D) ↓3%* ES (A-B)=- 0.3 ES (A-C)=- 0.24 ES (A-D)=- 0.29
Oliveira et al. [39]	33/F/24.2	(A) 30 min Pre-M (B) 30 min Post-M 2 groups (a) Winner	(a) a= b (A-B): +0.38 ,A=B b) (A-B): +1.86, A=B	(a) a > b* (A-B): +2.70, B>A*, b) (A-B): +2.19, B> A*		

		(b) Loser CM: saliva				
Moreira et al. [40]	22/M/23	(A) 10 min Pre-M (B) 10 min Post-M 2 groups (a) Team 1 (b) Team 2 CM: saliva	(a) a = b (A) 7.6 ± 4.4, (B) ↑59.4%* ES(A-B)= 0.8 (b) (A) 8.8± 3 (B) ↑16.9%* ES (A-B)= 0.3			**RPE** (a) (A) 7.6± 4.4 (B) ↑86%* ES (A-B)= 0.86 (b) (A) 8.8± 3.0 (B) ↑16%* ES (A-B)= 0.3
Souglis et al. [17]	18/M/25.6	(A) 8 h- Pre-M (B) +0 h (C) +13 h (D) +37 h CM: plasma	A < B* (B) ↑105%* ES (A-B)= 0.95 A= C= D			
Silva et al. [41]	7/M/22-31	(A) Pre-M (B) +24 h (C) +48 h (D) +72 h CM: plasma	(A) 50.5 ± 2.6 (B) ↑25.9%* (C) ↑59.4%* (D) ↑3.5%* ES (A-B)= 4.6 ES (A-C)= 4.95 ES (A-D)= 0.62	(A) 5.94 ±0.37 (B) NS (C) NS (D) NS	(A) 12.5 ± 0.6 (B) ↓ 12.4 %* (C) ↓14 %* (D) ↓8%* ES (A-B) = -3.4 ES (A-C) = -4 ES (A-D)= -1.4	**CMJ (cm)** (A) 43.83 ± 2.40 (B) ↓7%* (C) NS (D) NS ES (A-B)= -1.4

Thorpe et al. [16]	7/M/25	(A) 1 h Pre-M (B) +0 h CM: saliva	(A) 10.09 ± 4.04, (B) NS	(A) 0.26 ± 0.07 (B) ↑44%* ES (A-B)= 0.64	(A) 0.028 ± 0.008 (B) NS ES (A-B)= 0.14	
Acute training sessions **Mascarin et al. [43]**	13/F/18.8	(A) Pre-SSG: 16 × 4 min, 3 min rec) (B) +10 min (C) +24 h (D) +48 h (E) +72 h CM: serum	(A) 16.3± 3.66, (B) NS (C) ↓32.6%* (D) ↓8.9%* (E) ↓3.3% ES (A-C)= -3.4 ES (A-D)= -0.4 ES (A-D)= 0.14	**FT** (A) 32.4± 9.9. (B) NS (C) ↓41%* (D) ↓9%* ES (A-C)= -1.4 ES (A-D)= -0.3	**FT/C** (A) 2.11± 0.81 (B) NS (C) NS (D) NS (E) NS	

Sparkes et al. [15]	16/M/21	(A) Before SSG (24 × 29 m) 7 × 6 min, 2 min rec (B) +0 h (C) +2 h (D) +24 h CM: saliva	(A) 0.54± 0.2 (B) ↓16.5%* (C) ↓71.8 %* (D) ↓21.3 %* ES (A-B) = - 0.3 ES (A-C) = -2.2 ES (A-D) = -0.5	(A) 181± 6 (B) ↑11.1%* (C) ↓33.9%* (D) NS ES (A-B) = 0.32 ES (A-C) = -1.16		**CMJ** (A) 37.1± 4.4 (B) ↓59 %* (C) NS (D) NS ES (A-B)= -0.6 **[Lactate]** (A) (B) ↑100%* (C) ↓38.4%* (D) NS ES (A-B)= 1.6 ES (A-C) = -1.4
Sparkes et al. [42]		(a) SSG (24 × 29 m): 7 × 6 min, 2 min rec. (b)Same SSG + 1 h strength training (4 × 4 min, 85% of 1RM, 4 min rec). (A) Pre-training (B) +0 h (C) +24h CM: saliva	(a) (A-B): -0.035 ± 0.225*, A > B, C* (A-C): -0.078 ± 0.152*, (b) (A-B): - 0.022 ± 0.234 (A-C): - 0.006 ± 0.143, A= B =C	(a) (A-B): +3.6 ± 26.3, A=B=C (A-C): -0.8 ± 61.7 (b) (A-B): +3 ± 27.9, A=B (A-C): -16 ± 54.5*, C< A*	(a) (A-B): +138.3 ± 336.8*, A< B, C* (A-C):+53.6 ± 93.9*, (b) (A-B): +74 ± 190.2*, A< B* (A-C): -43± 69.5* , A > C*	**CMJ (cm)** (a) (A-B): -1.6 ± 2.7, B< A* (A-C): -0.5 ± 2.7, A= C (b) (A-B): -1.7 ± 3.8, B< A* (A-C): -0.8 ± 1.9, C< A*

Alovini et al. [45]	10/F/23.8	(A) Pre-STS (3 × 25 min of SSG, 5 min rec + 10 min stretching) (B) Post-STS (C) +4 h (s): Experimental (c): Control CM: serum	(s) (A) (B) ↑7.9%, NS (C) ↓52.7%* (c) A > B, C* s > c* in (B)			
Chmura et al. [44]	18/M/17.2	(A) Pre-SSG (B) +0 min (C) +15 min (D) +30 min 2 groups (a) SSG: 6 × 30 sec (1 × 1), 2 min rec. (b) SSG: 6 × 45 sec (1 × 1), 3 min rec CM: serum	(a) (A) 147.4 ±32.25 (B) ↑22.3%* (C) ↑70.6%* (D) ↑38.2%* ES (A-B)=0.88 ES (A-C)= 0.92 ES (A-D)= 1 (b) (A)127.4 ± 38.6 (B) ↑45.7%* (C) ↑33.6% * (D) ↑6.3% ES (A-B)= 1.2 ES (A-C)= 0.7 ES (A-D)= 0.2	(a) (A) 5.0 ± 1.3 (B) ↑11.2%* (C) ↓20%*, (D) ↓22.3* ES (A-B)= 0.34 ES (A-C)= -0.4 ES (A-D)= -0.9 (b) (A) 4.6 ± 1.0 (B) NS , (C)↓12.2%* (D) ↓20.7%* ES (A-C)= -0.2 ES (A-D)= -1.1 **FT** (a)	(a) (A) 0.035 ± 0.011, (B) NS (C) ↓42.8%* (D) ↓34.2%* ES (A-C)= -1.2 ES(A-D)= -0.85 (b) (A) 0.089 ± 0.033, (B) NS (C) ↓34.8%* (D) ↓29.2%* ES (A-C)= -0.9 ES (A-D)= -0.8 **FT/C** (a) (A) 0.089 ± 0.033	

				(A)12.75 ± 4.73 (B) ↑15.8%* (C) NS , C=A (D) ↓14.1%*, ES (A-B)= 0.39 ES (A-D)= -0.3 (b) (A) 12.75 ± 3.13 (B) ↑8.3%* (C) NS (D) ↓9*, D < B ES (A-B)= 0.3 ES (A-D)= -0.4	(B) NS (C) ↓34.8% (D) ↓29.2% ES (A-C)= -1.1 ES (A-D)= -1.4 (b) (A) 0.110 ± 0.043 , (B) ↓30%* (C) ↓22.7% (D) ↓18.1% ES(A-B)= - 0.9 ES (A-C)= -0.74 ES (A-D)= -0.62	

SSG small sided games, **RM** repetition maximum, **rec** recuperation, **min** minute, **G** game, **M** match, **CM** collection method, **sec** second, **h** hour, **FT** free testosterone, **STS**: soccer training session, **FT/C** free testosterone: cortisol ratio**,***significant difference, **NS**: no significant difference, **ES**: effect size, **CMJ** countermovement jump. **RPE**: rating of perceived exertion

Table 8. Effects of long-term of training on hormonal markers.

Studies	**Participants (Num/sex/age)**	**Interventions**	**Hormonal parameters: Cortisol (C), Testosterone (T), and Testosterone: Cortisol ratio (T/C)**			
			Cortisol (ng/ml) or (nmol/l) or µg/dl	**Testosterone (ng/ml) or (nmol/l) or (µg/dl)**	**T/C ratio**	**Physiological markers and physical fitness**
da Silva et al [18]	18/M/23.8	(A): week 0 (B): week 12 CM: serum	(A) 442.9± 95.1, A < B (B) ↑ 38 %* ES (A-B)= 0.8	(A) 33.6± 7.3 (B) ↓30%*, ES (A-B)= -1.5	(A) 59 ± 19× 10^{-3}, (B) ↓27.1%*, ES (A-B)= -0.62	

Perroni et al. **[50]**	45/M/14	(A) Before and after(B) pre-season 2 groups: (a) Lupafrascati, (b) Albalong CM: saliva	(a) (A) 3.3±2.6, (B) ↓57.5%* ES (A-B)= -1.2 (b) (A) 7.7 ± 4.7, (B) ↓ 85.7%* ES (A-B)= -2.4	(a) (A) 0.07 ± 0.04, A= B (B) 0.08 ± 0.07 (b) (A) 0.08 ± 0.03 (B) ↓50%* ES (A-B)= - 1.3	(a) (A) 0.03±0.04 (B) ↑50%* ES (A-B)= 0.6 (b) (A) 0.01 ± 0.01, (B) ↑400%* ES (A-B)= 1.6	(a) **CMJ** ↑16.5%* ES (A-B) = 1.62 **$VO2_{max}$**↑4.7%* ES (A-B)= 1.22 (b) **$VO2_{max}$**↑8.13%* ES (A-B)=1.77
Saidi et al. **[20]**	16/M/19-22	Before (A) and after (B) 12 weeks training and competitions	(A)135.8 ± 15.2 (B) NS	(A) 6.1 ± 2.3 (B) ↓ 17.3%* ES (A-B)= -0.51	(A)0.044 ±0.016 (B) ↓31.8% ES (A-B)= -1.07	**YYIR1** ↓ 34.9%* ES (A-B) = - 2.5 **$RSSA_{mean}$** ↑ 3.6%* ES (A-B) = 1.15 **$RSSA_{best}$** ↑ 0.9% ES (A-B) = 0.47 **SJ** ↓ 3.4%* ES (A-B) = -0.24

Gorostiaga et al. [33]	21/M/16-18.5	(A) W 0 (B) W11 2 groups: (s) Experimental (c) Control CM: Serum	(s) (A) 431± 149 (B) ↓32.3%* ES (A-D)= -0.32 (c) (A) 476± 107 (B) ↑5.2%* ES (A-D)= 0.25	(s) , A to D } ↑ 7.5%* (c) , A to D } NS **FT** (s) (A) 93.6 ± 18 (B) NS (c) , (A) 95 ± 32 (B) NS	(s) (A) 0.07±0.03 (B) ↑28.5%* ES (A-D)= 0.44 (c) (A) 0.06± 0.02 (B) NS	**CMJ** ↑5.1%*
Opaszowski et al.[51]	20/M/23.6	(A) Before Training (B) +7weeks of training Before (a) and (b) after running test. CM: plasma	(a) (A) 555.3 ± 174.0 (B) ↓32.6%* ES (A-B)= -0.6 (b) (A) 596.7 ± 155.2, (B) ↓32.7%* ES (A-B)= -0.6	(a) (A) 18.3 ±6.16 (B) NS (b) (A) 22.5± 8.56 (B) NS	(a) (A) 3.2± 1.6 (B) ↑ 31.2%* ES (A-B)= 0.6 (b) (A) 3.8± 1.32, (B) ↑36.8%* ES (A-B)= 1	(B), (b) **[Lactate]** ↓23.6%* ES (A-B) =- 1.2
Freitas et al. [49]	17/M/16.0	(A) End of W1 (B) End of W4 CM: saliva	(A) 14 (D) ↓ 35.7%* ES (A–D)= -0.61			**RPE** (A) 4000 (B)↓ 62.5%* ES (A-B)= -3.87

Kraemer et al. [52]	25/M/18-20	(A) pre- competitive period (B) End of competitive period . 2 groups:(a)non starters(b) starters CM: serum	(a), a < b* A= B b) A= B	(a) a > b* (A) 14.9 (B) ↑23% (b) (A) 12.25 (B) ↑ 29%	(a), A < B* (b) A= B	**CMJ (cm)** (A) 53.5 (B) ↓4.5%*
Kamran et al.[30]	36/M/-	(A) Before training (B) After training 3 groups (a) complex training (b) contrast training (c) Control group CM: serum	(a) a < b* (A)10.7± 4.8 (B) ↓ 11%* ES (A-B)= -0.31 (b) (A)10.90± 5.43 (B)↓ 10 %* ES (A-B)= - 0.33 (c) (A) 9.09± 2.42 (B) 9.13± 2.43	**FT** a) a > b* (A) 18.64 ± 6.58 (B) ↑ 28%* ES (A-B)= 0.6 b) (A):18.03± 4.11 (B) ↑ 17%* ES (A-B)= 0.7 c) , c < b, a* (A) 18.67 ± 6 (B) 19.09 ± 6		(a) **CMJ** ↑ 7.3% ES (A-B)= 0.77 **20 m sprint** ↓7.1%* ES (A-B)= - 0.8 **T-test** ↓4.8%* ES (A-B)= -0.5 (b) **CMJ** ↑7.65%* ES (A-B)= 0.68 **20 m sprint** ↓ 3%* ES (A-B)= - 0.8 **T-test** ↓ 2.2%* ES (A-B)= -0.46

Requena et al. [37]	19/M/19-30	(A) Mid-season (B) after off season CM: serum	(A) 20.1 ± 5.6 (B) NS	(A) 5.8 ±1.1, (B) ↑4.15* ES (A-B)= 0.20		**MAS** ↓ 4.5%* ES (A-B) = 0.8 **VJ** ↓17.5* ES (A-B) = -0.2
Silva et al. [19]	14/M/25.7	(A):before Pre-season (B): end of season CM: plasma	(A) 176.5 ± 35.3 (B) ↓48.7% ES (A-B)= -2.25	(A) 6.7± 1.3 (B) NS	(A) 3.9 ± 1.3 (B) ↑120%* ES (A-B)= 1.42	**T-test** ↓3.8%* ES (A-B) = -1.2%*
Moreira et al. [54]	45/M/12-13	(A) Pre-season (B) After the competitive period .		(A) 53.9± 44.3 (B) NS		
Filaire et al.[46]	17/M/23.7	(A) Start of season. (D) Mid season CM Saliva (a): 8 am, (b): 11.30 am, (c): 5 pm	(a) (A)13.0± 1.3 (B) NS (b) (A) 10.8 ±1.4 (B) ↑ 8% ES (A-B)= 0.69 (c) (A) 8.0 ± 0.8 (B) NS	(a) (A) 348.8± 18.1 (B) NS (C) NS (D) NS (b) (A) 343.5±19.3 (B) ↓14.2%* ES (A-B)= - 3.1 (c)	(a) (A) 25.7 ± 1.4 (B) NS (b) (A) 30.3 ± 2.6 (B) ↓12.5%* ES (A-B)= -1.3 (c) (A) 41.3 ± 6.2 (D) ↓45.7	

				(A) 338.8±17,0 (B) ↓17.4%* ES (A-B)= -4.2	ES (A-D)= -3.8	
Michailidis et al. [48]	25/M/-	(A) Pre-season (B) End of season CM Serum	(A) 19.18± 1.22, (B) ↑ 2.7% ES= 0.49	(A) 5.9± 0.4 (B) ↓1.3% ES (A-B)= -0.2	(A) 0.33± 0.03 (B) ↓ 9% ES (A-B)= -1.2	
Hammami et al. [10]	20/M/14.5	(A) baseline (B) End of may. 2 groups: (s) experimental (c) control CM: Plasma	(s) (A-B) ↓ 166.1%* (c) (A-B) ↓ 23.8 %* s ≠ c* in (C-D), (D-E), and (A-E)	(s) (A-B) ↑ 1.5%* (c) (A-B) ↑ 0.24% s > c* in (B-C), (C-D), and (A-E)	s) (A-B) ↑ 0.008%* (c) (A-B) ↑ 0.005%* s > c* in (A-B), (B-C), (C-D), and (A-E).	**CMJ** ↑ 12.6%* **SJ** ↑ 7.02%* **30 m sprint** ↓ 0.3%* **YYIR1 (m)** ↑1108* **$VO2_{max}$** ↑ 8.40%*
Arruda et al. [31]	39/M/14-17	(A) The Start competitive season. (C) End of the play-off phase. 2 groups (a) Under 15, (b) Under 17 CM: Saliva		(a) (A) 616 ±153 (B) ↓ 28.4%* ES (A-B)= -0.60 (b) (A) 775± 177 (B) ↓21.1%*, ES (A-B)= -0.4 b > a* , in (A), (B) and (C)		

Muscella et al. [5]	29/M/16.5	(A) pre-season (B) End of the season. CM: serum	(A) 16.8 ±3.8, (B) ↓11.9% * ES (A-D)= -0.2	(A) 610.3 ±38, (B) ↓18.2%* ES (A-B)= -3.1	(A) 36.5± 9.7, (B) NS	**CMJ (cm)** ↑15.2%* **SJ (cm)** ↑7.02%* **YYIR 1 (m)** ↑33%* **30 m sprint** ↑12.8%* **VO_{2max}**↑12%*
Walker et al. [36]		(A)pre-season (B) +16Ws CM: serum	(A) 637 ±276.5, (B) ↑ 74%* ES (A-B)= 0.9 **FC** (A) 19.87 ±6.90 (B) ↑88.2%* ES (A-B)= 3.5			**$VO2_{max}$** ↓ 6.7%* , ES (A-B)= -0.83* **VJ** ↓3%* ES (A-B)= - 0.28*
Koundourakis et al. [53]	67/M/25.5	(A) pre-season (B) End of the season. Three groups (a) Team A (b) Team B (c) Team C CM: Serum		(a) , a=b=c (A) 605.0 ± 141.9 (B) ↑ 62.5%* ES (A-B)= 0.8 (b) (A) 686.8 ± 124.3 (B) ↑9.4 %* ES (A-B)= 0.4 (c) (A) 622.3 ± 125.9 (B) ↑ 5.5%* ES (A-B)= 0.19 **FT**		(a) **$VO2_{max}$** ↑5.67%* ES (A-B)= 0.62 **SJ** ↑11.7%* ES (A-B)= 1.33 **CMJ** ↑11.5%* ES (A-B)= 1.15 **20 m sprint** ↓1.30%* ES= -2 (b) **$VO2_{max}$** ↑ 4%* ES (A-B)= 0.59 **SJ** ↑8.8%*

				(a) (A) 13.70± 5.80, (B) ↑13.7%* ES (A-B)= 0.31 (b) (A) 10.28± 5.8, (B) ↑ 14%* ES (A-B)= 0.21 (c) c < a, b (A) 9.26± 3.39, (B) NS		ES (A-B) = 1 **CMJ** ↑7.4%* ES (A-B)= 0.72 **20 m sprint** ↓1.40%* ES (A-B)= -0.6 (c) **VO2$_{max}$** ↑4.13%* ES (A-B)= 0.81 **SJ** ↑ 6.5%*, ES (A-B)= 0.68 **CMJ** ↑4.6%* ES (A-B)= 0.52 **20 m sprint** ↓0.9%* ES (A-B)= -0.46
Andrzejewski et al. [47]	18/M/18.1	(A) pre-season (B) +22 Ws CM: serum	(A) 206.92 ± 64.90, (B) ↑12.6 %* ES (A-B)= 0.43	(A) 7.31±1.19, (B) NS **FT** (A) 22.91± 4.07, (B) NS	(A) 3.6±1.6, (B) ↓ 10.5%* ES (A-B)= -0.29 **FT/C** (A) 11.51± 3.81, (B) ↓ 13.8%* ES (A-B)= -0.51	

CM: collection method. **FT**: Free testosterone. **FC**: Free Cortisol. **W**: Week. **W**: Weeks. **m**: meters. **FT/C:** Free Testosterone: Cortisol ratio.*Significant difference. **NS**: No significant difference. **ES**: Effect size. **YYIR1**: Yo-Yo intermittent recovery test level 1. **RSSA**: Repeated Shuttle Sprint Ability. **MAS**: Maximal aerobic speed. **SJ**: Squat Jump. **CMJ:** Countermovement jump. **VO2$_{max}$:** Maximal oxygen consomption.

2.3.4 Effects of acute (*e.g.*, soccer match or training session) and long-term soccer training on inflammatory and muscular damage markers

2.3.4.1 Acute and residual effects of soccer match and training session on inflammatory and muscular damage markers

Soccer match effect

Nine studies investigated the acute effects of soccer match [7, 13, 16, 17, 34, 35, 41, 55, 56] on inflammatory and muscle damage markers **(Table 9)**. Four studies analyzed IL-6 variations before and after the match [7, 17, 35, 56]. Four studies observed a significant increase for IL-6 immediately after the match (ES= 0.4 to 3.9) [7, 17, 35, 56]. Six studies investigated CRP change to soccer match [13, 17, 35, 41, 55, 56]. One study showed a small increase in CRP level immediately after the match (Δ%= 83.8; ES= 0.5) [13], and three studies observed no significant changes after the match [35, 55, 56]. Three studies demonstrated an increase for CRP levels 24 h after the match (Δ%= 130 to 200), [35, 41, 56]. One study observed an increase (Δ%= +290%) for CRP 13 h after the match [17]. Three studies analyzed TNF-α variation after a soccer game. These studies showed a significant increase for TNF-α level immediately after the match (Δ%= 149 to 242.8) [7, 17, 56]. Eight studies investigated CK variations after soccer match. Six studies observed an increase for CK levels immediately after soccer match (ES= 0.5 to 4.8) [13, 16, 17, 35, 55, 56]. Three studies showed a significant increase in CK level 24 h (Δ%=199.4 to 415) and 48 h (Δ%= 86.4 to 136.5) after the match [35, 41, 56]. One study showed a small increase for CK concentration 72 h after the match [41]. One study showed a very large increase for CK levels 2 h (ES= 6.5), 12 h (ES= 7.9), 36 h (ES= 11.1) and 60 h (ES= 6.3) after the match [34]. Three studies investigated LDH change to soccer match [13, 17, 55]. These studies showed a moderate to large increase immediately after the match (ES= 1.02 to 1.6). One study showed a large decrease in LDH concentration 18 h after the match (Δ%= -15.9; ES= -1.9) [13].

Physical performance declined after an acute soccer match. Indeed, a small to large decrease were observed in vertical jump performance (e.g., CMJ: ES= -0.3 to -1.4) [35, 41] and in the repeated sprint ability (RSA: ES= 1.9) [34].

Single training session effect

Three studies investigated acute effect of training session on muscle damage markers [15, 27, 35] **(Table 9).** One study showed a small increase immediately after training session (Δ%= 40.5; ES= 0.54), [15]. Two studies demonstrated an insignificant change in CK levels [32,

43]. One study showed a moderate increase 2 h (Δ%= 49.3; ES= 0.68), and 24 h (Δ%= 39.2; ES= 0.60) after training session based on small sided games [15]. One study demonstrated a significant decrease in CK level 24 h (Δ%= -19.5; ES= -0.4), 48 h (Δ%= -36.2; ES= -0.9), and 72 h (Δ%= -33.2; ES= -0.7) [43]. One study investigated LDH variations after training session, and showed a small increase immediately (Δ%=19.2; ES= 0.6) after training session followed by a small decrease 24 h (Δ%=-7.6; ES= -0.28), 48 h (Δ%=-5.7; ES= -0.20), and 72 h (Δ%= -13.6; ES= -0.5) after a soccer training session [43]. Additionally, physical performance (e.g., CMJ) decreased immediately (Δ%= -3.2; ES= -0.3), 2 h (Δ% =-2.6; ES= -0.24) and 24 h (Δ%= -3; ES= -0.29) after the training session [15]. In addition, the lactate concentration increased significantly after an acute training session (ES= 1.6) [15].

2.3.4.2 Effects of long-term soccer training on inflammatory and muscular damage markers

Eleven studies investigated the effects of long-term training on inflammatory markers **(Table 10)**.Two studies evaluated IL-6 variations [36, 57]. The first study showed a moderate increase (Δ%= +167.6%; ES= 0.8) after 16 weeks training periods in female professional soccer players [36]. The second study demonstrated an insignificant difference throughout nine weeks training periods in male professional soccer players [57]. One study showed a non-significant change for TNF-α level during training periods in professional soccer players [57]. Four studies evaluated CRP responses to different periods of training. Two studies observed a small to moderate increase for CRP level (ES= 0.4 to 1.1) [6, 19]. Two studies showed an insignificant change for CRP level training period [9, 57] in male professional soccer players. Eleven studies investigated CK changes to different periods of training. Four studies showed a small to moderate decrease for CK level (ES= -0.3 to -0.6) [6, 8, 58, 59]. Three studies observed a moderate to very large increase for CK level after periods of training (ES= 0.9 to 9.9) [19, 36, 60]. Four studies showed an insignificant change for CK to different periods of training [9, 37, 57, 61]. Five studies examined LDH change to long-term training periods. Four studies observed a moderate to very large decrease for LDH level after different periods of training (ES=-0.7 to -3.3) [6, 37, 58, 59]. One study observed an insignificant change for LDH level after preparation period of the soccer season (2014-2015), and a moderate increase (ES=0.9) after the same period of the soccer season (2015-2016) in professional soccer players [9]. Parallel to the increase in inflammatory and/or muscle damage markers, most of studies showed a decrease in performance parameters (e.g., VO_{2max}: Δ%= -

6.7 to 40%, VJ: Δ%= -3%, T-test: Δ%= -3.8%) after periods of training or/and competitions [19, 36, 37]. In addition, another study, showed that the increase in aerobic performance (e.g., VO_{2max}: Δ%= 18.2%) coincided with a decrease in muscle damage markers after periods of training [58].

Table 9 Effects of acute soccer matches and training sessions on inflammatory markers and muscular damage

Study	**Participants (number/sex/ age)**	**Interventions/ Times of evaluations**	**Inflammatory parameters (interleukine 6 [IL-6], tumor necrosis factor [TNF-α] and C-reactive protein [CRP]) and Muscle damage markers (creatine Kinase [CK], lactate dehydrogenase [LDH])**					
Acute soccer matches			**IL-6**	**CRP**	**TNF-α**	**CK**	**LDH**	**Physiological markers and physical fitness**
Gravina et al. [13]	28/F/18-30	(A) Pre-M (B) +0 h (C) +18h		(A) 0.06 ± 0.05 (B) ↑83.8%* (C) ↑166 %* ES (A-C)= 1.1 ES (A-B)= 0.5		(A) 150 ± 65 (B) ↑62%* (C) ↑121.3%* ES (A-B)= 0.8 ES (A-C)= 0.99	(A) 317 ± 39 (B) ↑28.3% (C) ↓15.9%* ES (A-B)= 1.6 ES (B-C)= -1.1	
Jamurtas et al. [34]	18/M/12-24	(A) Pre-M (B) +2h (C) +12h (D) +36h (E) +60h 2 groups (s) Experimental (c) Control				(s) s> c* (A) 163.5 ± 28.4 (B) ↑201%* (C) ↑352%* (D) ↑561.7%* (E) ↑175.7%* ES (A-B)= 6.5 ES (A-C)= 7.9 ES (A-D)= 11.1 ES (A-E)= 6.33 (c) , A=B=C=D= E		**RSA (s)** (s) (A) 4.56 ± 0.1 (B) ↑4%* (C) ↑5%* (D) ↑5.4%* ES (A-B)= 1.9 ES (A-C)= 1.5 ES (A-D)= 1.53 (c) A=B=C=D= E
Silva et al. [41]	7/M/22-31	(A) Pre-M (B)+24 h		(A) 1.04 ± 0.2 (B) ↑131.7%*		(A) 300.6 ± 33.5 (B) ↑199.4%*		**CMJ (cm)** (A) 43.8 ± 2.4

		(C) +48 h (D) +72h		(C) ↑57.6%* (D) ↑2.8% ES (A-B)= 3.91 ES (A-C)= 2 ES (A-D)= 0.15		(C) ↑86.4%* (D) ↑6.5%* ES (A-B)= 9.2 ES (A-C)= 5.4 ES (A-D)= 0.59		(B) ↓7%* (C) NS (D) NS ES (A-B)= -1.4
Romagnoli et al. [35]	22/M/17-20	(A) Pre-M (B) +30min (C) +24h (D) +48h	(A) 1.9± 2.7 (B) ↑92%* (C) NS (D) NS ES (A-B)= 0.4	(A) 0.08 ± 0.09 (B) NS (C) ↑200%*, (D) NS ES (A-C)= 1.2		A < B, C, D* (B) ↑180%* (C) ↑203%* (D) ↑134%*		**CMJ (cm)** (A) 49.6 ± 5.1 (B) ↓3.2%* (C) ↓2.6%* (D) ↓3%* ES (A-B)=- 0.3 ES (A-C)=- 0.24 ES (A-D)=- 0.29
Souglis et al. [17]	18/M/25.6	(A) 8 h pre-M (B) +0h (C) +13h (D) +37h	A < B, C* (B)↑440%* D=A	A < B* (C) ↑290%* D =A	A < B, C* (B) ↑240%* D =A	A < B, C, D* (B) ↑370%*	A < B, C, D* (B) ↑115%*	

Anderson et al. [7]	10/F/23	(A) Pre-M1 (B) +15-20 min (C) +21 h (D) +45 h (E) Pre-M2 (F) +15-20 min 2 groups (a) Active rec-post-M1 (b) Passive rec-post-M1	(a) (A) 2.1± 0.8 (B) ↑438%* (C) NS (D) NS (E) 3.9± 1.9 (F) ↑171%* ES (A-B)= 3.6 ES (E-F)= 2.8 (b) (A) 5.4 ±1.5 (B) ↑81%* (C) NS (D) NS (E) 4.3±2.5 (F) ↑134.8%* ES (A-B)= 2.9 ES (E-F)= 2		(a) (A) 7 ±1 (B) ↑242.8%* (C) NS (D) NS (E) 10 ±2 (F) NS ES (A-B)= 8 (b) (A) 9 ±1 (B) ↑178%* (C) NS (D) NS (E) 15 ±5 (F) NS ES (A-B)= 3			
Thorpe et al. [16]	7/M/25	(A) 1 h- pre-M (B) +0h				(A) 345 ± 360 (B) ↑84± 61%* ES (A-B)= 0.52		
Devrenja et al. [55]	43/M/16.8	(A) Pre-M (B) +0h		(A) 1.0 ± 1.1 (B) 0.94 ± 1.1, A= B		(A) 215 ± 268 (B) ↑68.8%* ES (A-B)= 1.18	(A) 208.7 ± 43.7 (B) ↑ 25.4%* ES (A-B)= 1.02	

Souglis et al. [56] **Acute training sessions**	43/M-F/22.9-23.1	4 groups (a) Male-experimental (b) Female-experimental (c) Male-control (d) Female-control 4 times of evaluation (A) Pre-M (B) +0h (C) +24h (D) +48h	(a) a= b (A)1.11 ±0.38 (B)↑390%* (C) NS (D) NS ES (A-B)= 3.9 a >c*in (B) a < c* in (A),(C), (D) (b) (A)1.23 ±0.71 (B)↑313% (C) NS (D) NS ES (A-B)= 2 b >d* in (B) b < d* in (A), (C), (D)	(a) a= b (A)1.5 ± 1.2 (B) NS (C) ↑130%* (D) NS a > c* in (C) (b) (A)1.3 ± 1.3 (B) NS (C) ↑130%* (D) NS B > d* in (C)	(a) a > b*in (B) (A) 1.77 ± 0.46 (B) ↑200%* (C) NS (D) NS ES (A-B)= 1.6 a > c* in (B) a < c* in (A),(C), (D) b) (A) 1.89 ± 0.53 (B) ↑149%* (C) NS (D) NS ES (A-B)= 1.4 b > d* in (B) b < d* in (A),(C), (D)	a) , a= b (A) 177 ±19 , (B) ↑112.4%* (C) ↑343.5%* (D) ↑106%* ES (A-B)= 4.8 ES (A-C)= 6.1 ES (A-D)= 2.4 a > c* in (B), (C) and (D) b) , b > d* in (B), (C),(D) (A) 145 ± 22 , (B) ↑80.6%* (C) ↑ 415%* (D)↑ 136.5%* ES (A-B)= 3 ES (A-C)= 3.8 ES (A-D)= 1.9		
Sparkes et al. [15]	16/M/21	(A) Before SSG(24×29m): 7×6 min, 2 min recup-59 min (B)+0h				(A) 239 ± 174 (B)↑40.5%* (C)↑49.3%* (D)↑39.2%*		**CMJ (cm)** (A) 49.6 ± 5.1 (B) ↓3.2%* (C) ↓2.6%* (D) ↓3%*

		(C) +2h (D) +24h				ES (A-B)= 0.54 ES (A-C)= 0.68 ES (A-D)= 0.60		ES (A-B)=- 0.6 ES (A-C)=- 0.24 ES (A-D)=- 0.29 **[Lactate]** (A) (B) ↑100%* (C) ↓38.4%* (D) NS ES (A-B)= 1.6 ES (A-C)= -1.4
Mascarin et al. [43]	13/F/18.8	(A) Pre-SSG: 16×4min, 3 min rec) (B)+10min (C)+24 h (D) +48 h (E)+72 h				(A) 231,62 ± 127.44 (B) NS (C) ↓19.5%* (D) ↓36.2%* (E) ↓33.2%* ES (A-B)= 0.2 ES (A-C)= -0.4 ES (A-D)= -0.9 ES (A-E)= -0.7	(A)397.38 ± 127.87 (B) ↑19.2%* (C) ↓7.6* (D) ↓5.79%* (E) ↓13.6%* ES (A-B)= 0.6 ES (A-C)= -0.2 ES (A-D)= -0.2 ES (A-E)= -0.5	
Mello et al. [32]	22/M/26.5	(A) Pre-STS: 2×40min, 10 min rec) (B): +5 min				(A) 300 (B) NS		

SSG small sided game, **rec** recuperation, **h** hour, **min** minute, **G** game, **M** match. **sec** second. **h** hour, **STS** soccer training session.**M** match.**NS** non significant change, *significant change, **ES** effect size, **RSA** repeated sprint ability, **CMJ**countermovement jump

Table 10 Effects of long term soccer training on inflammatory markers and muscular damage

Study	**Participants (number/sex/ age)**	**Interventions/ Times of evaluations**	**Inflammatory parameters (interleukine 6 [IL-6], tumor necrosis factor[TNF-α] and C-reactive protein[CRP]) and Muscle damage markers (creatine kinase [CK], lactate dehydrogenase [LDH])**					
			IL-6	**CRP**	**TNF-α**	**CK**	**LDH**	**Physiological markers and physical fitness**
Silva et al. [61]	15/M/23.4± 2.5	(A) W0 (B) W12				(A) 113.2± 3.2 (B) NS		
Requena et al. [37]	19/M/19-30	(A) Mid-season (B) After off season				(A) 286.2 ± 181.2 (B) NS	(A) 334.3 ± 46.9 (B) ↓14.5%* ES (A-B) = -1.08	**MAS** ↓4.5%* ES (A-B) = 0.8 **VJ** ↓17.5* ES (A-B) = -0.2
Coppalle et al. [9]	26/24/M/26.2 - 25.9	Two pre-season period (a) 2014-2015 (b) 2015-2016 (A) Before and After (B) preseason period.		(a) (A) 0.4 ± 0.4 (B) NS (b) (A) 1.2± 1.9 (B) NS		(a) (A)339.9 ± 178 (B) NS (b) (A) 256.1± 170.7 (B) NS	(a) (A) 188.9± 30 (B) NS (b) (A) 174.4± 25 (B) ↑21.9%* ES (A-B)= 0.9	

Reinke et al. [57]	10/M/20-36	(A) End of playing-season (B) End of recovery (C) End of pre-season	A=B= C	A= B= C	A =B=C	(A) 293.7± 163 (B) 348 ± 182 (C) 332.1± 148, C=B=A		
Gharahdaghi et al.[58]	12/M/21.8	(A) Pre and (B) Post training program (a) Before and (b) After exhaustive exercise				(a) (A)193.7 ± 121.6 (B) ↓31%* ES (A-B)= -0.6 (b) (A) 234.3 ± 136.4 (B) ↓30%* ES (A-B)= -0.35	(a) (A)164.2 ± 27.8 (B) ↓12%* ES (A-B)= -0.7 (b) (A) 220.16 ±52.5 (B) ↓23%* ES (A-B)= -1.3	(b) **$VO2_{max}$** ↑18.2%
Andelkovic et al.[6]	19/M/17.6	(A) Baseline (B)+90 days		(A) 1.3 ± 1.3 (B) ↑57.1% ES (A-C)= 0.4		(A) 421± 537 (C) ↓38.3%* ES (B-C)= - 0.3	(A) 414± 17.5 (C) ↓13.2%* ES (A-C)= - 3.3	
Pimenta et al. [60]	26/M/26	(A) Before and (B) After pre-season training				(A) 65.5 ± 4.2 (B) ↑ 565.1%* ES (A-B)= 9.9		**$VO2_{max}$**↓40%*
Silva et al. [19]	14/M/25.7	(A) Pre-season (B) End of season		(A) 0.39 ± 0.25 (B) ↑76.9%* ES (A-B)= 1.18		(A) 166.3 ± 96.5 (B) ↑72.2%* ES (A-B)= 1		**T-test**↓3.8%* ES (A-B) = -1.2%*

Walker et al.[36]	25/F/20	(A) Pre-season (B) +16Ws	(A) 1.2 ± 0.8 (B) ↑167.7%* ES (A-B)= 0.8			(A) 138.0 ± 148.3 (B) ↑134.5%* ES (A-B)= 0.6		**VO_{2max}** ↓6.7%* ES (A-B)= -0.83 **VJ** ↓3%* ES (A-B)= -0.28
Aquino et al.[59]	15/M/15.2	(A) W0 (D) W22				(A) 350 (B) ↓65.7%*	(A) 350 (B) ↓60 %*	
Heisterberg. [8]	24/M/26.3	(A) Mid-december (B) Late may				(A) 292 ± 27 (B) ↓4.5%* ES (A-B)= -0.4		

W week, **Ws** weeks, **NS** non significantchange,*significant change, **ES** effect size, **$VO2_{max}$** maximal oxygen consumption, **MAS** maximal aerobic speed, **VJ** vertical jump.

2.3.5 Relationships between biological markers (hormonal, inflammatory and muscle damage markers) with physical fitness performance after an acute (soccer match or training session)

Tables 11 and 12 summarized relationships between Δ% biological markers and Δ% physical fitness performance after an acute (soccer match or training session). No significant relationship was found between hormonal, inflammatory and muscle damage markers with physical fitness after an acute soccer match. Of note, we observed asignificant positive correlation between Δ% T and Δ% CMJ was found after training session (r=0.90; p=0.001). In addition, a negative correlation between Δ% CK and Δ% CMJ were observed after an acute training session (r= -0.99; p= 0.0001).

11 Relationships between biological markers (hormonal, inflammatory and muscle damage markers) with physical fitness performance after an acute soccer match

Biological markers/physical fitness	**C**	**IL-6**	**CRP**	**TNF-α**	**CK**	**LDH**
CMJ	r= 0.98 p=0.10	r=0.99 p=0.08	r=0.85 p=0.35	r=-0.57 p=0.61	r= 0.99 p=0.08	r=0.36 p=0.76

C cortisol. **IL-6** interleukine 6, **CRP** C-reactive protein**, TNF-α** tumor necrosis factor**, CK** creatine Kinase. **LDH** lactate desydrogenase, **CMJ** countermovenemnt jump.

12 Relationships between biological markers (hormonal and muscle damage markers) with physical fitness performance after acute soccer training

Biological markers/physical fitness	**C**	**T**	**CK**
CMJ	r= 0.80 p=0.40	r =-0.90 p=0.001*****	r=-0.99 p=0.0001*

CMJ countermovenemnt jump, **C**cortisol. **T** testosterone, **CK** creatine kinase.
*significant correlation.

2.3.6 Relationships between biological markers (hematological, hormonal, inflammatory and muscle damage markers) with physical fitness performance after long term of soccer training

Table 13 summarizes relationship between percentage changes of hematological, hormonal, inflammatory and muscle damage markers with percentage change in physical fitness performance. No significant relationships between percentage change of hematological parameters and physical fitness performance (VJ and VO_{2max}) were observed. In addition, a positive correlation between Δ% FT and Δ% SJ performance (r=0.98; p=0.01) were observed after long term of soccer training. We found that the increase in T/C ratio were related to the increase in SJ performance (r= 0.90; p= 0.03). Also the change in CRP concentration were related to change in $VO2_{max}$ (r=-0.99; p= 0.01).

13 Relationships between biological markers (hematological, hormonal, inflammatory and muscle damage markers) with physical fitness performance after long term of soccer training

Biological markers/physical fitness	**PVV**	**Ht**	**Hb**	**MCV**	**MCHC**	**C**	**T**	**FT**	**T/C**	**CRP**	**CK**	**LDH**
VJ	r= 0.96 p= 0.17	r= 0.58 p= 0.60	r= -0.33 p= 0.86	r= -0.23 p= 0.85	r= 0.75 p= 0.45							
VO_{2max}	r= -0.69 p= 0.50	r= -0.64 p= 0.55	r= 0.97 p= 0.14	r= -0.94 p= 0.21	r= 0.58 p= 0.60					r= -0.99 p= 0.01*	r= -0.19 p= 0.87	r= -0.92 p= 0.24
CMJ						r= 0.06 p= 0.84	r= -0.30 p= 0.33	r= -0.85 p= 0.14	r= -0.31 p= 0.35			
SJ						r= -0.72 p= 0.17	r= -0.02 p= 0.96	r= 0.98 p= 0.01*	r= 0.90 p= 0.03*			
20 m-sprint						r= -0.61 p= 0.27	r= -0.39 p= 0.74	r= 0.60 p= 0.39	r= 0.44 p= 0.45			
T-test						r= -0.64 p= 0.55	r= -0.39 p= 0.74	r= -0.11 p= 0.92	r= 0.03 p= 0.97			

PVV plasma volume variations**, Ht** hematocrit**, Hb** hemoglobin, **MCV** mean corpuscular volume**, MCHC** mean corpuscular hemoglobin concentration. *significant correlation**, MCH** mean hemoglobin concentration**. C**cortisol, **T** testosterone, **FT** free testosterone, **T/C** testosterone: cortisol ratio, **IL-6** interleukine 6, **CRP** C-reactive protein**, TNF-α** tumor necrosis factor**, SJ** squat jump**, CK**creatine kinase, **LDH** lactate desydrogenase, **CMJ** countermovenemnt jump, **VJ** vertical jump, **$VO2_{max}$** maximal oxygen consumption.

2.4 Discussion

This systematic literature review demonstrated that acute soccer match and training session appears to induce significant alterations (increases or decreases) in select hematological, hormonal, and inflammatory, muscle damage markers as well as physical performance in soccer players. It appears that the T and CK concentrations were related to CMJ performance after training session. Additionally, we observed that the long-term soccer training induced increased/decreased in hematological, hormonal, inflammatory, muscle damage markers and physical performances.The changes in hormonal parameters (FT and T/C) were related to SJ performance alteration after long-term of soccer training and/or competition.

2.4.1 Effects of acute (e.g., soccer match or soccer training session) and long-term soccer training on hematological parameters

2.4.1.1 Acute and residuals effectsof soccer match andsoccer training sessionon hematological parameters

Soccer match effect

Acute hematological effects to soccer match and soccer training session were assessed in soccer players with different level of expertise using measures of PVV, Ht, Hb, MCV, MCHC and MCH **(Table 5)**. Indeed, Romagnoli et al. [35] observed a significant increase for PV immediately (+7.9%), 24 h (+9%) and 48 h (+7.2) after the match and a significant decrease in vertical jump performance (e.g., CMJ) immediately (-3.2%), 24 h (-2.6%) and 48 h (+3%) after the game in male professional soccer players. Thus, playing the match induced significant hemodilution and a reduction in neuromuscular efficiency that lasted 48 h after the game in male professional soccer players. In contrast, Jumurtas et al. [34] observed an insignificant change of plasma volume, after a soccer match. The authors of this study explain the negligible change of plasma volume and consequently the unaltered Hb, and Ht level by the fact that participants were allowed to consume water ad libitum during the game and that the first post-game measurement occurred 2 h after the game. Differences between these two studies could be explained by experimental factors such as the level of participants' expertise, psychological factors, the time of measurement, and physical efforts during the match. In addition, in the study of Romagoli et al. [35] the soccer game was played during the competitive period, while the soccer match was played one week following the completion oftheir regular season in the study of Jumurtas et al. [34]. This may also be a reason for the different outcomes in the two studies.

Single training session effect

In this systematic review, one study reported alteration in hematological parameters after soccer training session [14]. The authors of this study demonstrated a small decrease for Hb (Δ%= -2.5%; ES= -0.2) and MCV (Δ%= -3%; ES= - 0.3). This decrease coincided with a large increase in lactate concentration (Δ%=148%; ES= 1.8) after 90 minutes training session. The authors of this study explain these changes by the increase in shear rate that occurs at the end of training as well as hemodilution which is a middle term effect of exercise. Our systematic observation of hematological parameters reveals a trivial to small alteration after a soccer game or training session. These results must be viewed with caution because only a few studies investigated the acute effect of soccer match or training session on hematological parameters, therefore more studies are necessary to better understand the magnitude of variations in hematological parameters after acute soccer exercise.

2.4.1.2 Long-term effects soccer training on hematological parameters

Hematological responses to long term period of soccer training were assessed in different levels of soccer players. **Table 6** illustrate that hematological markers present a significant variation after a long period of training. Indeed, Silva et al. [21] showed a moderate decrease in PV (Δ%= -12%, ES= -1.2), and MCV (Δ%= -3.7%, ES= -1.2) after 12 weeks training periods. Additionally, The Hb and MCHC concentrations increased (Hb: Δ%= 5.4%, ES= 0.8; MCHC: Δ%= 5%, ES= 2.7) parallel to an increase in aerobic performance parameters (Anaerobic threshold: Δ%= -12%). In contrast, Saidi et al. [4] showed a moderate increase for PV (Δ%= 6.1%; ES= 0.7) and a moderate decrease for Hb (Δ%= -4.6%, ES=-0.8) and Ht (Δ%= -3.1; ES= -0.6) after six weeks intensive period of training in elites soccer players, when the players are exposed to a high number of official match (10 matches/6 weeks). Theses hematological variations coincided with a decrease in endurance performance (e.g., YYIR1: Δ%= -34.9%, ES= -2.5), repeated sprint ability (e.g., $RSSA_{mean}$: Δ%= 2.6%, ES= 0.7; $RSSA_{best}$: Δ%= 3.8%, ES= 0.7) and vertical jump ability (e.g., SJ: Δ%= -2.5, %, ES= -0.26). The specifics of the soccer training program and/or participant's level could explain the contradictory finding. In addition, the mean weekly and training lasted volume in the study of Silva et al. [21] was higher (14.7-16.3h/week, 12 weeks) than that the study conducted by Saidi el al.[4] (9 h/week, 6 weeks).

The variations in intensity and volume and match exposure presented over 6-month period program led to significant alterations in hematological markers and physiological markers of

aerobic performance in professional soccer players [8]. The Significant increases for the MCV level (Δ%= 2.3%, ES= 1) and a significant decrease in Hb (Δ%= -2.1%, ES= -2), MCHC (Δ%= -2.3%, ES= -1), MCH concentration (Δ%= -1.6%; ES= -1) coincided with VO_{2max} decreases (Δ%=-2.3%; ES=-1) at the end of the competition season, after a period characterized by frequent match exposure. More recent studies have also examined the effects of long-term period of training on hematological makers [6, 37]. Reports from these studies indicated significant change in hematological parameters following different training periods. Requena et al. [37] showed an increase in Hb (Δ%= 3.5%; ES= 0.7) and Ht (Δ%= 2.9%; ES= 0.6), and a decrease in MCV (Δ%= -1.7%; ES= 0.4), MCHC(Δ%= -1.1%; ES= -0.4), MCH (Δ%= -2.6%; ES=0.4) after six weeks off season period when the volume and intensity of training were greatly reduced as compared to mid-season period. The increase of Ht and Hb observed in this study reflects a blood hemoconcentration process by a possible loss of plasma volume after the off-season period. Moreover, this blood hemoconcentration may also be related to the decrease of aerobic performances (e.g., maximal aerobic speed: Δ%= -3.1%). These results are in contradiction with that found by Silva et al. [21] who have shown that increased hemoglobin was associated with increased aerobic performance. The analysis of variation in hematological and performance parameters should be treated with caution. The relation between its parameters is not causal; it is perhaps a simple coincidence. Additionally, in young soccer players aged 17-18 years, Andelkovic et al. [6] observed a significant increase in PV by 7.5% and a decrease in Ht (Δ%= -4.1%, ES= -1.3) and Hb levels (Δ%= -3.1%, ES= -1.6) after the first 90 days of regular training. In fact, PV increased from baseline to 90 days of training, which explain that the decrement in Hb and Ht occurs because the plasma volume expansions. Thus, decreasing Ht and Hb values during periods of intense training or competitions could be explained by “sports anemia” concept [11]. In this concept, the absolute Hb concentration is increased, mainly because of exercise induced stimulation of erythrocytosis; however, this mechanism is suppressed by the far greeted rise in plasma volume [57]. Furthermore, Walkers et al. [36] showed a moderate decrease in Ht level (Δ%= -6.3%, ES= -0.9) and performance parameters ($VO2_{max}$: Δ%= -6.7%; ES= -0.83, VJ: Δ%= -3%, ES= -0.28) after 16 weeks training period in female professional soccer players. The authors of this study explain that the decrease in Ht and physical performance level could be caused by a fatigue state associated to higher number of practices and training load increases.

2.4.2 Effects of acute (soccer match or training session) and long-term soccer training on hormonal markers

Acute soccer match, training session and long term of soccer training effects on hormonal markers was assessed via examining C and T levels. Together these hormones identify anabolic and catabolic relations, the T/C ratio. A decreased ratio has been correlated with tiredness, lethargy, exhaustion and even negative performance [62]. Researchers who have investigated how the levels of theses hormones vary over the course of a soccer season, after a single soccer match and after a soccer training session.

2.4.2.1 Acute and residuals effects of soccer match and training session on hormonal markers

Acute soccer match and training session effects on C and T concentrations are illustrated in **Table 7**.

Acute soccer match

Thorpe et al. [16] showed an increased for salivary T level (Δ%= +44%; ES= 0.64) and an insignificant change for salivary C level and T/C ratio immediately after a soccer match in semi-professional soccer players. The authors explain the increase in salivary T by the fact that players performed up to 69 sprints in 90 minutes of intermittent- type exercise. This linked with the substantial muscle damage, might have provided a stimulus for the testosterone increases by mechanism more associated with strength exercise than aerobic exercise. Additionally, Oliveira et al. [39] examined salivary C and salivary T variations before and after a match in two groups of female professional soccer players; a winner and loser group. It showed an increase in salivary T (+2.7) thirty minutes after the match in the winner group and a decrease in the loser group (-2.19). A possible explanation for the differences in T responses between winners and loser was that winner group had higher physical exertion than did loser. Fothergill et al. [38] investigated variations of salivary C and T in elite soccer players, before and after two games; one home game and one away game. They showed that the home C level was significantly higher than away. In addition, the C level increased significantly after the match (Δ%= +56.2%; ES= 0.5). The T was not different at home than away and before than after the soccer match. In this study the authors attributed the rise of saliva C level when playing at home could be caused by the higher level of psychological stress and a greater effort, pressure and energy expenditure during home competitions. Moreira et al. [40] showed an increase for saliva C by 49.5% and 16.9%

respectively for the Team A (ES= 0.8), and Team B (ES= 0.3), ten minutes after a soccer match. The increase of C concentration coincided with an increase in RPE values for the two teams (Team A: ES= 0.86, Team B: ES= 0.3).The results of the last study are in agreement with that demonstrated by Souglis et al. [17] which have shown a significant increase immediately after the match for the C level in elite soccer players (Δ%= 105%; ES= 0.95). Another recent study showed that serum C and FT concentrations were found to be diminished 24 h (C -36%; T -25%) and 48 h (C -32%; T -30%) after the soccer match [35]. These hormonal alterations coincided with a significant decrease in vertical jump performance (e.g., CMJ) immediately (-3.2%), 24 h (-2.6%) and 48 h (+3%) after the game in male professional soccer players. The authors of this study found decreased C, T and vertical jump performance, which are usually related with fatigue [35]. In addition, Silva et al. [41] observed that the C level increased by 25.9% and 59.4%, respectively 24 h and 48 h after an official soccer match. The T/C ratio decreased significantly 24 h (Δ%= - 6.5 to 12.4%; ES= -2.3) and 48 h (Δ%= -4.2 to 14%; ES= -4) after the match. In addition, vertical jump ability declined immediately after the match (e.g., CMJ: Δ%= - 7%; ES= -1.4).

Acute training session

Sparkers et al. [15] investigated C and T responses to soccer training session based on small sided games (4 vs 4+goalkeepers; 6×7-min, 2 min inter-set recovery). They showed that saliva C level decreased immediately after SSG (Δ%= -16.5%; ES = -0.3), before large decreased at +2 h (Δ%= -71.8%; ES= -2.2), which persisted for 24 h (likely-small; Δ%= -21.3%, ES= -0.5). For the T level, a small increase (Δ%= 11.1%; ES= 0.3) was observed immediately after training session, before a likely moderate decline at + 2 hours (Δ%= -33.9; ES= -1.16). Vertical jump performance (e.g., CMJ) decreased immediately after the training session (Δ%= -59; ES= -0.6) return to pre- match value 2 h after the match. The investigators in this study explained the hormonal change by the natural changes in the player's circadian rhythm, where T and C in men has been shown to peak in the early morning followed by progressive reduction (30-40%) throughout the day. Additionally, Sparkes et al. [42] compared hormonal change to a single session (small sided games; (4 vs 4+goalkeepers; 6×7-min, 2 min inter-set recovery) versus double training session (small sided games +strength training sessions). No change in saliva T and a small decrease in C have shown after a single training session. In addition, no change and a small decrease in saliva T were shown 24 hours after a double training session. The decreased of T level coincided with a decrease in vertical jump performance. In the same study, the authors demonstrated higher T concentrations

following the single compared to the double training session. There were higher concentrations in C following the double compared to the single session (pre and +24 h, 0 h and +24 h). After a small sided game (SSG: 6 × 30 s × (1vs1), 2 minutes recuperation between sets) in youth soccer players, Chmura et al. [44] observed an increase in serum C concentration immediately (Δ%= +22.3%; ES= 0.88), 15 min (Δ%= 70.6%; ES= 0.92) and 30 min (Δ%= 38.2; ES= 1) after SSG. In addition, T and FT was not changed immediately and 15 min after SSG but decreased significantly 30 min after SSG. In the same study and after other forms of SSG (6× 45s × (1 vs 1), 3 min recuperation between sets). The C increased immediately (Δ%= +45.7%; ES= 1.2) and 15 min after SSG (Δ%= +33.6%; ES= 0.7). The authors felt the increased of C immediately and 15 min after SSG by the high intensity of exercise, mental stress and the higher activation of the sympathetic nervous system. Additionally, intensive the SSG session lead to reduced T/C and FT/C, 15 and 30 min after the two forms of SSG. Also, Mascarin et al. [43] found serum C and FT were unchanged 10 min and 72 h after SSG (4×4 min, 3 min-passive recuperation between sets). In contrast, C and FT concentrations were found to be diminished 24, and 48 h after the SSG (C [32.6 - 8.9%]; T [-41- 9%]). The authors of this study conclude that training based on SSG does not seem to produce a significant change in C and FT levels. Consequently, no significant alterations were observed in the T/C ratio. Alovini et al. [45] observed an insignificant change for the C level immediately after a soccer specific training session (duration 2 h; intensity 75% of HR_{max}). A decrease by 52.7% for C level has been shown 4 h after training session. These findings illustrate that training based on SSG and soccer specific does not seem to produce the same significant responses to the stimulus as the competitive soccer match. Additionally, we observed that the soccer match induces a greater acute magnitude (match end) of hormonal and physical fitness alterations as compared to soccer training session. In addition, the time taken to return to baseline level (pre-match or training session) was higher after the match compared to that after training session.

2.4.2.2. Long-term-effects of soccer training on hormonal markers

Table 8 illustrates the long-effects of soccer training on hormonal markers. Silva et al. [18] showed that plasma C increased (Δ%= 38%; ES= 0.8) after 12 weeks training in professional soccer players. In contrast, the T level and T/C were found to be diminished (T: Δ%= -30%; ES= -1.5, T/C: Δ%= 27.1%; ES= -0.62). These authors explained the C increases and T decreases by the increment of training volume and intensity during training periods. During 36 weeks of soccer season, in professional soccer players, Silva et al. [19] demonstrated a

significant decrease in C level (Δ%= -48.7%; ES= -2.25) and an increase in T/C ratio (Δ%= 120%; ES= 1.42) at the end of season. Filaire et al. [46] investigated C, T and T/C between the start and the middle of season, when three saliva sample taken at different times (resting values 8:00 AM), before breakfast (11.30 am), and between 4:00 and 6:00 PM. They showed that C increased significantly (Δ%= 8%; ES= 0.69) only at 11.30am. The authors explain this result by the fact that this type of training could therefore induced a prolonged increase in the diurnal C secretion at 11.30.

Additionally, The T and T/C ratios were found to be diminished in mid-season period at 11.30am (T: Δ%= -14.2%; ES= -3.1, T/C: Δ%= -17.4%; ES= -4.2) and between 4.00-6.00 PM (T: Δ%= -12.5%; ES= -1.3, T/C: Δ%= -45.7%; ES= -3.8). These changes of T and T/C level could be due to interactive modifications of various endocrines parameters, in particular to modifications of the hypothalamic-pituitary axis effects on the testicles and adrenal glands [63]. Gorostiga et al. [33] showed a significant decrease by 32.3% (ES= -0.32) for the serum C concentration after 11 weeks training periods (explosive strength and soccer training) in young soccer players. In addition, a significant increased by 7.5% and 28.5% respectively for T and T/C have been shown after 11 weeks training periods. The results of this study could suggest a training induced in anabolic androgenic activity may improve the neural adaptations that occur for strength gain [64]. Consequently, the vertical jump performance (e.g., CMJ) increased by 7.5% [33]. Another study showed a significant decrease for the C in elite soccer players for two different clubs (Club 1: Δ%= -57.5%; ES= -1.2, Club 2: Δ%=-85.7%, ES= -2.4), and an increase for T/C (Club 1: Δ%= 50%; ES= 0.6, Club 2: Δ%= 400%; ES= 1.6) after pre-season period [50]. These authors demonstrated a physical fitness increases in parallel to hormonal variations (CMJ: Δ%= 16.5; ES= 1.62, VO_{2max}: Δ%= 4.7-8.13; ES=1.2-1.7).The increase in the T/C value and physical performance shown during this study, strongly indicating a good training setting. The results found by Perroni et al. [50] were similar to that observed by Opaszowski et al. [51] who showed a decrease in C (Δ%= -32.6 – 32.7%; ES= -0.6) and an increase in T/C (Δ%= 31.2 - 36.8%; ES= 0.6 -1), T (Δ%= 85.7; ES= 2.4) after 7 weeks preparatory period, in semi-professional soccer players. Theses hormonal changes coincided with a decrease in blood lactate after running test (Δ%= -23.6; ES= -1.2). Michailidis et al. [48] evaluated the changes in C, T and T/C ratio after 43 weeks of training. The authors of this study, showed a small increase for the C (Δ%= 2.7%; ES = 0.49), a small decrease in T (Δ%= -1.3%; ES= -0.2) and moderate decrease in T/C ratio (Δ%= -9%; ES=-1.2). The C increased and a T/C decreased could be explained by tiredness due to the high number of match played, a higher training load, and psychological pressure during training

period. More recently, Hammami et al. [10] investigated hormonal changes with two season periods of intense training in elite young soccer players. The plasma C decreased by -166.1% between the start of the first season and the end of the second season. In contrast, T concentration and T/C value increased during the different periods during two soccer seasons. The authors of this study demonstrated a physical performance improves (VO_{2max}: Δ%= 8.4%, CMJ: Δ%= 12.62%, SJ: Δ%= 7.02%, YYIR1: Δ%= +1108 meters, T-test: Δ%= -2.2 to -4.8%, 30m-sprint: Δ%= -0.27%). In contrast, Saidi et al. [20] showed a significant change in T (Δ%= 8.4%; ES= -0.51) and T/C ratio (Δ%= -31.8%; ES= -1.07) after 12 weeks of training and competitive periods. The authors of this study explain this decrease by the accumulated fatigue caused by the high number of match played during the congested competitive schedule (10 matches played during the lasted six weeks). Also, the decrease in T and T/C ratio after the congested period of match play were associated to physical fitness decreases (YYIR1: ES= -2.54; $RSSA_{mean}$: ES= 1.15: $RSSA_{best}$: ES= 0.47; SJ: ES= -0.24). Another recent study investigated the effects of training on hormonal concentrations after 36 weeks period of training and competitions, in young soccer players [5]. The C level decreased (Δ%= -11.9%; ES= -0.2) and the T concentration decreased (Δ%= -18.2%; ES= -3.1). In addition, physical performance increased (VO_{2max}: Δ%= 12%, CMJ: Δ%= 15.2%; SJ: Δ%= 7.02, YYIR1: Δ%= 33%, 30m-sprint: Δ%= 12.8%). Kraemer et al. [52] investigated hormonal concentration change over 11 weeks training periods in starters and non-starters groups of elite soccer players aged 18-20 years. They showed an insignificant change in C concentration in the starters and non-starters groups during the evaluation period. In contrast, the concentration of T increased by 23% in the starters group and 29% in the non-starters group between the beginning and the end of the competitive period. The T/C increased only in the starters group at the end of 11 weeks training periods. In addition, Kamran et al. [30] compare the effects of a 6 weeks complex versus contrast training regimen on hormonal changes in professional soccer players. They showed an increase in FT concentration in the complex training group (Δ%= 28%; ES= 0.6) and contrast training group (Δ%= 17%; ES= 0.7). In addition, the C concentration decreased similarly in both training groups compared to the control group (complex training group: Δ%= -11%; ES=-0.31, contrast training group: Δ%= -10%; ES=-0.33). The C decreases and the FT increases coincided with physical performance improves (complex training group: CMJ: Δ%= 7.3%; ES=0.77, T-test: Δ%= -4.8%; ES=-0.5, sprint-20meters:Δ%= -7.1%; ES=-0.8, contrast training group: Δ%= 7.6%; ES=0.68, T-test: Δ%= -2.2%; ES=-0.46, sprint-20 meters: Δ%= -3%; ES=-0.8). Koundourakis et al. [53] examined the effects of three seasonal training programs, largely different in strength volume: high

(Team A), moderate (Team B) and low volume (Team C), on androgen levels in professional soccer players. They showed an increase in T concentration in the team A (Δ%= 62.5%; ES = 0.8), in the team B (Δ%=+9.4%; ES= 0.4 and in the team C (Δ%= 5.5%; ES= 0.24) between the beginning of the pre-season period and the end of season. Physical performance improved (Team A: VO_{2max}: Δ%= 5.6%; ES=0.62, CMJ: Δ%= 11.5% ; ES= 1.15, SJ: Δ%= 11.7%; ES= 1.3, 20 m-sprint: Δ%= -1.90%; ES=-2, Team B: $VO2_{max}$: Δ%= 4% ; ES=0.59, CMJ: Δ%= 7.4% ; ES= 0.72, SJ: Δ%= 8.8% ; ES= 1, 20 m-sprint: Δ%= -1.40% ; ES=-0.6, Team C: VO_{2max}: Δ%= 4.1% ; ES=0.81, CMJ: Δ%= 4.6% ; ES= 0.52, SJ: Δ%= 6.5%; ES= 0.68, 20 m-sprint: Δ%= -0.9% ; ES=-0.46). These changes appear to be mainly related to the volume of strength training since they both responded in proportion to volume. Thus, the higher volume of strength training in (Team A) was associated in the higher testosterone concentration and physical performance. Walker et al. [36] showed a significant increase for the C (Δ%= 74%; ES= 0.9) and the FC level (Δ%= 88.2%; ES= 3.5) after 16 weeks training period. These hormonal changes coincided with performance decline ($VO2_{max}$: Δ%= -6.7%; ES= -0.83; VJ: Δ%= -3%; ES= -0.28). The authors of this study explain that the increase of C and FC and the decrease of performance were associated to higher number of practices and training load increases. Another recent study investigated salivary T changes over a 1-year competitive season in elite under 15 (U15) and under 17 (U17) soccer players [31]. Higher T concentration was observed for the U17 players when compared with the U15 players. There was a decrease in T concentration at the end of the competitive season compared with the beginning of the season for both age categories (U15: Δ%=-28.4%; ES= -0.6, U17: Δ%= -21.1%; ES= -0.4). The reduction in T concentration in both the U15 and U17 groups over a season could be attributed to variation in the training content and perhaps could be related to an inappropriate balance between stress and recovery. Requena et al. [37] showed a small increase in serum T (Δ%= 4.15%; ES= 0.2) after 6 weeks off season period when the volume and intensity of training were greatly reduced as compared to the middle of season. More recently, Andrzejewski et al. [47] investigated anabolic-catabolic hormonal responses during six-month training and competitive periods in youth soccer players. They showed a small increase in C level (Δ%=12.6% ES= 0.43), and a small decrease for T/C (Δ%=-10.5% ES= -0.29), and FT/C ratio (Δ%=-13.8% ES= -0.51). These results could be explained by a fatigue state after 6-month periods of training and competitions. Freitas et al. [49] investigated the effects of a two-week overloading training phase followed by two week tapering phase on salivary C in male young soccer players. They showed a significant decrease in salivary C concentration in weeks 4 when compared with week 1 (Δ%= -35.7%). The magnitudes of

difference (effect size) for C concentration between the first week and week 4 (–0.61) were both moderate. The decrease in salivary C level during the final 2 weeks of the investigation (tapering) indicates an adaptive response of the hypothalamic-pituitary-adrenal (HPA) axis to the reduction in training intensity (RPE: Δ%= -62.5%; ES= -3.8). In conclusion, there is evidence that C and T concentrations were altered (increases or decreases) after long term of training. These alterations could be associated with physical performance changes. The divergence of these results could be explained by different soccer training programs (frequency, duration and intensity) and/or participant's level. Moreover, these hormones appear to be sensitive to intensity and volume of soccer training, and to other factors (fatigue, mood state…) providing an effective tool for monitoring workload and physical fitness.

2.4.3. Acute and long-term effects of soccer training on inflammatory and muscular damage markers

2.4.3.1. Acute and residuals effects of soccer training on inflammatory and muscular damage markers

Acute effects of soccer match and training session on select inflammatory parameters (IL-6, TNF-α, and CRP) and muscle damage markers (CK and LDH) are illustrated in **Table 9**.

Acute soccer match

Romagnoli et al. [35] investigated soccer match effects on IL-6, CRP and CK in professional soccer players. They showed a significant increased (Δ%=92%, ES=0.4) for the IL-6 thirty minutes post-match, to return to pre-match level 24 h and 48 h after the match. CRP concentration did not change immediately after the match and increased significantly 24 h (Δ%=+200%; ES= 1.2) after the match. In addition, the CK concentration increased by 180% immediately after the match and remained significantly elevated 24 h (CK ~ 203 %) and 48 h after the match (CK ~ 134 %). The increased in inflammatory and muscle damage markers were associated to CMJ performance decline immediately (-3.2%), 24 h (-2.6%) and 48 h (+3%) after the soccer match. The study of souglis at al. [17] showed an increased by 440%, 290%, and 240% respectively for the IL-6, CRP, and TNF-α immediately after a soccer match, to return to pre-match value 37h post-match. Additionally, significant increases by 370% and 115% respectively for CK and LDH after the match have been shown. The level of CK and LDH remained elevated 13 h and 37 h post-match in elite soccer players. Anderson et al. [7] investigated changes in inflammatory cytokines in elite female soccer players following

two 90-min games separated by a 72-h active or passive recovery. They found no differences in the IL-6 and TNF-α responses between the active recovery group and the passive group after two soccer game. IL-6 increased significantly after the first soccer game (active group: Δ%= +438%, ES= 3.6, passive group: Δ%= +81%; ES= 2.9) and remained at baseline levels until the beginning of the second game (+45 h post-match). Immediately after the second game IL-6 level increased significantly (active group: Δ%= +171%; ES= 2.8, passive group Δ%= +134.8%; ES= 2). The increase in inflammatory cytokines after the soccer match could be explain by the fact that high physical exercise increased catecholamines and C levels, together with changes in metabolic activities, the occurrence of membrane disruptions in muscle cells and increased free radical production [65, 66] can lead to the activation of the immune system. Additionally, TNF-α increased immediately after the first game (active group: Δ%= 242.8%; ES= 3, passive group: Δ%=178.8%; ES= 8). Another recent study, showed an insignificant change for CRP level immediately after soccer game lasting 90 minutes [55]. Also, the authors of this study demonstrated a significant increase by 68.8% and 25.4% respectively for the CK and LDH immediately after a soccer match in young soccer players. Jamurtas et al. [34] showed a significant increase in CK (Δ%= +201%; ES= 6.5) two hours after the match. In addition, CK was found to be increased 12 h (Δ%= +352%; ES= 7.9), 36 h (Δ%= +561.7%; ES= 11.1), and 60 h (Δ%= +175.7%; ES= 6.33) after the soccer match. The repeated sprint ability (RSA) decreased 2 h (Δ%=4; ES= 1.9), 12 h (Δ%= 5%; ES= 1.5), and 36 h (Δ%= 5.4%; ES= 1.53) after the soccer match. In addition, Gravina et al. [13] observed an increase for CK immediately (Δ%= +62%; ES= 0.8.), and 18 h (Δ%= +121.3%; ES= 0.99) after a match involving elite female soccer players. The LDH concentration increased immediately after the match (Δ%= +28.3%; ES= 1.6) following by a decrease 18 h post-match (Δ%= -15.9%, ES= -1.1). Additionally, the CRP level increased immediately (Δ%= +83.8%; ES= 0.5) and 18 h (Δ%= +166%; ES= 1.1) after the match. These investigators indicated that the eccentric component of movements in football and the number of impacts received by the soccer players during a match, which contribute to muscle breakdown, may be responsible for the observed alteration in inflammatory and muscle damage markers. Another study, investigated the inflammatory responses in male and female soccer players for a period of 48 h after an official match when the average intensity was similar for the two groups [56]. IL-6 increased immediately after the match (male players: Δ%= +390%; ES= 3.9, female players: Δ%= +313%; ES= 2) and remained to pre-match value 24 h and 48 h after the match. Also, the CRP level increased significantly 24 h after the match

(male players: Δ%= +130%; female players: Δ%= +130%). The TNF-α concentration increased immediately after the match (male players: Δ%=+200%; ES= 1.6, female players: Δ%= +149%; ES= 1.4) and remained to baseline level 24 h and 48 h after the match. In the same study, authors showed that CK increased immediately after the match (male players: Δ%= + 112.4; ES= 7.8, female players: Δ%= +80.6%; ES= 3) and peaked 24 h after the match (male players: Δ%= +343.5%; ES= 6.1, female players: Δ%= +415%; ES= 3.8). CK did not return to baseline values 48 h after the match (male players: Δ%= +106%; ES= 2.4, female players: Δ%= +136.5%; ES= 1.9). Thorpe et al. [16] showed a significant increase (Δ%= +84 ± 61%; ES= 0.52) in CK immediately after a soccer match.

Acute training session

In the other types an acute soccer activity (SSG: 4×4 min, 3 min-passive recuperation between sets). Mascarin et al. [43] showed an insignificant change for CK ten minutes after training flowed by a significant decrease 24 h (Δ%= -19.5%; ES= -0.4), 48 h (Δ%= -39.2%; ES= -0.9) and 72 h (Δ%= -53.2%; ES= -0.7) after training session. More recently, Sparkes et al. [15] investigated the CK variations to soccer training session based on small sided games (4 vs 4+goalkeepers; 6×7-min, 2 min inter-set recovery). The CK concentration increased immediately (+40.5%), 2 h (+49.3%) and 24 h (+39.3%) after the soccer match. These CK increases coincided with physical performance decreases (e.g., CMJ) immediately (-3.2%), 2 h (-2.6%) and 24 h (-3%). In addition, Mello et al. [32] observed an insignificant change for the CK concentration between pre-and post-moderate intensity soccer training session in professional soccer players. These findings illustrate that training based on SSG and soccer specific does not seem to produce the same significant alterations showed after a soccer match. We observed that the soccer match induces a greater acute magnitude (match end) of inflammatory and muscle damage markers in reference to soccer training. In addition, the time taken to return to baseline level (pre-match or training session) was higher after the match compared to that after training session. These differences could be explained by difference in mental stress and the amount of effort produced between soccer training session and soccer match.

2.4.3.2 Effects of long-term soccer training on inflammatory and muscular damage markers

Table 10 illustrates that inflammatory and muscle damage markers present a significant alteration over a long period of training. Indeed, Walker et al. [36] showed a significant increased for IL-6 (Δ%= 167.7%; ES=0.8) and CK (Δ%= 134.5%; ES= 0.6) after 16 weeks training period in female professional soccer players. In addition, they showed a decrease in performance (VO_{2max}: Δ%= -6.7%; ES= -0.83; VJ: Δ%= -3%; ES= -0.28). This latter study showed that the increased of IL-6 and CK and the decline of physical performance could be caused by the higher number of practices and training load. Another study investigated inflammatory and muscle damage variations during a soccer season [19]. Authors of this study showed a moderate increase for IL-6 (Δ%= 76.9%; ES= 1.18) and CK (Δ%= 72.2%; ES=1.0) at the end of season. The increase of inflammatory and muscle damage markers were associated to physical performance decline (e.g., T-test: Δ%= -3.8%; ES=-1.2). More recently, Coppalle et al. [9] evaluated effects of two pre-season periods (2014-2015; 2015-2016) on CRP, CK and LDH levels in professional soccer players. Insignificant differences were showed in CRP and CK from the beginning to the end of each pre-season period ($p > 0.05$). However, LDH increased significantly (Δ%= +21.9; ES= 0.9) during the pre-season period (2015-2016). The investigators speculated the small variations in inflammation and muscle damage markers by the little variability between the different training weeks. Additionally, Requena et al. [37] observed that CK remained unaltered after off season period. In contrast, LDH concentration decreased significantly (Δ%= -14.5%; ES= - 1.08). The decrease in LDH after off season period could be related to significant decrease in training volume and intensity. Aquino et al. [59] investigated the effects of 22 weeks periodization training focused in technical–tactical ability on CK and LDH levels. The CK and LDH were significantly reduced ($p<0.05$) throughout the periodization training (CK: Δ%= -65.7%; LDH: Δ%= -60%). The decrement of CK and LHD suggested that the body adapted positively to the training model provided to the athletes. Pimenta et al. [60] showed a significant increase in CK level (Δ%= 565.1%; ES= 9.9) and a significant decrease in VO_{2max} value between the beginning and the end of the pre-season period. In contrast, Silva et al. [61] observed an insignificant change for CK level during 12 weeks training period in professional soccer players. Additionally, Reinke et al. [57] showed that the levels of IL-6, TNF-α, CRP and CK were not altered during 9 weeks training period. Similarly, Heisterberg et al. [8] showed a small decrease of CK level at the end of season (Δ%= -4.45%; ES= -0.4). Likewise,

Andelkovic et al. [6] observed a significant decrease in CK and LDH plasma activity during the 90 days training periods (CK: Δ%= -38.3%; ES= -0.3, LDH: Δ%= -13.2%; ES= -3.3). The CRP increased significantly after 90 days of training (Δ%= +57.1%; ES= 0.4). In elite soccer players, Gharahdaghi et al. [58] investigated effects of one-month high intensity aerobic training in CK and LDH level at rest and after exhaustive exercise. They showed reduction of serum LDH level in rest (Δ%= -12%; ES=-0.7) and exhaustion time (Δ%= -23%; ES= -1.3%). As to CK, the meaningful reduction was (Δ%= -31%; ES= -0.6) and (Δ%= -30%; ES= -0.35) respectively at rest and after exhaustion periods. In conclusion, there is evidence that the different training periods and training protocol could be caused alterations (increases or decreases) in inflammatory and muscle damage markers, which may be used to monitor workload and fatigue in soccer players with different level of expertise.

2.4.4 Relationships between biological markers (hematological, hormonal, inflammatory and muscle damage markers) and physical fitness performance after an acute soccer match or training session.

For the relationships between hematological parameters and physical fitness parameters, we could not do the correlation test as the data was too limited in number **(Table 11)**. For example, only two studies have described associations between hematological and physical performance parameters after acute (match or training session). Romagnoli et al. [35] showed that the increases in plasma volume (Δ%= 7.9%) immediately after a football match was associated with a decrease in CMJ performance (Δ%= -3.2%). In addition, Karakoc et al. [14] found that the decreases in Hb (Δ%= -2.5%) and MCV (Δ%= -3%) were related to increase in lactate concentration after soccer training session (Δ%= 148).

Here in, we demonstrated a significant positive correlation between Δ% T and Δ% CMJ performance after acute training session (r=0.90; p= 0.001).Also, we observed that the increases in CK level was associated to the decline in vertical jump performance (r=-0.99, p=0.0001) **(Table 12)**. It can be speculated that soccer training could influence the increase of inflammation and muscle damage markers [67, 68]. Activity during soccer matches and soccer training include high amounts of jumping, sprinting, and eccentric muscle contractions [69, 70]. This type of muscle activity will result in micro tear of structural and contractile components in the muscle fibers [71] leading to increased CK level in the blood [72]. It can reasonably be supposed that the alteration in CK concentration might affect explosive-type performance.

2.4.5 Relationships between biological markers (hematological, hormonal, inflammatory and muscle damage markers) and physical fitness performance after long-term of soccer training

In the present review, percentage change in hematological parameters was not related to physical fitness performance after long soccer training. These results, however, should be viewed with caution. The number of study and data are limited. In the other hand, we observed a significant positive correlation between FT and T/C with SJ performance **(Table 12)**. The increase in FT and T/C ratio coincided with physical performance improves. A positive association between anabolic hormones (T and FT) changes and performances were showed in several studies [10, 30, 33, 53]. In contrast, C concentration was not related to physical fitness performance. This suggests that it may be more useful to follow variations in T and FT (anabolic hormone) than variations in C (catabolic hormone) to monitor physical performance in vertical jump tests (e.g., SJ and CMJ). No significant correlations were found between inflammatory and muscle damage markers with physical fitness performances. Thus, the increases in CK and/or CRP concentration were associated to the decrease in physical fitness performance (VJ, T-test, and VO_{2max}) in several studies [19, 36, 60]. Also the decrease in CK and LDH concentrations after a long term period of training was associated to the increase in aerobic performance (e.g., VO_{2max}; $\Delta\%= 18\%$) [58]. In contrast, another recent study showed that the decrease in LDH concentrations after off season period were associated to physical performance decreases (e.g., VJ and VAM), [37]. These differences in findings can primarily be explained by psychological factors, players' diet, [9, 15], and/workload parameters.

In addition, the interpretation of the relationship in inflammatory and muscle damage markers with physical fitness performance should be treated with caution too. The relation between these parameters could not be causal; but just a simple coincidence. The performance is multifactorial and the decrease in performance during the study of Requena et al. [37] may be due to a de-training after off-season period. Moreover, increases in CK level without impairments in muscle performance have been reported [67].

2.5 Limitations

The results of the current systematic review have some limitations to note. The studies reviewed exhibited great heterogeneity. In fact, the populations studied differed in terms of sample size, sex, and age and country recruitment. Concerning the studies investigated, the

acute effect (soccer match or training session), some of them, do not precisely report the time point of blood collection at match-end or training session (e.g., 10, 20, 30 minutes post) that may have influenced the observed the level and level of the analyzed biomarkers. In addition, the different protocols used to characterize the games and training sessions performed in different environmental conditions, and with players of different training status and gender, are not well delineated. Additionally, the studies investigated the effect of long-term period of soccer training in biomarkers level, we aggregated different evaluation method for the training load between studies. Soccer training programs varied largely regarding their frequency, duration and intensity. Consequently, large differences were observed regarding the duration and the number of investigations times in the study' protocols. Finally, some studies did not provide information on player dropout rates in response to the study's intervention programs.

2.6. Conclusions

This systematic review of the literature report the effects of acute (soccer match or training session) and long-term of training on hematological, hormonal, inflammatory and muscular damage markers in soccer players. In addition, we investigated the relationship between percentage change in biological markers and physical fitness performance. The current findings show that soccer players display significant alterations in hematological parameters, hormonal, inflammatory and muscle damage markers after acute soccer match play (e.g., soccer match vs. training session). The changes in T and CK concentrations were related to changes in CMJ performance. Additionally, we observed that the long-term of soccer training induced greater alterations (increases or decreases) in hematological, hormonal, inflammatory, muscle damage markers and physical performance. These different alterations could be explained by different factors (e.g., training load, duration of training, psychological factor, mood state). The changes in our select hormonal parameters (FT and T/C) were related to SJ performance alterations. These findings might suggest anabolic hormones and muscle damage markers could be used as a useful tool to identify physical performance alteration after acute and long term of soccer training. Future studies are needed examining the correlations between biomarkers alterations, workload, and psychological factors, thereby attempting to determine to how high degree changes in blood biomarkers are reflected in workload and mood state of soccer players.

2.7. Practical applications

Our review suggests that the biomarkers and physical performance alteration after acute soccer match, acute training session cannot only reflect training intensity and volume applied in the players but also different factor related to recovery strategies. The inadequate recovery after acute soccer exercise activities (e.g., soccer match or training session) and during long training periods could be caused a fatigue state and a critical change of biological markers and physical performance. Medical and technical staffs are called to implements methods that may optimize players' physiological and psychological state. Particularly, evaluating change in anabolic hormone (e.g., testosterone) and muscular damage (e.g., CK) after acute soccer training session seems to constitute the better strategy for monitoring muscle recovery and vertical jump performance (e.g., CMJ). The relationship between the alterations in FT and T/C ratio with SJ performance change reinforce the use of anabolic hormones analyses to assess the vertical jump performance and the recovery of quadriceps and hamstring contractile properties. Thus, training status optimization by including, among others, strength training may be a key to optimize recovery of muscle function and reducing injury risk.

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3 PREAMBULE AUX ETUDES

La revue de la littérature a permis d'examiner les effets aigus (match de football et/ou une séance d'entrainement spécifique au football) de l'entrainement et de compétition sur certains marqueurs biologiques et la performance physique chez des footballeurs de différents niveaux d'expertise. Les paramètres hématologiques, hormonaux, inflammatoires, de dommages musculaires et la performance physique ont été altérés après un match de football et/ou une séance d'entrainement (Romagoli et al., 2016 ; Sparkes et al., 2018). Ainsi, ces altérations pourraient avoir un impact direct sur la diminution de la performance observée au cours des heures et jours qui suivent le match et/ou la séance d'entrainement (Romagoli et al., 2016 ; Sparkes et al., 2018). Plusieurs études ont montré que plus de soixante-douze heures sont nécessaires après le match afin que les marqueurs de performance physique, hématologiques, hormonaux, de dommage musculaires et d'inflammation se normalisent (Silva et al. 2013 ; Jamurtas et al ; 2015 ; Mascarin et al., 2018). **Les interrogations subsistent quant aux effets de la congestion des matchs sur les différents marqueurs biologiques, et les paramètres de performance physique chez les footballeurs élites.**

La revue de la littérature a également examiné les effets à long terme de l'entrainement et de compétition de football sur les marqueurs biologiques et la performance physique. Nous avons observé une altération des paramètres hématologiques, hormonaux, inflammatoires, des marqueurs de dommage musculaire et de la performance physique (Hammami et al, 2017 ; Walker et al., 2017 ; Perroni et al., 2019). Des différences importantes peuvent être observées entre les études quant à l'amplitude des changements (augmentations ou diminutions) mesurés après la période d'entrainement et de compétition. Ces différences s'expliqueraient par une importance variabilité de la durée de l'entrainement, de la charge d'entrainement, de la charge compétitive, et de l'état d'humeur des footballeurs.

Dans cette thèse, ce sont les effets de la congestion des matchs au cours d'une longue période d'entrainement et de compétition qui vont nous intéresser. Par ailleurs, peu d'informations sont actuellement disponibles montrant les effets de la période compétitive congestive sur les marqueurs biologiques, psychologiques et les paramètres de performance physique chez des footballeurs élites. Ainsi, il semble important de clarifier et de comprendre les effets de cette période intensive surtout avec l'augmentation importante de la congestion des matchs durant les différents championnats à cause de la pandémie du COVID-19.

Par conséquent, les trois travaux originaux de cette thèse auront pour objectifs d'étudier les effets la période compétitive congestive sur les marqueurs biologiques, psychologiques, la charge d'entrainement et la performance physique chez des footballeurs élites.

Dans **la première étude,** nous allons nous intéresser à étudier la variation du volume plasmatique, des marqueurs hématologiques, de la charge d'entrainement et de la performance physique après six semaines d'entrainement et de compétition intense (période congestive).

Dans **la deuxième étude,** nous étudierons les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs hormonaux, psychologiques et la performance physique.

Dans **la troisième étude**, nous allons étudier les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs inflammatoires, les dommages musculaires, les états de bien être et la performance physique chez des footballeurs élites.

De plus, les relations entre les marqueurs biologiques, les paramètres psychologiques, la performance physique et la charge d'entrainement vont être examinés.

4 Matériels et Méthodes

Méthodologie générale

Les participants

Vingt quatre footballeurs professionnels masculins (âge: 19 - 22 ans) d'une équipe de football de la première division tunisienne ont participé à cette étude. Au cours de l'étude, quelques joueurs se sont blessés et n'ont pas pu terminer toutes les séances d'entraînement. Les joueurs qui ont un taux de participation inférieur à 85% durant les séances d'entraînement et les matchs ont été exclus de l'étude. Au total, 18 joueurs étaient éligibles pour être inclus dans cette étude. Les joueurs surveillés ont compris trois arrières centraux, trois arrières latéraux, six milieux de terrain et quatre attaquants. Tous les joueurs ont participé à des entraînements de football et à des compétitions nationales pendant au moins 8 ans. Chaque participant a donné son consentement écrit pour participer après avoir été pleinement informé de toute la procédure expérimentale. L'étude a été réalisée conformément à la dernière version de la Déclaration d'Helsinki et a été approuvée par le comité éthique du conseil scientifique de l'université de rennes 2, France et le personnel médical du club de football (Jeunesse Sportive Kairouannaise, JSK).

Procédure expérimentale

L'évaluation des footballeurs a commencé, six semaines après la trêve hivernale. Pendant cette période, les joueurs se sont entraînés cinq fois par semaine et ont joué un match par semaine. Tous les joueurs ont été évalués trois fois au cours de l'étude; à T1, T2 et T3. T1 était prévu au milieu de la période régulière non congestive (semaine 0; début mars). T2 était à la fin de la période congestive (avant la période congestive ; semaine 6; mi-avril). T3 était après la période congestive (semaine 12 ; fin mai jusqu'au début juin – fin de la saison) (voir figure 1).

L'analyse des marqueurs sanguins (hématologique, hormonaux, inflammatoires et de dommages musculaires) et de la performance physique ont été effectués sur trois jours au cours des sessions de test T1, T2 et T3. Le volume plasmatique et les paramètres hématologiques ont été évalués uniquement pendant la période congestive. Le premier jour, des échantillons de sang ont été prélevés pour déterminer les différents marqueurs sanguins au repos. Le deuxième jour, les participants ont effectué trois tests d'évaluation de la performance physique ; un test de répétitions des sprints (repeated shuttle sprint ability, [RSSA]), un test de squat jump (SJ) et un test de saut de contre-mouvement (CMJ). Le troisième jour du test, les participants ont effectué un test intermittent (yoyo intermittent recovery test Level 1 [YYIR1]). Tous les tests d'évaluation physique ont été effectués l'après-

midi, trois heures après avoir pris un repas léger. Les joueurs ont été invités à standardiser et à suivre le même plan nutritionnel 24 heures avant chaque session de test. Les paramètres psychologiques ont également été évalués trois fois au cours de l'étude à l'aide du questionnaire de l'état d'humeur (Profil of Mood state [POMS]), (MacNair et al., 1971). Avant chaque séance d'entraînement et chaque match, l'indice Hopper a été évalué pour surveiller le sommeil, le stress, la fatigue et les douleurs musculaires (DOMS). L'évaluation de l'effort perçu (sRPE; échelle de Borg) à été faite quotidiennement afin de quantifier l'impulsion d'entraînement (TRIMP= sRPE × temps en minutes), la monotonie et la contrainte (Foster et al., 1998).

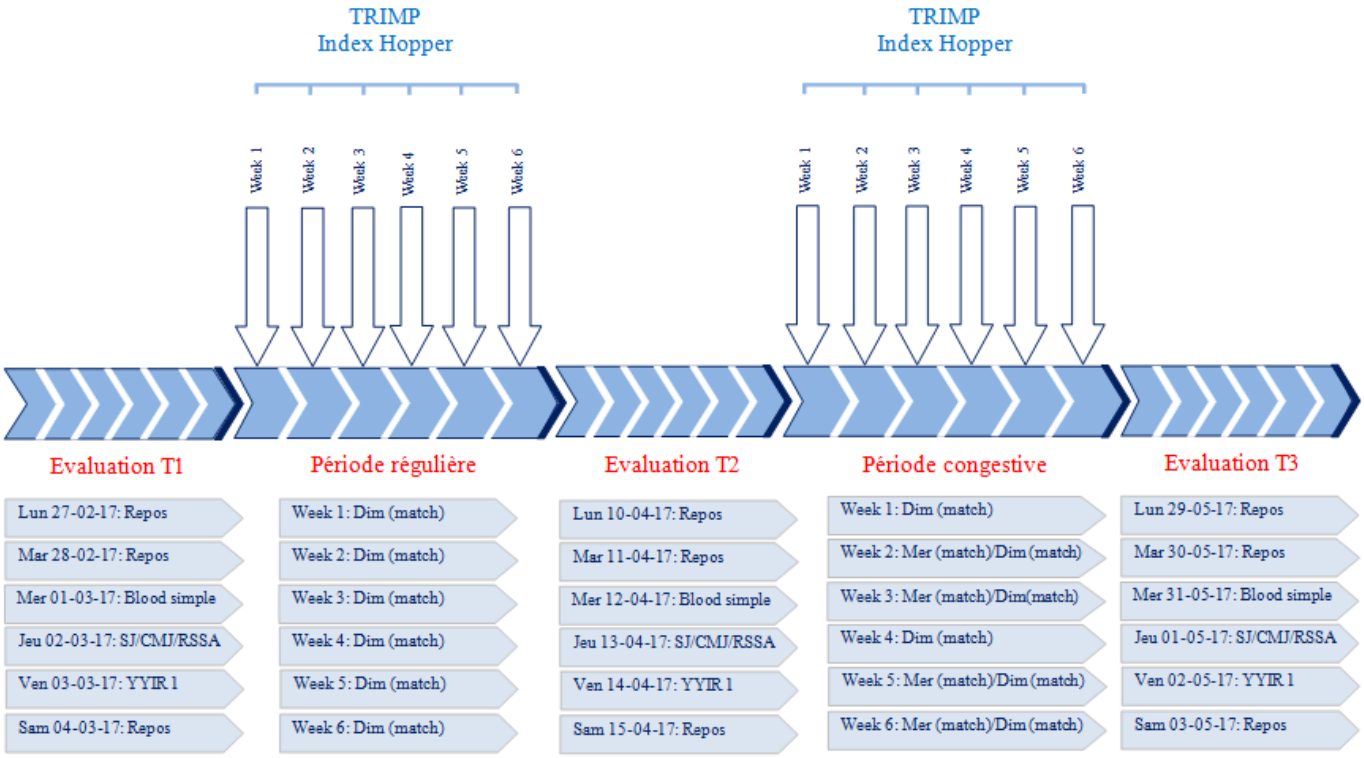


Figure 1. Procédure expérimentale.

Lun: Lundi.**Mar**: Mardi. **Mer**: Mercredi, **Jeu**: Jeudi. **Ven**: Vendredi, **Sam**: samedi, **Dim**: dimanche.**RSSA**: Repeated sprint shuttle ability. **SJ**: Squat jump **CMJ**: Countermovement jump.**YYIR1**: yoyo intermittent recovery test.

Programme d'entraînement pendant la période de compétition non congestive et congestive

Au total, 16 matches ont été joués au cours de la période d'intervention.

La figure 1 résume la distribution des matchs pendant la période non congestive et congestive. Six matches ont été joués pendant les six semaines de la période non congestive, et 10 matches ont été joués pendant les six semaines de la période congestive. Le nombre et la

durée des séances d'entraînement étaient les mêmes pour tous les joueurs et s'élevaient à 56 séances. La durée de chaque séance d'entraînement était de 90 minutes pour tous les joueurs. Les séances d'entrainement ont été réalisées dans l'après-midi (15h30 à 17h00).

Toutes les séances d'entraînement ont été précédées d'un échauffement standardisé de cinq à quinze minutes. La période non congestive comprenait cinq séances d'entrainement par semaine, qui ont eu lieu le mardi, mercredi, jeudi, vendredi et samedi. De plus, un match a été joué le dimanche. Au cours des semaines 7 et 10 de la période congestive, cinq séances d'entraînement ont été programmées par semaine et se sont déroulées le mardi, mercredi, jeudi, vendredi et samedi. Pendant les semaines 8-9-11-12, quatre séances d'entrainement ont été exécutées par semaine ; le lundi, mardi, jeudi et vendredi. De plus, deux matchs ont été joués par semaine le mercredi et le dimanche. Un jour de repos complet était toujours prévu le samedi (voir le tableau 1).

La charge d'entrainement hebdomadaire a été évaluée du lundi au dimanche. Le programme d'entraînement contenait un travail aérobie d'intensité faible/modérée (50-70% de la fréquence cardiaque maximale [FC_{max}]), un entrainement par intervalle de haute intensité (90-100% de la FC_{max}), entrainement de vitesse sur 10-30 mètres (courte distance) et 30-60 mètres (longue distance) avec et sans ballon, entraînement tactique, entraînement technique (c.-à-d., attaque contre défense), jeux réduits, et un entraînement de force.

Le programme d'entraînement de force appliqué pendant la période compétitive était différent en termes de durée et d'intensité par rapport à celui réalisé pendant la période de pré-saison. En effet, les séances d'entraînement de force duraient 25 minutes et se déroulaient une seule fois par semaine. L'entrainement était sous forme d'un circuit training contenant des exercices de résistance de haute intensité et un travail pliométrique pour améliorer la puissance musculaire.

Trois joueurs remplaçants ont eu moins de temps de jeu que le reste de la cohorte. Il est à noter que la charge d'entraînement des joueurs remplaçants a été augmentée le lendemain du match pour atteindre un niveau similaire à celui des joueurs ayant joué tout le temps du match.

Le tableau 1 résume les caractéristiques de l'entraînement pendant la période non congestive et congestive.

Tableau 1. Programme d'entrainement durant la période non congestive et congestive.

Jour	**Programme d'entrainement hebdomadaire durant la période non congestive et congestive en jouant un match par semaine (semaines 1 à 6, et semaines 7-10)**	**Programme d'entrainement hebdomadaire durant la période congestive en jouant deux matches par semaines (semaines 8-9-11-12)**
Lundi	Repos	Echauffement, 15 min Entrainement aérobie d'intensité faible/modéré, 30 min Entrainement technique, 30 min Tactique, 35 min
Mardi	Echauffement, 15 min Entrainement technique, 30 min Entrainement aérobie d'intensité faible/modéré, 25 min Jeux réduit, 15 min	Echauffement, 15 min Entrainement tactique, 30 min Entrainement intermittent à haute intensité, 10 min, Jeux réduit, 15 min
Mercredi	Echauffement, 15 min Entrainement de force, 30min Entrainement technique et tactique, 20 min Jeux réduit, 15 min	Match
Jeudi	Echauffement, 15 min Entrainement technique, 30 min Entrainement de vitesse (courte distance), 20 min Jeux réduit, 15 min	Entrainement aérobie d'intensité faible/modéré, 40 min Entrainement spécifique, 30 min
Vendredi	Echauffement, 15 min Entrainement technique, 30 min Entrainement de vitesse (long distance), 20 min Entrainement spécifique, 20 min	Echauffement/ technique, 25 min Entrainement de vitesse (courte distance), 15 min Jeux réduit, 25 min
Samedi	Echauffement/technique, 25 min Entrainement de vitesse (courte distance), 20 min Entrainement spécifique, 30min	Repos
Dimanche	Match	Match

min:minutes

Les mesures anthropométriques

La masse corporelle à été mesurée à l'aide d'une balance mécanique (Mi-525, Médical robe-France). Les participants portent des vêtements légers et sans chaussures. La taille en (m) a été déterminée à l'aide d'un ruban de mesure fixé à un mur. La masse corporelle a été

appréciée à 0,1 kg prés et la taille à 0,5 cm prés. L'indice de masse corporelle (IMC) a été calculé (Kg/m²).

Le pourcentage de graisse corporelle (MG%) a été estimé par la mesure d'épaisseurs des 4 plis cutanés (bicipital, tricipital, sous-scapulaire, supra-iliaque) à l'aide d'un caliper (CZ-Store, France). La masse maigre (MM à été calculé à l'aide de l'équation de Forsyth et al. (1973):$MM(kg) = Masse\ corporelle - (MG\ [\%] \times Masse\ corporelle\ /100)$

Les prélèvements sanguins

Pour chaque joueur de football, des échantillons de sang ont été prélevés au même moment de la journée (entre 08h00 et 10h00) pour déterminer les paramètres hématologiques, hormonaux, inflammatoires, et de dommages musculaires. Une période de repos-récupération de 12 h était prévue avant la veille du prélèvement.

Le jour du test, 15 ml de sang ont été prélevés en position assise via la veine ante brachiale médiane dans des tubes vacutainer (Vacuette®, Greiner bioOne, France) sans anti-coagulant et renfermant de l'EDTA (éthylènediaminetétracétique). Le sang a été immédiatement centrifugé à 30 000 tours/minutes pendant 10 minutes pour éliminer le plasma. Le plasma a été stocké à -80 °C jusqu'aux moments des différents dosages. Tous les échantillons ont été prélevés et manipulés dans un seul laboratoire.

Analyse du sang

Les paramètres hématologiques

Les paramètres hématologiques; érythrocytes (M/mm3), hématocrite (Ht [%]), hémoglobine (Hb [g/dl]), volume globulaire moyen (VGM [fl]), teneur corpusculaire moyenne en hémoglobine (TCMH [pg]) et la concentration corpusculaire moyenne en hémoglobine (CCMH [%]), ont été déterminé par la technique de cyométrie en flux (Celltac ES, Japon).

L'hématocrite et l'hémoglobine mesurés ont été utilisés afin de calculer la variation du volume plasmatique (ΔVP) exprimés en (%):

$$\%\Delta VP = 100 \times \left[\frac{Hb_B}{Hb_A} \times \frac{(1-Ht_A \times 10^{-2})}{(1-Ht_B \times 10^{-2})}\right] - 100$$ (Dill et costill., 1974)

Où B désigne les valeurs d'Hb et d'Ht au repos (avant la période congestive), et A désigne les valeurs d'Hb et d'Ht après la période congestive.

Les paramètres hormonaux

Les concentrations du cortisol et de testostérone plasmatique ont été analysés avec des kits de test commerciaux VIDAS (réf.30418) (kit Vidas®, France) en utilisant la technique ELFA (Enzyme Linked Fluorescent Assay).

Les paramètres inflammatoires et de dommages musculaires

La protéine C-réactive (CRP) a été déterminée en utilisant la méthode d'immunoturbidimétrie (système CRP Latex / Beckman Coulter AU 480). L'activité de la créatine kinase a été déterminée par la méthode UV (IFCC) en utilisant la N-acétyl-cystéine (EC 2.7.3.2/ Beekman Coulter, système AU 480). La créatinine a été déterminée par une modification cinétique de la procédure Jaffe, dans laquelle la créatinine réagit avec l'acide picrique à pH alcalin pour former un complexe jaune orangé (OSR6678 / Beckman Coulter, AU 480).

Tests d'évaluation de la performance physique

Le Yo-Yo intermittent recovery test level 1 [YYIRTL1]

Le YYIRT1 a été utilisé pour évaluer la capacité des joueurs de répéter des efforts de haute intensité (Bangsbo et al., 2008). Pour ce faire, des cônes ont été placés sur deux lignes distantes de 20 m et 5 m derrière la ligne de départ. Les joueurs ont exécuté deux navettes de 20 m après un signal sonore, séparées par une période de récupération de 10 secondes. Le temps entre les bips a diminué au cours des paliers et les joueurs ont continué le test jusqu'à ce qu'ils soient incapables de maintenir le rythme requis pour deux bips successifs. Les joueurs ont été invités à placer un pied sur ou derrière la ligne de 20 mètres à la fin de chaque navette. La vitesse finale (km/h) atteinte à la fin du test et la distance totale parcourue (m) ont été enregistrées pour une analyse approfondie.

Repeated Shuttle Ability Sprint test [RSSA]

Le test RSSA a été utilisé pour évaluer la capacité des footballeurs de répéter des sprints. Ce test comprend un changement de direction, ce qui représente un modèle de mouvement spécifique en football. Après un échauffement de 15 minutes, les joueurs ont effectué 6×40 sprints maximum entrecoupés de 20 secondes de récupération passive. Le meilleur temps de sprint a été enregistré pour une analyse plus approfondie ($RSSA_{best}$). En outre, les variables dépendantes comprenaient le temps moyen obtenu durant les six sprints ($RSSA_{mean}$) et la décrémentation de la performance : $RSSAdecrement\ (\%) = ([RSSAmean]/[RSSAbest] \times 100) - 100$, (Impellizeri et al., 2008)

La concentration de lactate sanguin a été déterminée à la fin du test en utilisant un analyseur portable de lactate (Lactate Pro2, Matsport, France).

Les tests de contermovement jump (CMJ) et de squat Jump (SJ)

Le SJ vise à mesurer la détente sèche non pliométrique et sans étirement : le sujet commence donc le test en position fléchit à 90° (articulation des genoux), pour effectuer une poussée maximale vers le haut. Les mains sont sur les hanches pour éviter une participation des bras.
Le CMJ s'effectue dans les même conditions, mais cette fois le sujet est autorisé à effectuer une flexion préalable à l'extension (il s'agit donc, cette fois d'un test pliométrique). La différence entre la CMJ et le SJ rend compte de la qualité élastique du muscle et de la coordination du sujet. Le CMJ, peut s'effectuer également avec l'aide des bras, ce qui permet une action d'étirement musculaire supérieure, mais ce qui exige également une bonne coordination des bras. Tous les joueurs ont effectué trois sauts verticaux dans le même ordre (SJ suivi de CMJ) avec un repos de 60 s entre chaque essai. La moyenne des trois meilleurs essais a été prise pour une analyse plus approfondie (Claudino et al., 2017).

Quantification de la charge d'entraînement

La charge d'entraînement a été quantifiée quotidiennement en utilisant l'évaluation de l'effort perçu (RPE) à l'aide de l'échelle de Borg 0-10 adaptée (Foster et al., 1998). Pour s'assurer que l'évaluation de l'effort perçu reflète la totalité de la séance, les données ont été collectées environ quinze à vingt minutes après chaque séance. L'impulsion d'entraînement (TRIMP) a été calculée: sRPE × temps (min) pour chaque séance. La monotonie a été calculée comme la charge hebdomadaire moyenne divisée par l'écart-type de la charge. La contrainte a été calculée comme la charge d'entraînement multipliée par la monotonie (Foster et al., 2001). Il a déjà été suggéré que ses indices étaient négativement liés au surmenage et/ou au surentraînement (Foster et al., 2001).

Tests psychologiques et évaluation des indicateurs du bien être

Le questionnaire de Profile of Mood State (POMS) (profil des états d'humeur)

Le POMS comporte soixante-cinq items repartis pour évaluer les six états d'humeur des footballeurs pendant la période expérimentale ; la tension, la dépression, la colère, la fatigue, la confusion la vigueur. Le score totale de la perturbation de l'humeur « Total Mood Disturanbance (TMD)» est mesuré en ajoutant toutes les autres mesures et en soustrayant la vigueur (McNair et al., 1971). Ce questionnaire a été utilisé pour surveiller les états d'humeur

et la performance des athlètes dans plusieurs sports d'équipe (Filaire et al., 2001, Hoffman et al., 1999).

Le questionnaire de Hopper

Le questionnaire Hooper, composé de 4 items (stress, fatigue, sommeil et DOMS); Hopper et Mackinnon, 1995) a été administré chaque matin avant le début des séances d'entraînement. L'échelle varie de 1 (très, très faible) à 7 (très, très élevé) pour les scores de stress, fatigue et DOMS. Dans le cas spécifique de la qualité du sommeil, 1 = très, très bon et 7 = très, très mauvais. Le bien étre géneral ou l'indice Hooper est la somme des 4 scores. Le même chercheur a appliqué le questionnaire et les réponses ont été enregistrées individuellement. La somme des valeures hebdomadaires pour chaque période de compétition (période non congestive et congestive) a été calculée et utilisée pour des analyses ultérieures.

Analyses statistiques

Les données sont exprimées en moyennes et écarts types (ET). La distribution normale des données a été testée et confirmée à l'aide du test de Kolmogorov-Smirnov. Des tests t appariés pour des échantillons dépendants ont été utilisés pour identifier les différences dans les paramètres de charge d'entrainement (TRIMP, monotonie, contrainte) entre la période non congestive et congestive. Les données ont été exprimées sous forme de moyennes et écarts types (ET). Une analyse unidirectionnelle de la variance (ANOVA) pour des mesures répétées a été calculée pour déterminer les différences entre tous les paramètres mesurés au cours de la période non congestive vs période congestive. Les relations entre les paramètres ont été évaluées à l'aide du coefficient de corrélation produit-moment de Pearson (r). L'ampleur des coefficients de corrélation a été considérée comme triviale ($r < 0,1$), petite ($0,1 < r < 0,3$), modérée ($0,3 < r < 0,5$), grande ($0,5 < r < 0,7$), très grande ($0,7 < r < 0,9$), presque parfait ($0,9 < r < 1$), et parfait ($r = 1$), (Hopkins et al., 2009). La signification statistique de toutes les analyses est acceptée pour $p < 0,05$. Les intervalles de confiance (IC) à 95% et la taille d'effet (ES) ont été calculés pour comparer la différence des valeurs moyennes pour tous les paramètres analysés au cours des différentes périodes d'entraînement et de compétition (non congestive vs. congestive). Lors du calcul de la taille d'effet, les écarts-types groupés (SD) ont été utilisés car aucun groupe témoin n'était disponible ; (Cohen's $d = (M1-M2) / SD_{pooled}$). Les valeurs de la taille d'effet (ES); 0,2, 0,5 et 0,8 ont été considérés respectivement comme ; légère, moyenne et large (Cohen, 1988). La significativité est accepté pour $p < 0,05$.

5 ETUDES

ETUDE 1

Effet d'une période compétitive congestive de six semaines sur la variation du volume plasmatique, les paramètres hématologiques, la charge d'entrainement, et la performance physique chez des footballeurs élites

Effects of a six-week period of congested match play on plasma volume variations, hematological parameters, training workload and physical fitness in elite soccer players

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Résumé

Objectifs: Les objectifs de cette étude étaient d'évaluer les effets de six semaines d'entraînement et de compétition intense (période congestive) sur la variation du volume plasmatique (VP), les paramètres hématologiques, et la performance physique chez des footballeurs élites. De plus, nous avons analysé les relations entre la charge d'entraînement, les paramètres hématologiques et les paramètres de performance physique chez des footballeurs élites.

Méthodes: Dix-huit footballeurs élites ont été évalués avant (T1) et après (T2) une période d'entrainement et de compétition de six semaines contenant dix matchs de football. A T1 et T2, les joueurs ont effectué les tests physiques suivants: Yo-Yo intermittent recovery test level 1, [YYIR1], repeated shuttle sprint ability, [RSSA]), squat jump (SJ) et saut en contre mouvement jump [CMJ]. De plus, la variation de volume plasmatique (VP [%]) et les paramètres hématologiques : les érythrocytes [M/mm3], l'hématocrite (Ht [%]), l'hémoglobine (Hb [g/dl]), le volume globulaire moyen (VGM [fl]), la teneur corpusculaire moyenne en hémoglobine (TCMH) [pg]) et la concentration corpusculaire moyenne en hémoglobine (CCMH [%]) ont été évalués. L'évaluation quotidienne de l'effort perçu (RPE) a été utilisée afin de quantifier la charge d'entraînement interne.

Résultats: De T1 à T2, une baisse significative de la performance a été observée dans les tests; YYIR1 ($p<0,001$, taille d'effet [ES]= 0,5), RSSA ($p<0,01$; ES= 0,6) et SJ ($p<0,046$, ES= 0,7). Cependant, aucun changement significatif de la performance n'a été observé dans le test CMJ ($p= 0,86$; ES= 0,1). Le lactate sanguin mesuré après le test RSSA ($p < 0,012$, ES= 0,2) et le VP ($p<0,01$, ES= 0,7) a été augmenté de manière significative. Une diminution significative a été observée pour la valeur érythrocytaire ($p<0,002$; ES= 0,5) et la concentration d'hémoglobine ($p<0,018$; ES= 0,8) en T2 par apport à T1. Le taux d'hématocrite était significativement plus faible ($p<0,001$; ES= 0,6) en T2 par apport à T1. Le volume globulaire moyen, la teneur corpusculaire moyenne en hémoglobine et la concentration corpusculaire moyenne en hémoglobine n'étaient pas statistiquement différents entre T2 et T1. Aucune relation significative n'a été observée entre les paramètres de la charge d'entraînement et le taux de variation (Δ%) des paramètres hématologiques. Cependant, une relation significative a été observée entre la charge d'entraînement et les changements de la performance en RSSA ($r= -0,60$; $p <0,003$).

Conclusions: Une période compétitive intensive de six semaines a considérablement compromis la performance physique des footballeurs élites. Ces changements n'étaient pas liés aux altérations des paramètres hématologiques.

Mots clés: entrainement, hématologie, fatigue, surentrainement, récuperation

Abstract

Objectives: The aims of this study were to investigate the effects of a six-week in-season period of soccer training and games (congested period) on plasma volume variations (PV), hematological parameters, and physical fitness in elite players. In addition, we analyzed relationships between training load, hematological parameters and players' physical fitness.

Methods: Eighteen elite players were evaluated before (T1) and after (T2) a six-week in-season period interspersed with 10 soccer matches. At T1 and T2, players performed the Yo-Yo intermittent recovery test level 1 (YYIR1), the repeated shuttle sprint ability test (RSSA), the countermovement jump test (CMJ), and the squat jump test (SJ). In addition, PV and hematological parameters (erythrocytes [M/mm3], hematocrit [%], hemoglobin [g/dl], mean corpuscular volume [fl], mean corpuscular hemoglobin content [pg], and mean hemoglobin concentration [%]) were assessed. Daily ratings of perceived exertion (RPE) were monitored in order to quantify the internal training load.

Results: From T1 to T2, significant performance declines were found for the YYIR1 ($p < 0.046$; ES = 0.7). However, no significant changes were found for the CMJ ($p = 0.86$, ES = 0.1). Post-exercise, RSSA blood lactate ($p < 0.01$; ES= 0.7) increased significantly from T1 to T2. A significant decrease was found from T1 to T2 for the erythrocyte value ($p<0.002$, ES = 0.5) and the hemoglobin concentration ($p < 0.018$, ES = 0.8). The hematocrit percentage rate was also significantly lower ($p < 0.001$, ES = 0.6) at T2. The mean corpuscular volume, mean corpuscular hemoglobin content and the mean hemoglobin content values were not statistically different from T1 to T2. No significant relationships were detected between training load parameters and percentage changes of hematological parameters. However, a significant relationship was observed between training load and changes in RSSA performance ($r = -0.60$; $p < 0.003$).

Conclusions: An intensive period of “congested match play” over 6 weeks significantly compromised players’ physical fitness. These changes were not related to hematological parameters, even though significant alterations were detected for selected measures.

Key words: training, hematology, overtraining, overreaching, recovery

Introduction

In contemporary professional soccer, teams are confronted with a large number of matchesthroughout the season, including league, cup,and international matches [1].These tight timetables frequently demand that teams play consecutive matches with less than three to four days of recovery in-between. It has been recommended that the recovery period between matches should be at least 72 h to avoid injuries and excessive residual fatigue [2]. Previous studies examined the effects of such a "congested period" of match play on physical and physiological performances in elite soccer players [3]. These authors demonstrated that running activity wasnegatively affected during match congestion periods. Of note, skill-related performance can also be affected by a decline in soccer players running capacity [4,5]. These studies provide important insight into the demands of congested match play, but they are limited in scope and few in number. Therefore, more research is warranted to better examine the negative effects of different congested periods on physical fitness, technical and tactical performance in elite soccer players. Furthermore, little is known on how an intensified in-season soccer period affects different physiological markers such as hematological parameters and how these parameters may affect and explain the impairment of physical performance during periods of match congestion in soccer players.

Excessive Physical exercise may influence body homeostasis and may result in impaired immune function and changes in hematological variables. This could lead to signs of overreaching which ultimately affects physical performance. To avoid injury, and to protect health and at the same time optimizing training in terms of performance development, it is mandatory to monitor players' physical fitness together with physiological markers during different periods of a soccer season. More specifically, blood samples could be taken to evaluate changes in hematological variables (e.g., plasma volume, Hematocrit, Hemoglobin) [6].

It is well known that most common hematological variables (e.g., plasma volume [PV], hemoglobin [Hb], hematocrit [Ht], and Red blood cells [RBC] count are linked to the development of aerobic capacity [7]. Previous research suggested that physical stress affects PV and hematological parameters, which may negatively influence players' physical performance. [8,9]. PV variation (PVV) is considered a form of body fluid adaptation in response to different training loads [10] and exercise intensity [11]. For example, Hbvalues determine the oxygen transport and consumption [12], which is linked to physical performance through aerobic capacity [13]. Ht levels are usually associated with

enhancements in oxygen transport capacity [14]. The main function of Red blood cells [RBC] is oxygen transport and consumption. Thus, the longitudinal monitoring of Hb, Ht and Red blood cells [RBC] over 6 months revealed increases in hematological parameters at the end of the pre-season period in professional football [6]. According to Silva et al. [15], Ht was significantly increased after 3 months of training while it was decreased during the more intense periods of training in Brazilian soccer players. Moreover, Heisterberg et al. [16] monitored these hematological parameters in players from the best Danish league, and found a decrease in Hb and Ht as the competitive season progressed. In addition, the highest value of Ht and the lowest value of Hb were obtained after a period characterized by frequent matches [16]. Blood parameter variations are related to the amount of aerobic and anaerobic training, strength training, and the number of matches per week [16]. Given the importance of these measurements for physical fitness, it is imperative to better understand the concurrent effects of a congested period of matches on physical and hematological parameters fora better management of training and competitive workloads.

To our knowledge, no studies have examined PVV and hematological parameters of elite soccer players from the same team, before and after acongested period of match play. In addition, there are no studies available that quantified the relationships between training load parameters, hematological parameters and physical fitness. Thus, the aims of this study were twofold: 1) to investigate the effects of a congested fixture period on PV, hematological parameters, and physical fitness in elite soccer players; and 2) to examine therelationships between training load, hematological parameters and physical fitness of elite soccer players. It was hypothesized that successive matches played during a short time period could negatively affect PV, hematological parameters which may have an impact onplayers' physical fitness [1, 16].

METHODS

Eighteen elite Tunisian soccer players with a mean age of 20.1 ± 0.4 years from the same first division Tunisian football team volunteered to participate in this study. Participants included three central backs, four fullbacks, seven midfielders, and four attackers. Thirteen players performed all matches. The other 5 players trained with the first team and played their matches with the reserve team. The first and the reserve team had the same match schedule.

All players were evaluated before and after a period of match congestion during the season's play-off period. The first test session (T1) took place inthe middle of April, while the second

test session (T2) was performed at the end of the season (late May until early June), after 6 weeks of competitions. Evaluation of hematological parametersand physical fitnesswere performed over three-days. On the first day, a blood sample was collectedto determine resting plasma volume (PV) and hematological parameters. On the second day, participants performed three physical fitness tests in the following sequence: squat jump test (SJ), countermovement jump test (CMJ), and repeated shuttle sprint ability test (RSSA). On the third day, participants performed theYo-Yo intermittent recovery test level 1 (YYIR1).

The detailed description of the protocol can be found in the section dedicated to general methodology of this document.

Statistical analyses

Data were expressed as mean values and standard deviations (SD). All statistical tests were processed using SigmaStat 3.5 software (Systat, Inc, USA). Normal data distribution was tested using the Kolmogorov-Smirnov test and confirmed. Paired t-tests for dependent samples were used to compare all parameters measured before and after the period of match congestion. A one-way analysis of variance with repeated measures (ANOVA) was computed to determine the difference between the weekly training load during the period of match congestion. Relationships between parameters were assessed using Pearson's product-moment correlation coefficients(r). The magnitude of the correlations was defined according to Hopkins's scale (Hopkins et al., 2009): r<0.1, trivial; 0.1–0.3, small; >0.3–0.5, moderate; >0.5–0.7, large; >0.7–0.9, very large; >0.9, nearly perfect; and 1, perfect. Statistical significance for all analyses was set at $p < 0.05$. The 95% confidence intervals (CI) and effects sizes (ES) were calculated to compare differences in mean values for all analyzed parameters before and after the six-week in-season period. When calculating ES, pooled standard deviations (SD) were used because no control group was available (Cohen's d= (M1-M2)/SD_{pooled}). ES with values of 0.2, 0.5, and 0.8 were considered small, medium and large, respectively [24].

RESULTS

Anthropometric data

Changes in anthropometrics are illustrated in Table 2. No significant pre-to-post differences were found for these measures: body height (p=0.2; ES=0.3), body mass (p=0.1; ES=0.3), BMI (p=0.06; ES=0.4), BF (p=0.1; ES=0.1), LBM (p=0.4; ES=0.1).

Table 2. Effects of a congested period of match play on soccer players' body height, body mass index (BMI), body fat percentage (BF%), and lean body mass (LBM).

Anthropometric measures	Pre testing (T1)	Post testing (T2)	p-Value	Effect size (ES)
Body height (m)	1.78± 0.04	1.78± 0.05	0.3	0.2
Body mass(kg)	72.6± 5.1	72.2± 5.1	0.1	0.3
BMI(kg/m²)	22.7± 1.19	22.6± 1.14	0.06	0.4
BF(%)	8.2± 2.9	8.5± 2.4	0.1	0.1
LBM (kg)	66.6± 4.3	66.1± 4.7	0.4	0.1

BMI: Body mass index, **BF**: Body fat, **LBM**: Lean body mass.

Physical fitness data

In general, physical fitness declined over the 6-week in-season period (Table 3). More specifically, significant decreases were found for the YYIR1 (p≤0.05; ES=0.5), the RSSA (p≤0.05; ES=0.6) and the SJ (p≤0.05; ES=0.5). In contrast, CMJ performance did not change significantly (p=0.86). Blood lactate after the RSSA test increased significantly from T1 to T2 (p=0.04; ES=0.5).

Table 3. Changes in performance in physical tests after the period of match congestion.

Fitness measures	Pre testing (T1)	Post testing (T2)	p-Value	Percentage change	Effect size (ES)
YYIR1 (m)	2520±362.6	1640±326.6	<0.001*	34.9%	0.5
$RSSA_{mean}$ (s)	8.07±0.3	8.28±0.3	0.01*	2.6%	0.6
$RSSA_{best}$ (s)	7.4±0.4	7.7±0.4	0.001*	3.8%	0.7
$RSSA_{decrement}$(%)	7.8±4.7	6.6±3.1	0.3	0.06%	0.2
$[Lac]_{RSSA}$(mM/L)	18.2±3.2	20.3±2.3	0.04*	11.5%	0.5
SJ (cm)	36.5±3.8	35.5±3.9	0.01*	2.5%	0.5
CMJ (cm)	39.0±3.6	39.2±2.4	0.8	0.4%	0.1

YYIR1: Yo-Yo intermittent recovery test Level 1, **RSSA**: repeated sprint shuttle ability, **SJ**: squat jump, **CMJ**: countermovement jump.

Training load

Total weekly training load (TWTL), the monotony, and the strain over the six training weeks are illustrated in Figures (2A, 2B, 2C). The TWTL of the 6th week was higher than the TWTL during the first 4 weeks (p≤ 0.01), **(Fig 2A).** The monotony of the 6th week was higher than that of the 1stweek, the 3rdweek and the 5th week (p≤ 0.01).The monotony during the 3rd week, the 4th-week, and the 5th week were higher than that during the 1st week (p≤ 0.01) **(Fig 2B).**The strain during the 6th week was higher than that during the 1st, 2nd, 3rd, 4th, and 5th week (p≤ 0.01). The strain of the 3rd, 4th and 5th week was higher than that during the 1st week (p≤ 0.01) **(Fig 2C).**

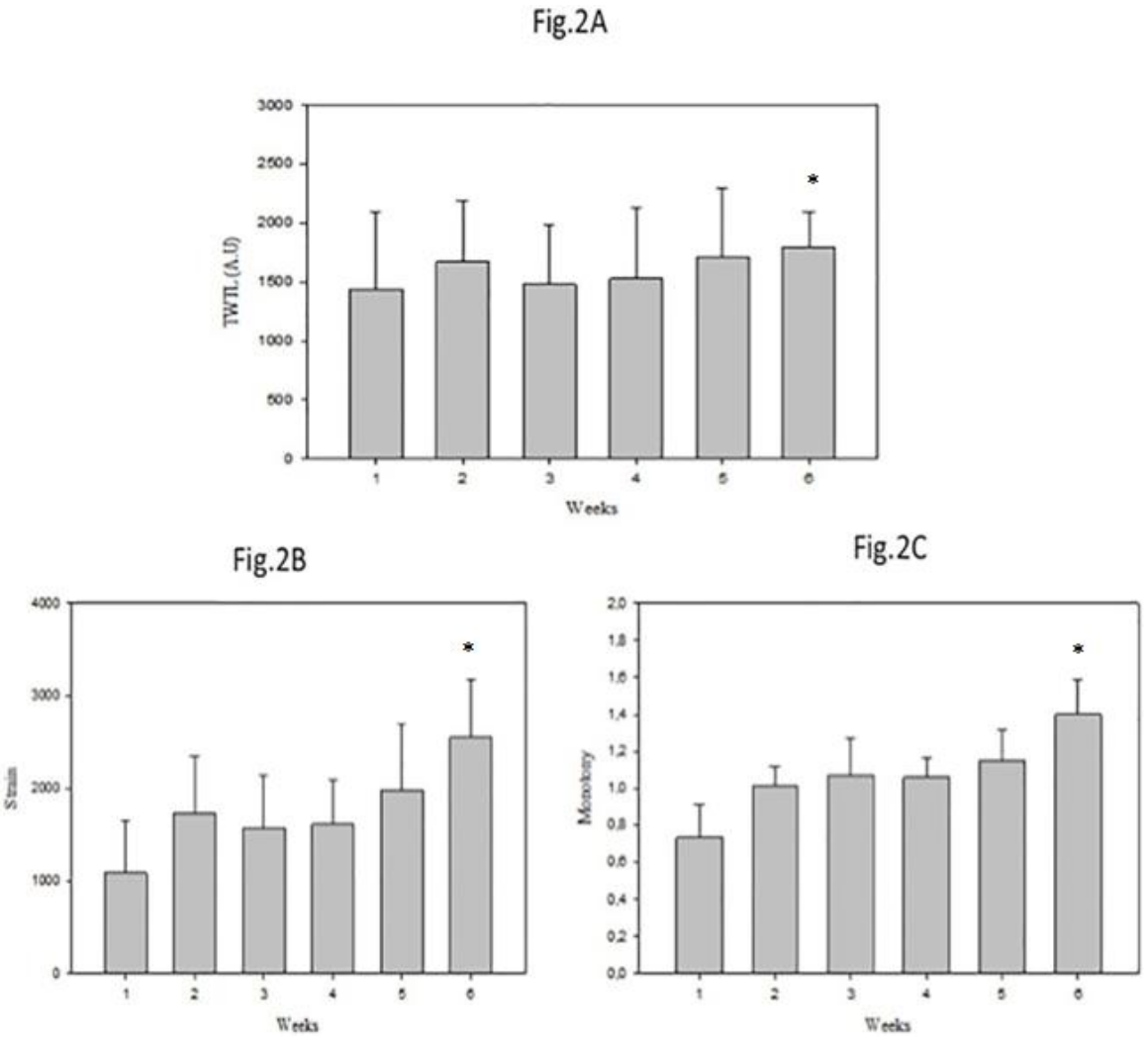


Figure 2. Total weekly training load (TWTL) (Fig 2A), the monotony (Fig 2B) and the strain (Fig 2C) of the six weeks training during the match congestion period.

*= Significant differences compared to week 1, week 3, and week 4 (p< 0.01). **TWTL** = Total weekly training load; A.U. = arbitrary units, monotony and strain.

Hematological data and plasma volume variation after the period of congested match play

Table 4 summarizes data of all analyzed hematological parameters. A significant decrease was found from T1 to T2 for erythrocyte count (p=0.018; ES=0.5), Hb (p=0.002; ES=0.8), and Ht percentage (p=0.006; ES=0.6). MCV, MCHC, and MHC values did not change significantly over the experimental period. PV increased significantly from T1 to T2 (6.1± 8.7%; p=0.01; ES= 0.5).

Table 4. Effects of the period of match congestion on soccer players' hematological parameters

Hematological parameters	**Pre-testing (T1)**	**Post-testing (T2)**	**p-Value**	**Effect size(ES)**
Erythrocytes (M/mm³)	5.3± 0.4	5.1± 0.3	0.018*	0.5
Hemoglobin (g/dl)	14.9± 1.04	14.3±0.7	0.002*	0.8
Hematocrit (%)	43.9±2.7	42.5±2.07	0.0006*	0.6
MCV(fl)	83.1±4.5	82.9±4.5	0.1	0.3
MCHC(pg)	28.05±1.7	28.09±1.6	0.4	0.1
MHC (%)	33.9±0.7	33.7±0.6	0.1	0.3
PVV (T1-T2) (%)	6.1±8.7%		0.01**	0.7

MCV: Mean Corpuscular Volume, **MCHC**: Mean Corpuscular Hemoglobin Content, **MHC**: Mean hemoglobin concentration, PVV (T1-T2): Plasma volume variation between T1 and T2; **YYIR1**: Yo-Yo intermittent recovery test Level 1, **RSSA**: repeated sprint shuttle ability, SJ: squat jump, CMJ: countermovement jump.

* = Significant difference between T1 (before the period of match congestion) and T2 (after the period of match congestion).

Relationships between PVV, hematological parameters, physical fitness, and training load parameters

Table 5 shows that there were no significant correlations between PVV, Δ% of hematological parameters, and Δ% of physical fitness. There were significant positive correlations between Δ% of SJ and Δ% of Ht (r=0.54; p=0.02), MCV (r=0.51; p=0.01), and MCHC (r=0.62; p=0.001) (Figure 3). In addition, there was a significant negative correlation between percentage change of SJ and PVV(r=-0.43; p=0.03).

Table 5. Relationships between PVV, percentage changes of hematological parameters and percentage change of physical fitness.

Hematological parameters / physical fitness	**YYIR1**	**RSSA mean**	**SJ**
Hemoglobin (g/dl)	p=0.12; r=0.21	p=0.08; r=0.15	p=0.07; r=-0.4
Hematocrit (%)	P=0.11; r=0.19	P=0,15; r=0.21	p=0.02*; r=0.54
PVV (%)	p=0.9; r=-0.01	p=0.4; r=0.20	p=0.03*; r=-0.42
Erythrocytes(M/mm³)	p=0.8; r=0.04	p=0.4; r=-0.12	r=0.4; p=0.06
MCV (fl)	p=0.6; r=0.15	p=0.3; r=-0.22	p=0.01*; r=0.51
MCHC (pg)	P=0.5; r=-0.12	p=0.3; r=-0.20	p=0.001*; r=0.64
MHC (%)	p=0.6; r=-0.14	p=0.2; r=-0.25	p=0.1; r=0.32

MCV: Mean Corpuscular Volume, **MCHC**: Mean Corpuscular Hemoglobin Content, **MHC**: Mean hemoglobin concentration, **PVV (T1-T2):** Plasma volume variation between T1 and T2. **YYIR1**: Yo-Yo intermittent recovery test Level 1, **RSSA**: repeated sprint shuttle ability, **SJ**: squat jump, **CMJ**: countermovement jump.*****Significant relationship between parameters.

Table 6 illustrates that there wasno significant relationship between training load parameters and Δ% of hematological parameters (Hb [r=0.12; p=0.6], Ht [r=-0.02; p=0.9], PVV [r=0.12; p=0.6], erythrocytes [r=-0.22; p=0.2], MCV [r=-0.05; p=0.9], MCHC [r=0.22; p=0.2], MHC [r=0.41; p=0.08]). There were no significant relationships between monotony and Δ% of hematological parameters (Hb [r=0.3; p=0.1], Ht [r=-0.12; p=0.6], PVV [r=-0.14; p=0.6], erythrocytes [r=-0.34; p=0.1], MCV [r=-0.12; p=0.6], MCHC [r=0.3; p=0.1], MHC [r=0.32; p=0.1]).

Moreover, there were no significant relationships between strain and Δ% of hematological parameters (Hb [r=0.14; p=0.5], Ht [r=-0.05; p=0.8], PVV [r=0.18; p=0.6], erythrocytes [r=-0.34; p=0.1], MCV [r=-0.08; p=0.7], MCHC [r=0.30; p=0.2], MHC [r=0.42; p=0.08].

There was no significant relationship between performance in the YYIR1 and training load parameters (r=0.14; p=0.5), monotony (r=-0.01; p=0.9), and strain (r=0.12; p=0.6).There was, however, a significant relationship between training load and strain, with RSSA (r=-0.64; p=0.003; r=-0.63; p=0.005, respectively).

Table6. Relationships between PVV, percentage changes of hematological parameters physical fitness, and workload parameters.

Hematological parameters/ Training load	**TWTL(Total Week Training Load)**	**Monotony**	**Strain**
Hemoglobin	p=0.6; r=0.12	r=0.30; p =0.1	p=0.5; r=0.14
Hematocrit	P=0.9; r=-0.02	p=0.6; r=-0.12	p=0.8; r=-0.05
PVV	p=0.6; r=0.12	p=0.6; r=-0.14	p=0.6; r=0.18
ERYTHROCYTES	p=0.2; r=-0.22	p=0.1; r=0.34	p=0.1; r=-0.34
MCV	p=0.9; r=-0.05	p=0.6; r=-0.12	p=0.7; r=-0.08
MCHC	p=0.2; r=0.22	p=0.1; r=0.30	p=0.2; r=0.30
MHC	p=0.08; r=0.41	p=0.1; r=0.32	p=0.08; r=0.42
YYIR1	p=0.5; r=0.14	p=0.9; r=-0.01	p=0.6; r=0.12
RSSA-mean	p=0.003*; r=-0.6	p=0.2; r=-0.22	p=0.005*; r=-0.63
SJ	p=0.8; r=-0.54	p=0.5; r=0.14	p=0.8; r=-0.03

MCV: Mean Corpuscular Volume, **MCHC**: Mean Corpuscular Hemoglobin Content, **MHC**: Mean hemoglobin concentration, **PVV (T1-T2)**: Plasma volume variation between T1 and T2. **YYIR1**: Yo-Yo intermittent recovery test Level 1, **RSSA**: repeated sprint shuttle ability, SJ: squat jump, **CMJ**: countermovement jump. *Significant relationship between parameters.

Discussion

The main purpose of this study was to analyze the effects of a 6-week period of congested match play on plasma volume variations, hematological parameters, training load and physical fitness in elite soccer players. In accordance with our research hypothesis, the main result of the current study was that PV, and selected hematological parameters, and physical fitness of the soccer players may be altered after an intense 6-week match congested fixture However, the changes observed in these parameters were not related to declines in physical fitness other than SJ performance.

In the current study, Hb and Ht values decreased after a 6-week congested period of match schedule. It is well established that training may alter homeostasis, including hematological parameters. Hb determines oxygen transport and consumption [25], "which is related to physical performance through aerobic capacity [12]". Hb is generally associated with an increase in blood oxygen transport capacity and aerobic performance, while an increase in Ht increases blood viscosity values [26]. The kinetics of variation of these hematological parameters may partly explain players' aerobic performance. Thus, it seems beneficial to assess soccer players' Hb and Ht.

Several studies have shown decreases in Hb and Ht values after periods of intense training or competition [6]. These declines are known as an adaptation to training and called sports pseud-anemia [27,6]. In the concept of sports pseudo-anemia, stimulation of the erythrocytosis during exercise induces an increase in the absolute concentration of Hb [6].This mechanism is masked by the rise in PV [15,28,29]. Sports anemia can be treated as a first sign of overtraining or regular overtraining that can alter aerobic performance [29]. To date, only few studies have evaluated Hb and Ht variations during a competitive season in soccer players [27, 15]. In a previous study by Silva et al. [15], it was observed that Hb and Ht of Brazilian soccer players were significantly increased after 12 weeks of soccer training. These authors postulated that these alterations occurred due to plasma volume changes. In fact, in this study [15], plasma volume decreased by 11.2% after thesoccer-training program and was significantly correlated to the alteration percentage of Hb and Ht.In contrast, Heisterberg et al. [16] recorded no significant changes in Hb and Ht levels over a 6-month periodin which the training and match load varied considerably.These discrepancies in results can be mainly explained by differences in the experimental protocols involved, and factors such as training intensities, durations, frequency, and player's level of training.

In this study, we hypothesized that the period of congested match play would negatively affect plasma volume and hematological parameters which, in turn, would lead to negative changes in players' 'physical fitness. Concerning the values of erythrocytes, the current results showed a significant decrease after the period of match congestion.In agreement with our results many studies suggest that erythrocytes number is decreased at the end of a competitive period in soccer [6]. In general, erythrocytes, Hb, and Ht decrease after endurance training [30]. This is mainly caused by PV expansion [31]. In contrast, Silva and al. [15] showed training-induced increases in erythrocytes after 12 weeks of training in Brazilian soccer players. A possible explanation for this increase might be plasma volume changes [15, 32]. In fact, Silva et al. [15] were able to show that the altered percentage of erythrocytes significantly correlated with plasma volume change during the 12-weeks soccer-training program.In the present study, no significant changes were observed in MCV, MHC and MCHC values after the period of match congestion. These results are not in accordance with findings from Meister et al. [33] who recorded a decrease in MCH and MCHC values and an increase in MCV values during the competitive period. These differences in findings can primarily be explained by psychological factors, players' diet [9,15], and/or differences between players' effective match time.

In the current study, PV measured after a 6-week period of match congestion was significantly higher (+6.1%) in T2. In contrast, Silva et al. [15] demonstrated a decrease in PV after 12 weeksof specific soccer training. The specifics of the soccer-training program and/or the expertise level of the subjects could explain the differences in findings. For example, in the current study, the mean weekly training volume was lower (= 9 h) than that of a previous study conducted with Brazilian soccer players (14.66 -16.33 h) [15]. Additionally, the study period lasted 6 weeks in our study, which is short from the study of Silva et al. [15] (12weeks). This might also be a reason for the different outcomes of the two studies. Previous studies showed that increases in PV and decreases in Ht were accompanied with improved endurance performance [34].This finding can be explained by auto-hemodilution, which is manifested by the decrease in blood viscosity [35,36].This decrease in Ht together with the increase in PV improves physical fitness performance by decreasing peripheral vascular resistance and ventricular post load [36].Thus, the beneficial effects of training were not a decrease in plasma volume and an increase in Ht, but rather the opposite.

In the present study, contradictory results concerning the effects of changes in hematological parameters on performance were observed compared to findings from a previous study

[15,34]. In fact, the decline in physical fitness performance of elite soccer player after the period of match congestion was accompanied by an increase in PV and a decrease in the values of Hb and Ht. Thus, our results confirm that the soccer players enrolled in this study did not show signs of sport anemia as was reported for endurance athletes [29]. A likely explanation for the different outcomes could be that training volume of endurance athletes is much higher compared with that of soccer players. In addition, the hypothesis that the soccer-training program presented in the current study included the development of specific physical qualities (endurance, strength, speed, power) could be explain that the soccer player do not have all amount of endurance training that will lead to the same adaptations observed in endurance athletes. In addition, the soccer training program can be the main reason for the observed changes in erythrocytes, Hb, Ht and plasma volume variations [15,16].

Interestingly, in this study, no significant correlations were observed between PVV, hematological parameters, and players' performance in the YYIR1 and RSSA tests. In contrast, several studies conducted with endurance athletes and soccer players have shown significant negative correlations between hematocrit and measures of physical fitness, suggesting increased performance with a decreasing hematocrit value [6,37]. In addition, there is evidence that hematocrit correlates well with the development of VO_{2max} in young soccer players [37]. Moreover, Abdderrahman et al. [34] showed a significant correlation between plasma volume variations and $VO2_{max}$ in physical education students. Furthermore, it has previously been demonstrated that gains in aerobic performance are related with blood volume increases, primarily through increased red cell mass.

Notably, Silva et al. [38] showed that the hematocrit increased with increasing training volume over a 6-week soccer-training program. This is indicative of the reactivity of hematological parameters to variations in training volume. In addition, Colombini et al. [39] reported that hematocrit values were higher after the match due to a decrease in plasma volume. In the present study, the changes observed in hematological parameters and training load were not related to declines in physical fitness other than SJ performance. These differences in findings may at least in part be explained by experimental factors such as the level of participants' expertise, the respective training period, the training protocol, and the testing methodology.

The decline in physical fitness in the current study could mainly be explained by fatigue which occurred after the heavy training load applied to the soccer players and the large

number of matches (10 matches for 6 weeks). The training load, the monotony and strain applied to soccer player in the last week was very higher than compared to the other weeks, which means that performance deterioration and alteration in hematological markers could be explained by the acute effect of the last week of competitions.

Similarly, Meister et al. [33] assessed the effects of a 3-week period of high match exposure (> 270 min during 3 weeks) compared with a low (<270 min during 3 weeks) match exposure period in German male elite football players on hematological markers. No relevant alterations in the analyzed parameters were observed in this previous study. It might be argued that the periods of high match exposure was too low to induce alterations in physical fitness. Thus, it seems plausible, that the training volume was intentionally reduced during this period to avoid excessive overload [33]. However the average match exposure in our study, was 1,3 match a week (= 900 min during 6 weeks).This indicates that the high match exposures times of elites football players could be explain in part deterioration performance. Contrary to the study by meister et al. [33], the training volume was very important during the congested period of match play. It did not change when playing a single or two matches a week has not changed. It has been noted that the team was having an intense training session containing an intense aerobic work that could be highly demanding given the inherent variability of the technical and tactical training. It can be concluded, that a 6-week period of high match exposure more the high volume of training could be explained blood markers alteration and performance decline in elite's soccer players.

In this study, physical fitness significantly declined over the 6-week experimental period. It therefore seems justified to suggest that physiological signs of fatigue or maybe overreaching were present after the period of match congestion when playing 10 matches over a 6-week period. Training load analysis showed the highest values of training load, monotony and strain in the last weeks as compared to other weeks which could be explain that physiological signs of fatigue was a reflect of the acute effects of the last weeks of competitions. In addition, we believe that the performance decline was a consequence of the high match exposure and the high training volume during the 6 weeks period which means that fatigue could be explain by the chronic effect of the congested period of match play. Thus, this explication has any physiological relevance is at this point difficult to answer and needs further testing. The distance covered during the YYIR1 decreased by 34.9%. A possible explanation for this decline could be impaired cell contractile properties. In fact, structural damage has been suggested to interfere with optimal muscle glycogen store replenishment which is required if

the player performs prolonged high-intensity intermittent exercise [38].The parameters of the repeated sprint schedule ability test (RSSA-test); $RSSA_{mean}$ and $RSSA_{best}$ increased by 2.6% and 3.8%, respectively. This decrease in performance may be explained by the increase by 15.8% in the lactate level measured at the end of test. The increase in lactate level can be explained by at least two hypotheses. The first is related to the increase in energy production by the lactic anaerobic system and a decrease in energy production by the anaerobic alactic system. Thus, soccer players could show decreases in stored phosphagen (ATP-CP) in the muscles at the end of intensive training periods [40]. The second assumption is the decrease in the players' ability to eliminate lactate as a result of the deterioration in the aerobic system performance [41]. We speculate that the decrease in performance in the first sprint during RSSA-test is a result of the degradation of the alactic anaerobic system performance. The increase in the time of the first sprint is probably related to the reductionof the ATP-CP energy reserves [41]. This however is speculative and needs to be verified in future studies.Also,the decline in RSSA and YIYR1 performances may result from mechanical (e.g., force generation) and metabolic (e.g., glycogen depletion) impairments [42].

Soccer players' SJ performance decreased by 2.5% while CMJ performance was not impaired. A potential explanation for this finding could be that alterations in peripheral neuromuscular function occurred which were induced by the reiteration of damaged skeletal muscle fibers [43]. These results confirm the fatigue state of elite soccer players after the congested period (10 matches for 6 weeks). However, it is important to note that in the current study, evaluation of physical fitness occurred 72 hours after the congested period. Therefore, it was possible that playing multiple games for a prolonged period (6 weeks) and the important increase of the values of the training load, monotony and during the last week could have further reduced the time required restoring neuromuscular system properties. It was possible that the change in physical performances was due to inadequate recovery during match congestion period, and after the last weeks period of competitions before testing which also reduced the rate of muscle protein, glycogen resynthesize, and the ability to generate forces [44]. The ability to generate forces may also be related to muscular performance measures such as speedand power ($RSSA_{mean}$, $RSSA_{best}$, and SJ).

Similarly, Bangsbo et al. [45] suggested that the alteration of physical performance of soccer playersduring the season might be due to the lack of in-week physical training caused by the mid-week matches. In this regard, Impelizzeri et al. [46] also reported that the soccer player's ability to jump, sprint and perform repeated high intensity exercise was impaired after 6-week

training period when playing two matches a week. Alternatively, reduced performance could be explained by delayed muscle soreness [47]. In general, muscle soreness peaks 24 to 48 hours after physical exercise. This exercise-induced phenomenon is called delayed onset muscle soreness (DOMS) [48]. In addition, the deterioration of physical performance may also be related to other psychological factors caused by the negative results of the team that won only 2 out of 10 games played during this period.

The present study does have a number of limitations that warrant discussion. First, we examined only one soccer team in this study. Hence, our sample size and the training regime of the subjects are limited. Second, only a limited number of physiological and physical fitness tests were applied. Thus, our findings are specific to these outcomes. Future studies should examine additional biochemical, immunological and psychological markers to further elucidate this research topic.

Conclusions and practical applications

This study examined the effects of a match congestion period on PV, hematological parameters and physical fitness in elite soccer players. The results of this study showed significant changes in various hematological parameters and physical fitness, which suggests potentially negative effects of the training load and 10-match exposure during the experimental period. The congested period of match play affected PV, hematological parameters and simultaneously resulted in declines in physical fitness that were related to TWTL.

This research may provide useful information for coaches and medical staff with regards to TWTL and physical fitness performances during an intensive period of congested match play to better manage the work strain and to avoid overreaching and overtraining, while reducing the risk of sustaining injuries. Our data which was assessed during a congested soccer period clearly illustrate the need for sufficiently long recovery periods at the end of the season. This is of particular importance for players who are nominated for their national teams and perform international tournaments after the regular season. “Its data also allows us to individualize the recovery of players according to the degree of participation during the competitive period”. In addition, our findings raise some concerns with regards to current practices in soccer clubs. Among the key questions are the uses of team rotation and recovery strategies to reduce the risk of injuries. If the goal is to develop soccer players’ physical performance over the course of a season, it is mandatory to monitor physical and physiological demands during periods of match congestion.

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Etude 2

Interaction entre la concentration plasmatique des hormones, la performance physique, la charge d'entrainement et le changement d'état d'humeur durant la période compétitive congestive chez des footballeurs professionnels.

The interplay between plasma hormonal concentrations, physical fitness, workload and mood state changes to periods of congested match play in professional soccer players

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Résumé

Contexte: L'évaluation régulière des marqueurs hormonaux et de l'état d'humeur dans le football professionnel pourrait être des bons indicateurs pour prévenir un état de surentraînement pendant les périodes d'entraînement et/ou de compétition intense.

Objectif: L'objectif de cette étude était d'analyser les paramètres hormonaux, psychologiques, la charge d'entrainement et la performance physique durant la période compétitive congestive chez des footballeurs élites.

Méthodes: Seize footballeurs élites évoluant dans la première ligue du football tunisien ont été évalués trois fois (T1, T2 et T3) durant douze semaines d'entrainement et de compétition. La période régulière (non congestive) a été de T1 à T2, et les joueurs ont joué six matchs durant six semaines. La période congestive a été de T2 à T3, et les joueurs ont disputé dix matchs en six semaines. De T1 à T3, les joueurs ont effectué les tests suivants : un test intermittent (Yo-Yo intermittent recovery test [YYIR1]), un test de répétitions des sprints (RSSA), un test de saut en contre-mouvement (CMJ) et un test de squat jump (SJ). Les concentrations plasmatiques du cortisol (C), de testostérone (T) et le rapport T/C ont été analysés en T1, T2 et T3. Les dimensions de l'humeur (tension, dépression, colère, vigueur, fatigue, confusion et score total de perturbation de l'humeur) ont été évaluées à l'aide du questionnaire Profile of Mood State (POMS). L'évaluation quotidienne de l'effort perçu (sRPE) a été utilisée afin de quantifier la charge d'entraînement interne, la monotonie et la contrainte.

Résultats: Une baisse significative de la performance (T1 <T2 <T3) a été observée dans le test de SJ ($p = 0,04$, taille d'effet [ES] $ES_{1-2} = 0,15$, $ES_{2-3} = 0,60$). La performance en YYIR1 a été significativement améliorée en T2 par apport à T1 et elle a diminué de manière significative en T3 par apport à T2 ($p= 0,001$, $ES_{1-2}= 0,24$, $ES_{2-3}= -2.54$). La performance $RSSA_{mean}$ a été significativement plus élevée ($p= 0,019$, $ES_{1-2}= -0.47$, $ES_{2-3}= 1,15$) en T3 par rapport à T2 et T1. La performance $RSSA_{best}$ a été significativement plus élevée en T3 par rapport à T2 et T1 ($p = 0,006$, $ES_{2-3} = 0,47$, $ES_{1-2} =- 0,56$), par contre, elle a été significativement plus faible en T2 par rapport à T1. La testostérone (T) et le rapport T/C a été significativement plus faible en T3 par rapport à T2 et T1 (T:$p = 0,03$, $ES_{3-2} = -0.51$, $ES_{3-1} = -0,51$, T/C: $p = 0,017$, $ES_{3-2}= -1.1$, $ES_{3-1}= -1.7$). Une diminution significative du score de la vigueur a été observé en T3 par rapport à T2 et T1 ($p = 0,002$, $ES_{3-2} = 0,31$, $ES_{3-1}= -1.25$).

Une augmentation significative du score de la fatigue à été observé en T3 par rapport à T1 et T2 (p = 0,002, ES1-2 = 0.43 ; ES2-3 = 0,81). Une augmentation significative a été observé pour le score de tension T1 <T2 <T3 (p = 0,002, ES_{1-2} = 1,1, ES_{2-3}= 0,2) et pour le score de la colère (p = 0,03, ES_{1-2} = 0,47, ES_{2-3} = 0,33) au cours de la période d'étude. Le score total de perturbation de l'humeur (TMD) a augmenté de manière significative (p = 0,02, ES_{1-2} = 0,91, ES_{2-3} = 1.1) de T1 à T3. Entre T1 et T2, des relations significatives ont été observées entre la charge d'entrainement et les variations de testostérone (r= 0,66, p= 0,003) et du rapport T/C (r= 0,62, p= 0,01). Il y avait des relations significatives entre le $RSSA_{best}$ et les paramètres de la charge d'entraînement (charge d'entrainement: r= 0,52, p= 0,03; monotonie: r= 0,62, p= 0,01; contrainte: r= 0,62, p= 0,009). Entre T2 et T3, il y a avait une relation significative entre Δ% du score total de perturbation de l'humeur et Δ% de YYIR1 (r= -0,54; p= 0,04), $RSSA_{best}$ (r= 0,58 ; p= 0,01), SJ (r= -0,55 ; p= 0,01), T (r= 0,53; p= 0,03) et T/C (r= 0,5; p = 0,04).

Conclusions: Une période compétitive congestive a considérablement compromis les performances physiques et mentales des footballeurs élites. Le changement des paramètres de performance étaient liés aux paramètres psychologiques. La surveillance de l'état d'humeur pourrait être un outil simple et utile pour déterminer le degré de préparation aux matchs pendant la période congestive chez des footballeurs professionnels.

Mots clés: entraînement, hormones, surentraînement, récupération.

Abstract

Background: The regular assessment of hormonal and mood state parameters in professional soccer are proposed as good indicators during periods of intense training and/or competition to avoid overtraining.

Objective: The aim of this study was to analyze hormonal, psychological, workload and physical fitness parameters in elite soccer players in relation to changes in training and match exposure during a congested period of match play.

Methods: Sixteen elite soccer players from a team playing in the first Tunisian soccer league were evaluated 3 times (T1, T2, and T3) over 12 weeks. The non-congested period of match play was from T1 to T2, when the players played 6 games over 6 weeks. The congested period was from T2 to T3, when the players played 10 games over 6 weeks. From T1 to T3, players performed the Yo-Yo intermittent recovery test level 1 (YYIR1), the repeated shuttle sprint ability test (RSSA), the countermovement jump test (CMJ), and the squat jump test

(SJ). Plasma Cortisol (C), Testosterone (T), and the T/C ratio were analyzed at T1, T2, and T3.Players had their mood dimensions (tension, depression, anger, vigor, fatigue, confusion, and a Total Mood Disturbance) assessed through the Profile of Mood State questionnaire (POMS). Training session rating of perceived exertion (sRPE) was also recorded on a daily basis in order to quantify internal training load and elements of monotony and strain.

Results: Significant performance declines (T1<T2<T3) were found for SJ performance (p=0.04, effect size [ES] ES_{1-2}= 0.15, ES_{2-3}= 0.60) from T1 to T3. YYIR1 performance improved significantly from T1 to T2 and declined significantly from T2 to T3 (p= 0.001, ES_{1-2}= 0.62, ES_{2-3}= 1.3). Mean RSSA performance was significantly higher (p= 0.019, ES_{1-2}=0.63, ES_{2-3}= 1.03) in T3 compared with T2 and T1. Best RSSA performance was significantly higher in T3 when compared with T2 and T1 (p= 0.006, ES_{2-3}= 0.52, ES_{1-3}= 0.71), but significantly lower in T2 when compared with to T1.T and T/C were significantly lower in T3 when compared with T2 and T1 (p=0.03, ES_{3-2}= 0.14, ES_{3-1}= 0.56, T/C: p= 0.017, ES_{3-2}= 0.04, ES_{3-1}= 0.61). Significant decreases were found for the vigor scores in T3 when compared to T2 and T1 (p= 0.002, ES_{3-2}= 0.16, ES_{3-1}= 0.47). A significant increase was found in fatigue scores in T3 as compared to T1 and T2 (p=0.002, ES_{1-2}= 0.1, ES_{2-3}= 0.65). A significant increase was found from T1< T2< T3 intension score (p=0.002, ES_{1-2}=1.1, ES_{2-3}=0.5) and anger score (p= 0.03, ES_{1-2}= 0.47, ES_{2-3}= 0.27) over the study period. Total mood disturbance increased significantly (p= 0.02, ES_{1-2}= 0.70, ES_{2-3}= 0.81) from T1 to T3. Between T1-T2, significant relationships were observed between workload and changes in T(r=0.66, p=0.003), and T/C ratio (r= 0.62, p= 0.01). There were significant relationships between performance in $RSSA_{best}$ and training load parameters (workload: r=0.52, p=0.03; monotony: r= 0.62, p= 0.01; strain: r= 0.62, p= 0.009). Between T2-T3, there was a significant relationship between Δ% of total mood disturbance and Δ% of YYIR1 (r= -0.54; p= 0.04), $RSSA_{best}$ (r= 0.58, p= 0.01), SJ (r= -0,55, p= 0.01),T (r= 0.53; p= 0.03), and T/C (r= 0.5; p= 0.04).

Conclusions: An intensive period of congested match play significantly compromised elite soccer players' physical and mental fitness. These changes were related to psychological but not hormonal parameters; even though significant alterations were detected for selected measures. Mood monitoring could be a simple and useful tool to determine the degree of preparedness for match play during a congested period in professional soccer.

Key words: training, hormones, overtraining, overreaching, recovery

Introduction

Contemporary elite soccer players are exposed to a high number of matches and confronted with a large number of fixtures throughout the season, including league cup and other competitions. With fixture, we refer to a schedule of matches that a football club must play over a season (Carling et al., 2012; Nédélec et al., 2012). Moreover, elite soccer players often play competitive matches with only 2-3 days of recovery in between (Arruda et al., 2015;Rowsell et al., 2011, Ekstrand et al., 2004).This means that soccer players are continuously subjected to a variety of physical and psychological stressors from both, training and competition (Ekblom et al.1986). As a consequence of the soccer-specific physiological demands, training programs should be specifically tailored and address physical qualities such as aerobic capacity, strength, power, and change-of-direction speed. Consequently, the goal of training is to provide adequate stimulito induce soccer-specific adaptations that will improve physical performance over time. Maintaining or improving performance is not only determined by training, but also the capacity of bodily systems (e.g., neuromuscular system, endocrine system) to recover and regenerate after a period of intense training or after psychological stress caused by high number of competitions during a congested period. During congested periods, the dynamic homeostatic balance between anabolic and catabolic processes in the muscle can ultimately influence players' physical fitness level (Moreira et al., 2016). The effects of soccer training on hematological variations (Saidi et al., 2019), immunological markers (Rebelo et al., 1998) muscle strength (Silva et al., 2011), physical performance (Dellal et al., 2015; Dupont et al., 2010), hormonal responses to single game (Thorpe et al., 2012), and long-term training have previously been reported. However, only a few studies exist that systematically tracked hormonal responses during different periods of competition over the course of a season (Silva et al., 2013). Indeed, biological and quantitative evaluations are now a common practice used for the evaluation of physiological responses during competition and training in many sports. A wide variety of biochemical, hematological, and physiological markers have been used for long-term monitoring of athletes (Silva et al., 2013; Heisterberg et al., 2013). In particular, cortisol (C) and testosterone (T) have been identified as reliable markers of training stress and could be considered two important hormones in the biochemical assessment of athletes (Adlercreutz et al., 1986; Hackney., 2020). In addition, the T/C ratio has additionally been used to evaluate soccer players' anabolic/catabolic status (Urhausen et al., 1995). A decline in this ratio has previously been associated with the overtraining syndrome (Viru et al., 2001). Reflective of

this study, Freitas et al. (2014) provided evidence that intensive periods of training may lead to an increase in salivary C concentrations in elite young soccer players. According to da.Silva et al. (2011), 12-weeks of training with professional soccer players resulted in increased serum concentration of C with concomitant decreases in T and T/C ratio. Thus, hormonal parameters are sensitive to both, training periods that differ in terms of volume and intensity (da.Silva et al., 2011), and frequency of matches during the competitive period (Silva et al., 2013). While the use of biochemical markers for long-term monitoring of exercise stress is widespread, the relationship between hormonal concentrations and physical performance standards during different periods of competitive season remains unclear. If the physical demands of training and competition are too great, one might assume that catabolic activities will predominate. If, however, the body is able to successfully cope with the demands, the anabolic metabolism can help maintain or improve the performance during different periods of the competitive season (Fry et al., 1992). However, many factors influence this delicate hormonal balance, not only training workloads, training schedules, competition factors but also psychological stress. The interplay of these factors can influence the overall physiological state and readiness of the player. Thus, Edwards et al. (2015) have shown that players with a positive mood state activated the parasympathetic nervous system and consequently cortisol secretion decreased. In contrast, negative mood states can activate the sympathetic nervous system, higher C secretion can take place. In this regard, physiological influences of stress and mood state may intervene and have rebound effects on physical fitness (Chenaoui. et al. 2016). This emphasizes the necessity to assess the interplay of hormonal, psychological, and physical fitnessin elite soccer players during different competitive periods of a soccer season.

Thus, in light of the above, the aim of this study was two-fold; 1) to examine elite soccer players' fluctuations in hormonal, psychological, and physical fitness parameters during the competitive period of the season, including a period of congested match play; and, 2) to examine the inter-relationships between all of these parameters. It was hypothesized that hormonal and psychological parameters may be altered after the congested periods of competition, which, in turn, could cause negative changes in players' physical fitness which would be evident through the associations between selected measures (Faude et al., 2011; Silva et al., 2013; Moreira et al., 2016).

Methods

Sixteen male professional soccer players aged 19 to 22 years from the same soccer team playing in the first Tunisian soccer division participated in this study (body height: 177 ± 4 cm; body mass: 72 ± 5.2 kg; BMI= 22.7± 1.1 kg/m²). The monitored players included three central backs, three fullbacks, six midfielders, and four strikers.All players were evaluated three times during the study; i.e., T1, T2, and T3. T1 was scheduled in the middle of the non-congested period (week 0; early March). T2 was at the end of the non-congested period (before the congested period; week 6; mid-April). T3 was completed after the congested period (week 12; late May until early June - after the season).

The detailed description of the protocol can be found in the section dedicated togeneral methodology of this document.

Results

Over the 12-week experimental period, 16 players were able to complete the study requirements according to the previously described study design and methodology. The Mean ICC (Intraclass Correlation Coefficients) for the different parameters was presented in **Table 2.**

Table 2. Intraclass correlation coefficient.

Parameters	**Mean Intraclass correlation coefficient (ICC)**	**95% confidence interval**	
		Lower bound	**Upper bound**
Cortisol	0.74	0.40	0.90
Testosterone	0.83	0.61	0.93
T/C	0.82	0.59	0.93
YYIR1	0.48	0.51	0.78
$RSSA_{mean,}$	0.61	0.15	0.84
$RSSA_{best}$	0.69	0.34	0.89
$RSSA_{decremen}$	0.34	-1.26	0.58
SJ	0.95	0.90	0.98
CMJ	0.98	0.77	0.95
TRIMP	0.77	-1.20	0.93
Monotony	0.10	-0.20	0.08
Strain	0.004	-0.51	0.13
TMD	0.21	-0.54	0.67

T/C : Testosterone / Cortisol ratio. **YYIR1**: Yo-Yo intermittent recovery test level 1. **RSSA**: Repeated Shuttle Sprint Ability. **SJ**: Squat Jump. **CMJ**: Countermovement Jump. **TRIMP**: Training Impulse. **TMD**: Total Mood Disturbance.

Training workload

In **Table 3**, we presented data from workload analyses in periods of congested and non-congested match play. All training parameters were significantly different between comparisons.

Table 3. Training load parameters during the 12 weeks training period (congested vs. non-congested period of match play).

Training periods	From T1 to T2: Non-congested period	From T2 to T3: Congested period	p-value	Effect size (ES)
TRIMP (A.U.)	8302.00±1239.39	10092.96±2362	<0.001*	0.81
Monotony (A.U.)	0.49±0.07	1.87±0.16	<0.001*	1.70
Strain (A.U.)	717.30±174.60	10975.30±2664.70	<0.001*	0.87

Data are presented as mean ± SDs. * Significant difference between congested and non-congested period with p<0.01. **TRIMP**: Training impulse, **A.U**: Arbitrary units.

Physical fitness data and team performance

Performance test scores are illustrated in **Table 4**. The YYIR1, $RSSA_{mean}$ and $RSSA_{best}$, and SJ performance were significantly different in T3 after the congested period. In addition, the YYIR1 performance increased from T1 to T2 after the non-congested period. The $RSSA_{best}$ were significantly lower in T2 as compared to T1. The CMJ, the $RSSA_{decrement}$, and blood lactate after the RSSA test did not change significantly from T1 to T3.

Table 4. Changes in physical fitness.

Training periods	T1	T2	T3	p-value	Effect size(ES) ES_{1-2} .ES_{2-3}
YYIR1 (m)	2536.2±382.7	2635.5±439.6	1650±335.14	0.001*£	0.62-5.30
$RSSA_{mean}$(s)	8.23±0.3	8.07±0.3	8.53±0.46	0.019*	0.63-1.03
$RSSA_{best}$ (s)	7.70±0.30	7.48±0.48	7.77±0.40	0.006*£	0.52-0.71
$RSSA_{decrement}$(%)	7.01±2.81	7.89±4.71	6.05±2.66	0.09	0.06 -0.26
$[Lac]_{RSSA}$(mM/L)	18.03±2.79	18.21±3.26	20.34±2.30	0.19	0.22-0.50

SJ (cm)	36.58±3.95	36.31±3.85	35.34±3.99	0.04*	0.15-0.60
CMJ (cm)	39.01±2.46	39.50±2.44	39.15±2.31	0.89	0.25-0.07

Data are represented as means± SDs.**YYIR1**:Yo-Yo intermittent recovery test level 1. **RSSA**:Repeated Shuttle Sprint Ability.**[Lac]$_{RSSA}$**: Lactate concentration measured afterRepeated Shuttle Sprint Ability- test. **SJ**: Squat Jump, **CMJ**: **C**ountermovement Jump.*****Significant difference between T3-T2 and T3-T1 with p< 0.05. **£** Significant differences between T2 and T1 with p< 0.05.

Team performance is illustrated in **Table 5**. Statistically significant differences were found between the congested and non-congested period. The percentage rate of winning matches amounted to 33% during the non-congested and20% during the congested period. (p<0.001, Z=-4). Match accumulated time was higher in the congested period as compared to the non-congested period (p<0.001, Z= 4).

Table 5. Team performance of the soccer team during the congested and non-congested period

Periods	**Matches played**	**Win (%)**	**Draw (%)**	**Lost (%)**	**Matches accumulated-time (minutes)**
From T1 to T2 Non-congested Period	6	33	0	67	540
From T2 to T3 Congested Period	10	20	0	80%	900

Hormonal data

The C levels were not significantly different during the different periods of training. On the other hand, T was significantly lower (p=0.03, ES_{3-2}= 0.14, ES_{3-1}= 0.56) in T3 (5.08± 1.83) when compared to T2 (6.14± 2.30) and T1 (6.14± 2.19). The T/C ratio was significantly lower in T3 when compared to T2 and T1 (p=0.017, ES_{3-2}= 0.04, ES_{3-1}= 0.61). The T level and T/C ratio were not significantly different in T2 as compared to T1 (**Table 6**).

Table 6. Hormonal parameters during the 12 weeks training period.

Hormonal data	**T1**	**T2**	**T3**	**p-value**	**ES ES_{3-2} -ES_{3-1}**
Cortisol (ng/ml)	135.75±15.19	134.56±14.31	135.75±10.58	0.89	0.07-0.17
Testosterone (ng/ml)	6.14± 2.30	6.14± 2.30	5.08± 1.83	0.03*	0.14-0.56

T/C ratio	0.044 ± 0.016	0.046 ± 0.019	0.03 ± 0.01	0.017*	0.04-0.61

Data are represented as means± SDs. **T/C**: Testosterone / Cortisol . * Significant differences between T3-T2 and T3-T1 with p< 0.05.

Psychological data

Changes in scores from the POMS questionnaire at different time points are presented in **Table 7**.

The vigor score assessed by the POMS questionnaire was significantly lower in T3 after the congested period than in the other time points (p=0.002, ES_{1-2}=0.16, ES_{2-3}=0.77).Moreover, the fatigue score increased in T3 as compared to T2 and T1 (p=0.002, ES_{1-2}=0.1, ES_{2-3}=0.65). The tension score increased progressively during the different competitive periods compared with T1 (p=0.002, ES_{1-2}=1.1, ES_{2-3}= 0.5). The anger score increased significantly (p=0.03, ES_{1-2}= 0.47, ES_{2-3}= 0.27) from T1to T2 and from T2 to T3.There were no alterations in the other psychological scores (depression and confusion).Finally, the Total Mood Disturbance increased significantly from T1 to T3 (i.e., T1<T2<T3; p= 0.001, ES_{1-2}= 0.7, ES_{2-3}= 0.81).

Table 7. Changes in psychological parameters.

Psychological scores	**T1**	**T2**	**T3**	**p-value**	**ES** ES_{1-2} - ES_{2-3}
Tension (A.U.)	9.31±1.74	11.87±2.75	12.37±2.08	0.002***£**	1.10-0.50
Depression (A.U.)	8.68±1.35	10.18±2.50	9.37±1.92	0.08	0.53-0.32
Anger (A.U.)	9.12±2.12	10.18±2.42	11.00±2.53	0.03***£**	0.47-0.27
Fatigue (A.U.)	9.12±1.58	10.06±2.72	12.31±2.79	0.002*****	0.10-0.65
Confusion (A.U.)	8.06±2.04	8.75±2.93	9.43±2.55	0.35	0.20-0.15
Vigor (A.U.)	18.75±2.38	19.43±3.88	14.93±3.41	0.002*****	0.16-0.77
Total mood disturbance	25.56±7.38	32.12±6.90	39.06±5.63	0.001*£	0.70-0.81

(A.U.)					

*****Significant differencesbetween T3 and T2 and between T3-T1 with p<0.05. **£** Significant difference between T2 and T1 with p <0.05. **A.U**.: Arbitrary units.

Relationships between hormonal parameters and physical fitness

Only a few significant correlations were found between hormonal data and physical fitness during the non-congested period (**Table 8**). The Δ% of testosterone correlated with Δ% $RSSA_{best}$ (r= -0.7, p= 0.02). In addition, there was a significant relationship between Δ% of T/C parameters and Δ% of $RSSA_{best}$ (r= -0.55, p= 0.02).

Table 8. Relationships between hormonal parameters, and psychological parameters with physical fitness.

		Non-congested period (T1-T2)					**Congested period of match play (T2- T3)**				
		YYIR1	**RSSA mean**	**RSSA best**	**SJ**	**CMJ**	**YYIR1**	**RSSA mean**	**RSSA best**	**SJ**	**CMJ**
C	**r**	-0.4	0.01	-0.3	0.008	0.11	-0.15	0.3	-0.4	-0.32	0.22
	p	0.07	0.9	0.14	0.97	0.12	0.62	0.1	0.12	0.91	0.49
T	**r**	0.13	0.20	-0.7	0.18	0.21	0.18	-0.1	-0.3	0.31	0.16
	p	0.60	0.43	0.02*****	0.48	0.33	0.44	0.64	0.2	0.22	0.54
T/C	**r**	0.12	0.23	-0.55	0.21	0.28	0.96	0.93	-0.1	0.10	- 0.43
	p	0.64	0.37	0.02*****	0.42	0.32	0.72	0.75	0.6	0.55	0.11
TMD	**r**	-0.2	0.3	-0.2	0.1	-0.7	-0.6	0.13	0.58	-0.55	0.18
	p	0.3	0.1	0.2	0.6	0.7	0.02*****	0.58	0.01*****	0.01*****	0.62

*Significant correlation between parameters. **TMD**: Total Mood Disturbance.**YYIR1:**Yo-Yo Intermittent Recovery test level 1. **RSSA**: Repeated Shuttle Sprint Ability. **SJ**: Squat Jump, **CMJ**: Countermovement Jump.**C**: Cortisol. **T:** Testosterone.**T/C**: Testosterone/Cortisol.

Relationships between hormonal parameters and psychological parameters

Several significant correlations were identified between hormonal parameters and psychological markers (POMS) during the congested period of match play **(Table 9)**. There was a positive correlation between Δ% of total mood disturbance and Δ% of testosterone (r= 0.53; p= 0.03) and between Δ% of total mood disturbance and Δ% of T/C (r= 0.50, p= 0.04).

Table 9. Relationships between hormonal parameters and psychological parameters.

		From T1 to T2 Non congested period			From T2-T3 Congested-Period		
		Cortisol	**Testosterone**	**T/C**	**Cortisol**	**Testosterone**	**T/C**
TMD	r	0.03	0.31	0.33	0.10	0.53	r= 0.50
	p	0.89	0.21	0.19	0.65	0.03*	p= 0.04*

*significant correlation between parameters. **T/C**: Testosterone / Cortisol. **TMD**: Total Mood Disturbance

Relationships between percentage change of psychological parameters and physical fitness

No significant relationship was found between Δ% of total mood disturbance and Δ% of physical fitness during the normal competitive period. Of note, we observed a significant relationship between Δ% of total mood disturbance and Δ% of physical fitness during the period of congested match play (r= -0.6; p= 0.02; r= 0.58, p= 0.01; r= -0.55, p= 0.01 respectively for YYIR1, $RSSA_{best}$ and SJ) (**Figures 2A, 2B, 2C**).

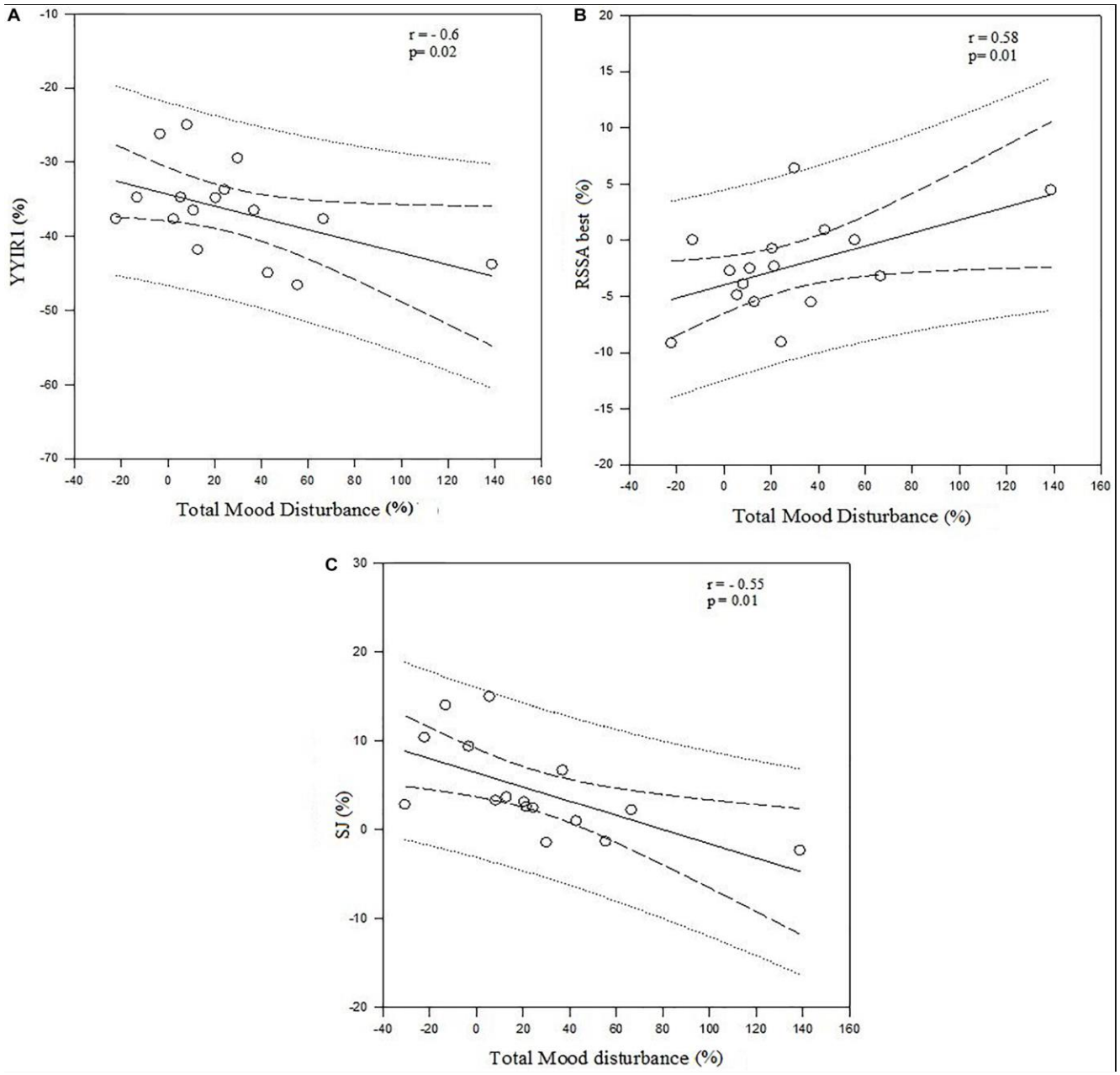


Figure 2. (A) Correlation between $\Delta\%$ Total Mood Disturbance and $\Delta\%$ YYIR1 during the congested period of match play.

YYIR1: Yo-Yo Intermittent Recovery test level 1. (B) Correlation between 1% total mood disturbance and $RSSA_{best}$ during the congested period of match play. **RSSA**: Repeated Shuttle Sprint Ability test. (C) Correlation between $\Delta\%$ total mood disturbance and SJ during the congested period of match play. **SJ**: Squat Jump.

Relationships between percentage change of hormonal parameters, psychological parameters, physical fitness and workload parameters

Table 10 summarizes relationships between percentage change of hormonal parameters, psychological parameters, physical fitness, and workload parameters during the study. We observed that changes in testosterone from T1 to T2 were related to workload (r= 0.6, p=

0.008). The Δ% of T/C ratio was related to workload from T1 to T2 (r= 0.62, p= 0.01). Also, the change in cortisol was related to monotony from T2 to T3 (r= 0.51, p= 0.01).In the non-congested period from T1 to T2, there was a significant relationship between training workload, monotony and strain, with physical fitness in $RSSA_{mean}$ (r=0.52, p=0.03; r=0.62, p=0.01; r= 0.62, p= 0.009, respectively).

Table10. Relationships between percentage change of hormonal parameters, psychological parameters, physical fitness and workload parameters.

		From T1 to T2 Non-Congested period			**From T2 to T3 Congested Period**		
		TRIMP	**Monotony**	**Strain**	**TRIMP**	**Monotony**	**Strain**
Cortisol	r	0.37	0.25	0.32	0.19	0.51	0.27
	p	0.12	0.35	0.22	0.42	0.01*	0.27
Testosterone	r	0.66	0.33	0.44	-0.13	-0.12	-0.1
	p	0.008*	0.20	0.08	0.5	0.6	0.4
T/C	r	0.62	0.39	0.47	-0.21	-0.23	-0.25
	p	0.01*	0.19	0.06	0.33	0.35	0.29
TMD	r	0.06	0.15	0.15	0.11	0.25	0.11
	p	0.79	0.56	0.56	0.63	0.31	0.66
YYIR1	r	0.04	0.04	0.28	0.08	0.06	0.05
	p	0.86	0.8	0.28	0.74	0.8	0.8
$RSSA_{mean}$	r	0.52	0.62	0.62	0.02	-0.1	-0.04
	p	0.03*	0.01*	0.009*	0.93	0.5	0.86
$RSSA_{best}$	r	-0.29	-0.07	-0.1	0.1	-0.09	0.08
	p	0.26	0.77	0.70	0.69	0.71	0.7
SJ	r	0.03	0.11	0.07	0.02	0.17	0.04
	p	0.9	0.66	0.79	0.94	0.51	0.86
CMJ	r	0.15	0.04	0.08	0.14	0.44	0.28
	p	0.57	0.88	0.76	0.59	0.08	0.28

TRIMP: Training Impulse. **T/C**: Testosterone/ Cortisol ratio. **YYIR1**: Yo-Yo intermittent recovery test level 1. **RSSA**: Repeated Shuttle Sprint Ability. **SJ**: squat jump. **CMJ**: Countermovement Jump. **TRIMP**: Training Impulse. **TMD**: Total Mood Disturbance.*significant correlation between parameters.

Discussion

This study examined elite soccer players' fluctuations in hormonal, psychological, and physical fitness parameters during the competitive period of the season, which included a period of congested match play. In addition, the relationships among the various parameters were measured. The results showed significant alterations in hormonal, psychological and physical fitness parameters during the congested period of match play. However, while the changes observed in hormonal parameters were not related to physical fitness performance, the changes in psychological parameters were related to performance declines in physical fitness **(Figure 3).**

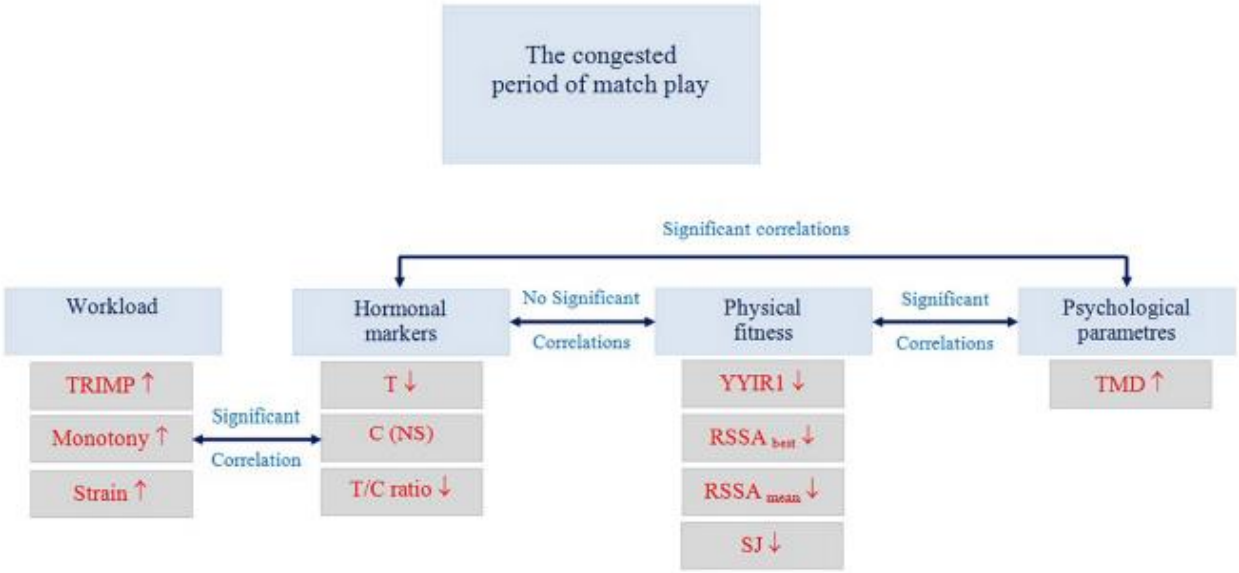


Figure 3. Summary of the main findings of this study.

TRIMP: Training Impulse. **C**: Cortisol. **T:** Testosterone. **T/C:**Testosterone/Cortisol ratio. **YYIR1**: Yo-Yo intermittent recovery test level 1. **SJ**: Squat Jump. **TMD**: Total mood disturbance.

Hormonal markers

In this study, plasma C concentrations were unchanged during the 12 week competitive period and were not altered when players were exposed to the period of congested match play. This result is in agreement with data reported by Moreira et al. (2016) who investigated the subsequent impact of a congested period of match play (7 matches played over a 7 day period) on resting hormonal parameters in elite young soccer players from a reference team. Given the current results and those reported by Moreira et al. (2016), it can be speculated that congested periods of different density do not significantly affect the hypothalamic-pituitary-adrenal axis responses of players, when considering their resting C values. The absence of a

significant change in resting C concentration may be, at least in part, attributed to the high fitness level of the players and to their habitual high daily and weekly training volume over the season. Further studies should verify the impact of congested periods of higher densities on hormonal responses.

Conversely, the current results disagree with the results of Handziniski et al. (2006) who showed an increase in plasma C concentrations after the end of the competitive period. Differences between studies can be partially explained by distinct experimental protocol designs and participants' characteristics. For example, the study of Handziniski et al. (2006) and the current study analyzed the soccer season at different time points (e.g. half-season and 12 weeks competitive periods). In addition, the different degree of competition during the in-season might also explain the differing results. Previously, Silva et al. (2011) showed a 43.2% increase in plasma C levels in professional soccer players after a 12-week training program. The authors suggested that the increment of training intensity played an important role in cortisol increases. It is suggested that an increase in training intensity is the determining factor behind increases in C concentrations. This can be explained by a hyper responsiveness of the HPA axis due to a physiological adaptation of the neuro endocrine system to training (Hackney., 2006). However, it is reasonable to suggest that the practical usefulness of using this approach to assess hormonal changes during highly intensive competition periods may be limited.

The C response to the competitive period during a congested schedule should be viewed with caution. In the current study, only resting plasma concentrations were examined, and on a limited basis (i.e., three time points). Some authors have suggested that evaluating hormonal responses to exercise rather than resting may provide a better scenario for regarding hormonal changes. For instance, during training, or after a competitive match, or during states of non-functional overreaching (Hugh et al., 2013; Silva et al., 2013; Meeusen et al., 2004), including the potential influence of circadian rhythms, and competitive vs. training workloads (Fernandez-Fernandez et al., 2015).

In contrast to C responses, a significant decrease in T concentrations was observed after the congested period of match play, and it was not recovered after a period with a normal match exposure (non-congested period). The current results suggest that the higher number of matches played in the last 6 weeks (10 matches) potentially affected resting T concentrations. Thus, the accumulated fatigue potentially caused by the congested competitive schedule seems to lead to a decrement in T. Previous research found that T concentrations were related

to tiredness and the magnitude of fatigue (Maso et al., 2004; Filaire et al., 2001; Silva et al., 2013). Maso et al. (2004) suggested that a low T level could be used as a marker of fatigue and might reflect the residual effect of previously accumulated fatigue, which could be linked to insufficient recovery intervals. In addition, Moreira et al. (2016) showed a decrease in T concentration measured after a congested match period (7 matches played in 7 days). Moreover, Filaire et al. (2001) showed a decrease in salivary T concentration measured after 7 weeks of high-intensity training. Based on previous results reported by Filaire et al. (2001) and Moreira et al. (2016), it appears that the T responses observed in the current study are associated with the accumulated fatigue generated during the congested period of match play.

In the present study, the soccer players showed significant reductions in the T/C ratio only after a period of congested match play, which is linked to the lowering in basal T levels. In fact, the decrease in T/C ratio of elite soccer players was accompanied by a reduction of physical performance. According to Gorostiaga et al. (2004), an impairment of physical performance could be accompanied by lower T/C values. A reduction in the T /C ratio is generally considered to be an indicator of anabolic-catabolic balance and it has previously been considered a useful tool for diagnosing overtraining (Urhausen et al., 1995). In the current study, it is possible that the decreased T/C ratio could mainly be explained by fatigue, which occurred after the heavy training load and the increased frequency matches played during the last 6-weeks of the competitive period. In addition, TRIMPs, monotony and strain values during the period of congested match play were higher compared with the non-congested period. This means that alterations in hormonal markers could be explained by the chronic effects of the last 6 weeks of competition. However, the average match exposure in our study was 1 match a week during the non-congested periods and 1.67 matches per week during periods of congested match play. In addition, match-accumulated time during the congested period was very high (=900min in 6 weeks) compared to that of the non-congested period (=540min in 6 weeks). This indicates that the higher match time exposures of elite soccer players during the last six weeks of competition could partially explain the observed deterioration in performance and hormonal changes. Thus, our data suggests that the accumulated match time during the competitive period of the season is associated with physical and hormonal parameters. That is, elite players presented a significant decrease in physical fitness (YYIR1, RSSA, and SJ) after a period with more match exposure.

Mood state changes

In the present study, the included elite players presented a typical "iceberg profile" after the 12-week competitive period (Morgan., 1980). In the iceberg profile, the athletes score below the 50^{th} percentile with regards to tension, depression, anger, fatigue and confusion, and above the 50^{th} percentile in terms of vigor. Previous data regarding the response to POMS assessment during 12 weeks competitive periods have suggested that negative changes of the mood state such a tension, fatigue and anger are a sign of staleness (i.e., a term typically used in psychology to represent overtraining) (O'Connor et al.,1989). Moreover, the positive score (vigor) of the POMS questionnaire was reduced, and the negative scores (fatigue, tension and anger) and the total mood disturbance, were increased after the congested period. In addition, Anger, tension scores and Total Mood disturbance increased during the non-congested periods. Moreover, it was hypothesized that players' mood was affected by the greater training load applied along with the high number of matches played during the period of congested match play. In addition to workload influences, our study showed that players' mood was affected by their performance and vice versa. During both, the congested and non-congested period, increased tension, anger, and decreased vigor coincided with poor performance. More specifically, the percentage rate of matches won fell below 50% for all played games (33% during the non-congested period versus 20% during the congested period). In this regard, Hoffman et al. (1999) showed when the team results are poor, the mood state vigor was reduced. As the teams' results improved (a greater winning percentage), vigor returned to its normal level. In agreement with Filaire et al. (2001), who showed that the tension and depression score increased during the period of the season when the team winning percentage fell below the 50% for game played.

Relationships between hormonal parameters and physical Fitness

In this study, no significant correlations were observed between hormonal parameters and players' performance in the YYIR1, RSSA, SJ and CMJ tests, with only negative correlations observed between T, T/C ratio and $RSSA_{best}$ during the non-congested period. These findings are in agreement with previous reports showing negative associations between changes in performance and T/C (Gorostiga et al., 2004), and improved performance with maintenance of higher T/C values (Kraemer et al., 2004). In the current study, the decrease in physical fitness after the congested period of match play may be interpreted by a decrease in plasma T levels and T/C values. The observed-congested period of match play induced decrease in testosterone concentrations reflect decreased anabolic activity (Kraemer, 2004). Hence,

Muscella et al.(2019) showed that the increased serum testosterone level together with serum cortisol decrement, would suggest a strong intensification of anabolic androgenic activity in young soccer players. It can reasonably be supposed that the alteration in testosterone concentration might affect explosive-type performance. In fact, positive associations between testosterone concentration and greater power performance were observed in young soccer players (Muscella et al., 2019). In addition, Hammami et al. (2018) demonstrated a positive correlation between T and T/C with physical fitness in SJ, CMJ and YYIR1 in elite soccer players. In contrast, Silva et al. (2013) have shown that a higher match exposure in the midseason was associated with higher C levels, a lower T/C, and better physical performances. These differences in results suggest that the analysis of the T/C ratio should be used with caution as noted by others (McLellane et al., 2010). Our results further suggest that the decrease in T/C ratio noted in T3 after the congested period of match play may reflect tiredness or a state of fatigue. To determine more precisely the influence of fatigue on these hormonal variations, it is necessary to address these two variables. To do this, we examined potential correlations between the total mood disturbance and hormonal data.

Relationships between mood state with hormonal parameters and physical fitness

In our study, C concentrations were not related to total mood disturbance during different competitive periods. In contrast, T concentrations showed a relationship with Total mood disturbance during the congested period suggesting the involvement of mood changes on T responsiveness. This is in accordance with previous studies (Maso et al., 2004; Elloumi et al., 2008). Moreover, we found a significant positive correlation between the T/C ratio and the Total mood disturbance. This suggests that it is more useful to follow variations in T (an anabolic hormone) than variations in C (a catabolic hormone) to determine the degree of tiredness and change in Mood state (Maso et al., 2004). In addition, there appears to be a negative relationship between physical fitness and total mood state during a period of congested match play. Thus, the increase of total mood disturbance was associated with physical fitness declines. These results cannot, however, be used to indicate whether changes in physical fitness are affected by changes in mood (T-score on fatigue, anger and tension increased and T-score on vigor decreased) or vice-versa. Our results confirm that physical performance depends simultaneously on physical and psychological characteristics. Thus, it seems that the participation in a high number of matches during the congested period induces psychological stress on soccer players. Most likely due to the need for sustained physical and cognitive demands during every match. In addition, anger, tension and total mood disturbance

were observed to increase, during the non-congested period, but these changes appeared to be independent of physical fitness. YYIR1 and best RSSA increased after the non-congested period. It could be assumed that the changes in mood states observed during this period are related to other factor than staleness. The financial problems experienced by the players in this study may have resulted in the observed changes in the mood traits anger and tension. However, soccer players were still able to perform during physical test well regardless of these changes in mood during the non-congested period. Moreover, this result could be explained by the professionalism of these players who must show your physical ability to coach in order to confirm our selection during the matches.

In this study, we showed a positive correlation between monotony and C variations during the congested period of match play. In addition, changes of T concentrations and T/C ratio showed a significant relationship with workload during the non-congested period. These results confirm that some hormones are sensitive to competitive periods and soccer training programs which is in agreement with the study of Silva et al. (2011) who showed that increased C and decreased C levels are related to increases in both, training intensity and volume. Thus, the hormonal levels can be used as potential markers for variations of volume and intensity of training.

While a few studies exist that assessed hormonal variation, mood changes and performance data in athletes (Filaire et al., 2001, Hoffman et al., 1999). Our study additionally examined the interplay between these factors. Findings from this study suggest that the observed changes in hormonal parameters during the congested period of match play were related to psychological but not physical fitness parameters. We acknowledge that the use of the POMS questionnaire is limited in team sport. Players from the starting line-up may exhibit a more positive mood state during the competitive period compared with substitutes because they know that they will get match time. Thus, different motivational levels of the team players may have affected the outcomes of the POMS questionnaire.

This study has a number of limitations that warrant discussion. First, the study cohort was composed of a single team only which is why findings are specific to the examined cohort and environment. Caution is needed when transferring our findings to female soccer or male soccer of different expertise level. Second, the sample size is rather small and the number of measurement points is limited which is why more research is needed to verify our findings.

Conclusions and practical applications

This study analyzed hormonal (plasma C concentration, plasma T concentration), psychological, physical, workload, and physical fitness parameters in elite soccer players, and their relationships with changes in training and match exposure (congested and non-congested periods of match play). The results of this study showed significant changes in various hormonal, psychological and physical fitness parameters during the final competitive period of the season (congested match play). Our results suggest that the congested period of match play alters various parameters in elite soccer players. While the observed changes in hormonal parameters were not related to declines in physical fitness, those in psychological parameters were. Our findings lead us to suggest the use of the POMS questionnaire as an appropriate and simple monitoring tool for identifying performance decrements in players exposed to high competitive loads during the congested period. This recommendation may provide useful information for coaches and medical staff for better monitoring workload and physical fitness to manage players' work-strain ratio to avoid overreaching - overtraining, and to reduce the risk of sustaining injuries.

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Etude 3

Marqueurs biochimiques et état de bien être durant la période compétitive congestive chez des footablleurs élites

Biochemical markers and wellness status during a congested match play period in elite soccer players

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Résumé

Contexte: L'évaluation régulière des marqueurs biochimiques et des indicatuers du bien être pourrait être des bons indicateurs pour prévenir un état surentraînement chez des footballeurs élites pendant les périodes d'entraînement et/ou de compétition intenses.

Objectif : Les objectifs de cette étude était (1) d'examiner les fluctuations des paramètres inflammatoires, des marqueurs de dommages musculaires, des indicateurs du bien être et de la performance physique pendant la période compétitive de la saison, y compris la période congestive ; (2) évaluer les relations entre les paramètres biochimiques, la charge d'entrainement, les indicateurs du bien être, et la performance physique chez des footballeurs élites.

Méthodes : Quatorze footballeurs élites évoluant dans la première ligue tunisienne de football ont été évalués trois fois (T1, T2 et T3) pendant douze semaines d'entrainement et de compétition. De T1 à T2, la période régulière (non congestive) a duré six semaines et a compris six matchs. De T2 à T3, la période congestive a duré six semaines et a compris dix matchs. De T1 à T3, les joueurs ont effectué les tests suivants: Le squat jump [SJ], Le countermovement jump [CMJ]), le test de répétitions des sprints (RSSA) et le test Yo-Yo intermittent recovery test. La protéine plasmatique C-réactive (CRP), la créatinine, et la créatine kinase (CK) ont été analysées en T1, T2 et T3. Les états de bien être (douleurs musculaires (DOMS), le stress, la fatigue, la qualité du sommeil) ont été mesurés quotidiennement à l'aide du questionnairede Hopper. L'évaluation quotidienne de l'effort perçu (sRPE) a été utilisée afin de quantifier la charge d'entraînement interne, la monotonie et la contrainte.

Résultats : La performance en SJ a diminué de manière significative en T3 par rapport à T1 et T2 (p= 0,01, taille d'effet [ES] ES_{1-2}= -0,15; ES_{2-3}= 1). La performance en YYIR1 est améliorée de manière significative en allant de T1 à T2 et a diminué de manière significative en allant de T2 à T3 (p = 0,01, [ES] ES_{1-2} = 0,5; ES_{2-3} = 1,38). Le $RSSA_{moyen}$ était significativement plus faible en T3 (p = 0,01, ES_{1-2} = 0,15; ES_{2-3} = 1,01) par rapport à T1 et T2. CRP et CK étaient significativement plus élevés en T3 par apport à T2 et T1 (CRP: p = 0,01, ES_{1-2}= 0,29; ES $_{2-3}$= 1,53, CK: p = 0,01, ES_{1-2}= 0,11 ; ES_{2-3} = 1,7).

L'indice Hopper, le stress, la fatigue, et les douleurs musculaires (DOMS) étaient plus élevé durant la période congestive [T2-T3] par rapport à la période régulière non congestive [T1-

T2], (stress, p= 0,001, ES= 2; fatigue, p= 0,008, ES= 0,81; DOMS, p= 0,001, ES= 2,3, Indice Hopper, p = 0,002; ES = 1,33). De T2 à T3, une relation significative a été trouvée entre le changement (Δ%) de CRP avec l'indice Hopper (r = 0,53, p = 0,04) et le score de fatigue (r = 0,64, p = 0,01). De plus, Δ% de la CK était corrélée avec l'indice Hopper (r = 0,63, p = 0,01). Il y avait une relation significative entre Δ% de la fatigue et Δ% YYIR1 (r = -0,49, p = 0,04). Le Δ% du score DOMS est corrélé à Δ% $RSSA_{best}$ (r = 0,51, p = 0,04). Une corrélation positive entre Δ% $RSSA_{mean}$ et Δ% de l'indice Hooper (r = 0,54, p = 0,04) a été observé durant la période congestive.

Conclusion : Une période compétitive intensive (congestive) a considérablement compromis la performance physique et les indicateurs du bien être chez des footballeurs élites. Le changement des indicateurs du bien être pourrait expliquer la baisse de la performance physique pendant la période congestive chez des footballeurs élites. Ainsi, la surveillance des indicateurs du bien être pourrait être un outil utile pour les entraîneurs et le staff médical afin de mieux gérer la charge d'entrainement et de prévenir les blessures. De plus, les changements de CRP et de CK étaient en corrélation avec les indicateurs du bien-être (fatigue, DOMS et Indice Hopper), ce qui suggére qu'il est utile de suivre les variations de la concentration de CRP et de CK pour déterminer le degré de changement de l'état de bien être et/ou vice versa.

Mots clés: entraînement, surentraînement, dépassement, récupération, congestion des matchs

Abstract

Background: The regular assessment of biochemical markers and wellness status are proposed to be good indicators of overtraining during periods of intense training and/or competition in elite soccer players.

Objective: The aim of this study was to analyze biochemical and wellness status together with workload and physical fitness parameters in elite soccer players in relation to changes in training and match exposure during a congested period of match play.

Methods: Fourteen elite soccer players from a team playing in the first Tunisian soccer league were evaluated three times (T1, T2, and T3) over 12 weeks. The regular period (i.e., uncongested) of match play was from T1 to T2, when the players played 6 games over 6

weeks. The congested period was from T2 to T3, when the players played 10 games over 6 weeks. From T1 to T3, players performed the vertical jump tests (squat jump [SJ] and countermovement jump test [CMJ]), the repeated shuttle sprint ability test (RSSA), and the Yo-Yo intermittent recovery test (YYIR1). Plasma C-reactive protein (CRP), creatinine, creatine kinase (CK) were analyzed at T1, T2, and T3. Wellness status was measured daily using Hopper questionnaires (delayed muscle soreness DOMS, stress, fatigue, sleep quality). Training session rating of perceived exertion (sRPE) was also recorded on a daily basis in order to quantify internal training load and elements of monotony and strain.

Results: SJ performance decreased significantly in T3 as compared to T1 and T2 (p= 0.01, effect size [ES] ES_{1-2}= -0.15; ES_{2-3}= 1). YYIR1 performance improved significantly from T1 to T2 and declined significantly from T2 to T3 (p= 0.01, ES_{1-2}= 0.5; ES_{2-3} = 1.38). Mean RSSA performance was significantly lower in T3 (p= 0.01, ES_{1-2}= 0.15; ES_{2-3}= 1.01) compared with T1 and T2. Best RSSA performance was significantly lower in T3 as compared to T1 and T2 (p= 0.01, ES_{1-2}= 0.15; ES_{2-3}= 1.01) but significantly higher in T2 when compared with T1. CRP and CK were significantly higher in T3 as compared to T2 and T1 (CRP: p= 0.01, ES_{1-2}= 0.29; ES_{2-3}= 1.53, CK: p= 0.01, ES_{1-2}= 0.11; ES_{2-3}= 1.7).
A significant increase was found in stress, fatigue, DOMS scores, and Hopper index during the congested period (between T2-3) as compared with the regular period (between T1-2), (stress, p= 0.001, ES= 2 ; fatigue, p= 0.008, ES= 0.8; DOMS, p= 0.001, ES= 2.3, Hopper Index, p= 0.002; ES= 1.33). Between T2-3, significant relations were found between the Δ% of CRP with the Hopper Index (r= 0.53, p=0.04) and the fatigue score (r= 0.64, p=0.01). In addition, the Δ% of CK was correlated with the Hopper Index (r=0.63, p=0.01). There was a significant relation between Δ% of fatigue score with Δ% YYIR1 (r= -0.49, p=0.04). The Δ% of DOMS score correlated with Δ% $RSSA_{best}$ (r=0.51, p=0.04). A positive correlation was found between Δ% $RSSA_{mean}$ and Δ% Hooper Index (r= 0.54, p= 0.04).

Conclusions: An intensive period of congested match play significantly compromised elite soccer players' physical fitnessand wellness status. Elite soccer players'wellness status reflects declines in physical fitnessduring a congested period of match play while biochemical changes do not. Thus, wellness status monitoring could be a useful tool for coaches and medical staff to manage workload and prevent injuries. Furthermore, CRP and CK changes correlate with wellness status (fatigue, DOMS, Hopper index), suggesting that it is useful to

follow variations in CRP and CK level to determine the degree of change in wellness status or vice versa.

Key words: training, congested calendar, overtraining, overreaching, recovery

Introduction

The physiological demands of soccer have increased over the past decades due to a higher density of soccer matches, and an increase in mean intensity of matches (Carling et al., 2012; Clemente et al., 2013). During periods when the schedule is particularly congested (i.e., two matches per week over several weeks), the recovery time between successive matches is only 3-4 days, which may be insufficient to allow full recovery of the players. As a result, players may experience acute and chronic fatigue potentially leading to underperformance and an increased injury risk (Dellal et al., 2015; Folgado et al., 2015; Saidi et al., 2020).

To prevent imbalances andovertraining during congested schedules, biochemical assessments are commonly used (Rowsell et al., 2009). This is well-established in soccer clubs and typically involves the assessment of blood markers indicating inflammation and muscle damage (Siva et al., 2008; Pascoal et al., 2018; Coppalle et al., 2019). Creatine kinase (CK), C-reactive protein (CRP), and creatinine are biochemical markers that areoften used to quantify muscle damage and inflammation (Djaoui et al., 2017). These biochemical parameters are sensitive to training periods differing in volume and intensity (Walker et al., 2017), and frequency of matches during the competitive period (Walkers et al., 2017; Heisterberg et al., 2013).

Coaches and sports scientists conceptualized soccer as a psycho-physiological complex sport (Coutts et al., 2008; Haddad et al., 2013) where subtle changes in training load could affect wellness status and, ultimately physical performance (Thorpe et al., 2015, Saidi et al.,2020). Therefore, several psychological monitoring of changes intraining-related stress, wellness, strain and recovery have been suggested to detectearly signs of tiredness and potential overtraining in high-performance sport programs (Coutts etReaburn, 2008; Hooper et Mackinnon, 1995). Recently, Thorpe et al. (2015) have reported that daily training load had affected these wellnessmeasurements in elite outfield soccer players of the English Premier League.Haddad et al. (2013) found that player's wellness (sleep, stress, fatigue, muscle soreness) are not major contributors to perceived exertion during standard endurancetraining in young soccer players.In addition to training load, there are various contextualaspects, particularly those related to environmental conditions such as social factors, family context, personality trait, religious practices, moment of the season,league ranking, and match importance, that could influence players' status and,hence, their perceived wellness level (Chamari et al., 2012; Fessi et al., 2016a; Boullosa et al., 2020).

However, to the best of our knowledge, there is little information concerning the relation between biochemical markers, players' wellness, and physical fitness in elite soccer players during different competitive periods of a soccer season.

Therefore, the aims of this study were to evaluate the effects of a period of congested match play on biochemical markers, wellness status (including perceived ratings of sleep, fatigue, stress, and muscle soreness),and physical fitness and to examine the inter-relation between all of these parameters. Based on relevant literature data (Haddad et al. 2013; Thorpe et al., 2015; Fessi et al., 2016a; Saidi et al., 2020; Boullosa et al., 2020), it was hypothesized that biochemical parameters and wellness status may be altered after the congested periods of competition, which, in turn, could cause negative changes in players' physical fitness which would be evident through the associations between selected measures.

Methods

24 elite soccer players from the same first division Tunisian club were enrolled in this study. However, complete data were obtained from 14 players (age: 20.9 ± 0.8 years; height: 177.6 ± 4.7 cm; mass: 72.0 ± 5.2 kg). The playing positions of the players were three central backs, three fullbacks, four midfielders, and four strikers. The study was carried out during 12 weeks of the competitive period, and was designed to analyze inflammation, muscle damage, workload, physical fitness, and wellness status to changes in training and match exposure during a congested period of match play. All players were evaluated three times during the study; T1 was in the middle of the regular period (uncongested) (week 1; early March); T2 was at the end of the regular period (before the congested period; week 6; mid-April). T3 was completed after the congested period (week 12; late May until early June - after the season)

Statistical analyses

Data are expressed as means and standard deviations (SD). Normal distribution of data was tested and confirmed using the Kolmogorov-Smirnov test. Paired t-tests for dependent samples were used to identify differences in workload parameters (TRIMP, Monotony, Strain and Hopper index) between the congested and non-congested periods of match play. We computed a one-way analysis of variance (ANOVA) for repeated measures to determine variations of biochemical and physical performance parameters measured during the congested vs.non-congested period of match play. Pearson's product-moment correlation coefficients (r) were computed to identify relations between all parameters. The magnitude of correlation coefficients was considered as trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($0.9 < r < 1$), and perfect ($r = 1$) (Hopkins et al., 2009). Statistical significance was set at $p < 0.05$ for all analyses. The 95% confidence intervals (CI) and effects sizes (ES) were calculated to compare differences in mean values for all analyzed parameters. When calculating ES, pooled standard deviations (SD) were used since no control group was available (Cohen's $d = [M1-M2]/SD_{pooled}$). ES with values of 0.2, 0.5, and 0.8 were considered to represent small, medium and large differences, respectively (Cohen, 1988). All statistical tests were processed using statistical software (Sigma Stat 3.5, Systat, Inc, USA).

Results

Training Workload

Table2 presents data from workload analyses in periods of regular (uncongested) and congested match play. All training parameters were significantly different between comparisons.

Table 2. Training load parameters during the 12 weeks training period (regular vs congested period of match play)

Training periods	**From T1 to T2 Regular period**	**From T2 to T3 Congested period**	**p-Value**	**Effect size (ES)**
TRIMP (A.U.)	8302.0 ± 1239	10092.96 ± 2362	<0.001*****	0.8
Monotony (A.U)	0.49 ± 0.7	1.85 ± 0.16	<0.001*****	3.1
Strain	817.7 ± 255.24	19843.9 ± 597345	<0.001	6.1

Data are presented as mean ± SDs. *Significant difference between regular and congested period with p <001. **TRIMP**: Training impulse, **A.U**: Arbitrary units.

Physical fitness data

Performance tests results are provided in **Table 3**. There were no significant changes in CMJ performance, $RSSA_{decrement}$, and blood lactate between the three testing sessions. However, The YYIR1,$RSSA_{mean}$, $RSSA_{best}$ and SJ performance were differed significantly in T3 after the congested period. Also, the YYIR1 performance increased significantly from T1 to T2 after the regular period. The $RSSA_{best}$ were significantly lower in T2 as compared to T1.

Table 3. Changes in physical fitness parameters.

Training periods	T1	T2	T3	p-value	Effect size (ES) ES_{1-2} -ES_{2-3}
YYIR1 (m)	2308,5± 363,5	2495,7± 339,0	1594,2± 295,1	<0.001*£	0.5_1.38
$RSSA_{mean}$(s)	8.2± 0.2	8.0±0.1	8.5±0.4	0.001*	0.15_1.01
$RSSA_{best}$ (s)	7.8±0.3	7.5±0.2	8.2±0.2	<0.001*£	0.96_0.41
$RSSA_{decrement}$(%)	7.0±3.1	7.2±3.8	5.9±3.0	0.93	0.03_0.05
$[Lac]_{RSSA}$ (mM/L)	18.4±3.2	18.2±2.8	20.2±2.3	0.07	0.05_0.10
SJ (cm)	37.9±3.8	37.4±3.2	34.2±3.2	0.01*	-0.14_1
CMJ (cm)	38.7±2.5	39.1±2.6	38.8±2.5	0.85	0.03_0.14

Data are represented as means± SDs.**YYIR1**: Yo-Yo intermittent recovery test level 1. **RSSA**: repeated shuttle sprint ability**.** **$[Lac]_{RSSA}$**: lactate concentration measured after repeated shuttle sprint ability- test. **SJ**: squat jump, **CMJ**: countermovement jump. *significant difference between T3-T2 and T3-T1 with p< 0.05. **£** significant differences between T2 and T1 with p < 0.05.

Biochemical data

Parameters connected to inflammation and muscular damage markers showed number of changes during the observation's periods, the CRP was significantly higher in T3 as compared to T2 and T1. The CK was significantly higher in T3 as compared to T2 and T1. However, CRP and CK did not change significantly between T1 and T2. In addition, there were no significant differences in creatinine during the observation period **(Table 4).**

Table 4. Changes in inflammation and muscle damage markers.

Parameters	T1	T2	T3	p-value	ES ES_{1-2} - ES_{2-3}
C-Reactive Protein (mg/L)	25.56± 7.38	32.12± 6.90	39.06± 5.63	0.001***£**	0.29_1.53
Creatine Kinase (UI/L)	268.5± 113.2	278.0± 133.4	417.6± 237.8	0.002***£**	0.11_1.7
Creatinine (mg/L)	10.0± 0.8	10.02± 0.7	10.34± 1.0	0.24	0.04_0.40

*Significant differences between T3 and T2 with p<0.05. **£** Significant difference between T3 and T1 with p <0.05.

Wellness status

Changes in wellness scores at different competitive periods are presented in **Table 5**. The stress score assessed by the Hopper questionnaire was significantly higher after the congested period than in the regular period (p=0.001, ES=2). Moreover, the fatigue score increased after the congested period as compared to the regular period (p=0.008, ES=0.81). The DOMS score increased during the congested period compared with the regular period (p <0.001, ES= 2.3). However, therewas no alterations in the sleep score during the different competitive periods. Finally, Hopperindex increased significantly after the congested period compared with the regular period (p<0.001, ES=1.3).

Relation between biochemical parameters and physical fitness

No significant correlation between biochemical parameters and physical fitness performance during the congested period of match play. Only a significant relationship between Δ% CRP and Δ% SJ (r= 0.55, p=0.03) during regular period were observed **(Table 6)**.

Table 6. Relationships between biochemical parameters and physical performance

		Regular period (T1-T2)					**Congested period of match play (T2- T3)**				
		YYIR1	**RSSA mean**	**RSSA best**	**SJ**	**CMJ**	**YYIR1**	**RSSA mean**	**RSSA best**	**SJ**	**CMJ**
C-Reactive Protein (mg/L)	r	0.07	0.07	0.09	0.55	0.14	0.04	-0.19	0.10	-0.29	0.26
	p	0.80	0.8	0.75	0.03*	0.62	0.86	0.51	0.71	0.31	0.37
Creatine Kinase (UI/L)	r	-0.05	-0.06	0.16	-0.32	-0.33	0.46	0.23	0.02	-0.02	-0.3
	p	0.85	0.81	0.56	0.25	0.24	0.09	0.41	0.99	0.99	0.15
Creatinine (mg/L)	r	-0.34	-0.12	-0.10	-0.29	0.14	-0.08	-0.13	0.07	-0.29	0.05
	p	0.23	0.68	0.72	0.30	0.62	0.77	0.64	0.79	0.30	0.85

YYIR1: yoyo intermittent recovery test level 1. **RSSA**: repeated shuttle sprint ability. **SJ**: squat Jump, **CMJ**: countermovement Jump. *significant correlation between parameters.

Relation between biochemical parameters and wellness status

No significant relationship was found between inflammatory and muscle damage parameters with wellness scores during the regular period **(Table 7).** Of note, we showed that the Δ% of CRP was correlated with Hopper Index (r= 0.53, p=0.04) and fatigue score (r= during the congested period (r= 0.64, p=0.01). In addition, the Δ% of CK was correlated with Hopper Index (r=0.63, p=0.01) **(Figure 2).**

Table 7. Relationship between biochemical parameters and wellness scores

		Regular period of match play (T1- T2)					**Congested period of match play (T2- T3)**				
		Sleep	**stress**	**fatigue**	**DOMS**	**HI**	**Sleep**	**stress**	**Fatigue**	**DOMS**	**HI**
C-Reactive Protein (mg/L)	**r**	0.37	-0.52	0.37	0.11	0.16	0.24	-0.07	0.64	0.13	0.53
	p	0.18	0.06	0.18	0.68	0.56	0.40	0.8	0.01*	0.64	0.04*
Creatine Kinase (UI/L)	**r**	-0.12	0.01	-0.20	-0.14	-0.12	-0.18	0.16	-0.42	0.63	0.22
	p	0.67	0.95	0.91	0.61	0.67	0.52	0.57	0.13	0.01*	0.31
Creatinine (mg/L)	**r**	-0.12	0.01	-0.20	0.06	-0.12	-0.53	0.13	0.02	-0.33	-0.2
	p	0.67	0.95	0.47	0.81	0.67	0.06	0.65	0.93	0.23	0.2

*Significant correlation between parameters. **DOMS**: Delayed onset muscle soreness. **HI**: Hopper Index

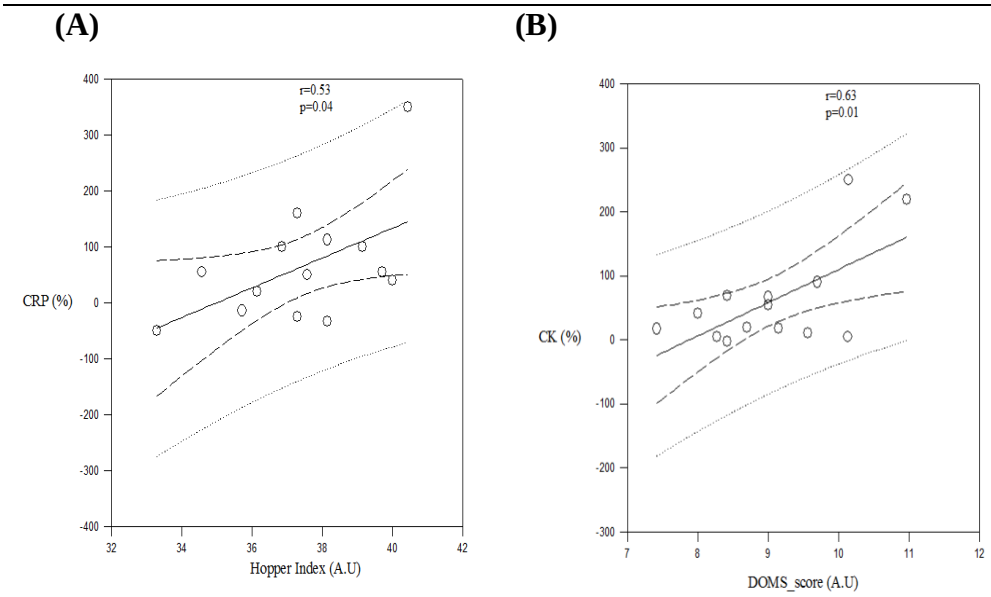


Figure 2. (A) Correlation between Δ% CRP and Hopper Index. (B): corrélation between Δ% CK and DOMS. **CRP**: C-reactive protein. **DOMS**: dayled onset muscle soreness.

Relation between wellness status and physical fitness

Several significant correlations were found between wellness scores and physical fitness during the congested period of match play **(Table 8).** The Δ% of fatigue score correlated with Δ%YYIR1(r= -0.49, p=0.04). In addition, the Δ% of DOMS score correlated with Δ%$RSSA_{best}$ (r=0.51, p=0.04). Moreover, we observed a positive correlation between Δ%$RSSA_{mean}$ and Δ%Hooper Index (r= 0.54, p= 0.04*)**(Figure 3).**

Table 8. Relationships between wellness scores and physical fitness

		Regular period of match play (T1- T2)					**Congested period of match play (T2- T3)**				
		Sleep	**stress**	**fatigue**	**DOMS**	**HI**	**Sleep**	**stress**	**Fatigue**	**DOMS**	**HI**
YYIR1 (m)	r p	0.41 0.14	-0.15 0.60	0.20 0.47	0.14 0.61	0.15 0.58	-0.03 0.90	0.7 0.78	-0.49 0.04*	0.48 0.07	0.25 0.38
$RSSA_{mean}$ (s)	r p	0.17 0.54	-0.32 0.25	0.30 0.29	0.40 0.15	0.21 0.46	0.09 0.72	0.25 0.37	-0.42 0.13	0.02 0.91	0.68 0.007*
$RSSA_{best}$ (s)	r p	0.27 0.33	-0.02 0.92	-0.27 0.33	-0.08 0.76	0.08 0.76	-0.45 0.10	0.25 0.37	-0.25 0.37	0.51 0.04*	0.20 0.47
SJ (cm)	r p	0.10 0.73	0.41 0.13	0.12 0.68	0.08 0.76	0.21 0.45	0.37 0.18	0.08 0.77	0.11 0.68	0.07 0.80	0.32 0.25
CMJ (cm)	r p	-0.08 0.78	0.11 0.69	-0.23 0.41	-0.21 0.47	-0.21 0.46	-0.34 0.22	0.28 0.32	0.45 0.10	-0.27 0.34	0.10 0.73

YYIR1: Yo-Yo intermittent recovery test level 1. **RSSA**: repeated shuttle sprint ability. **SJ**: squat jump, **CMJ**: countermovement Jump.
*significant correlation between parameters.**HI**: Hopper Index

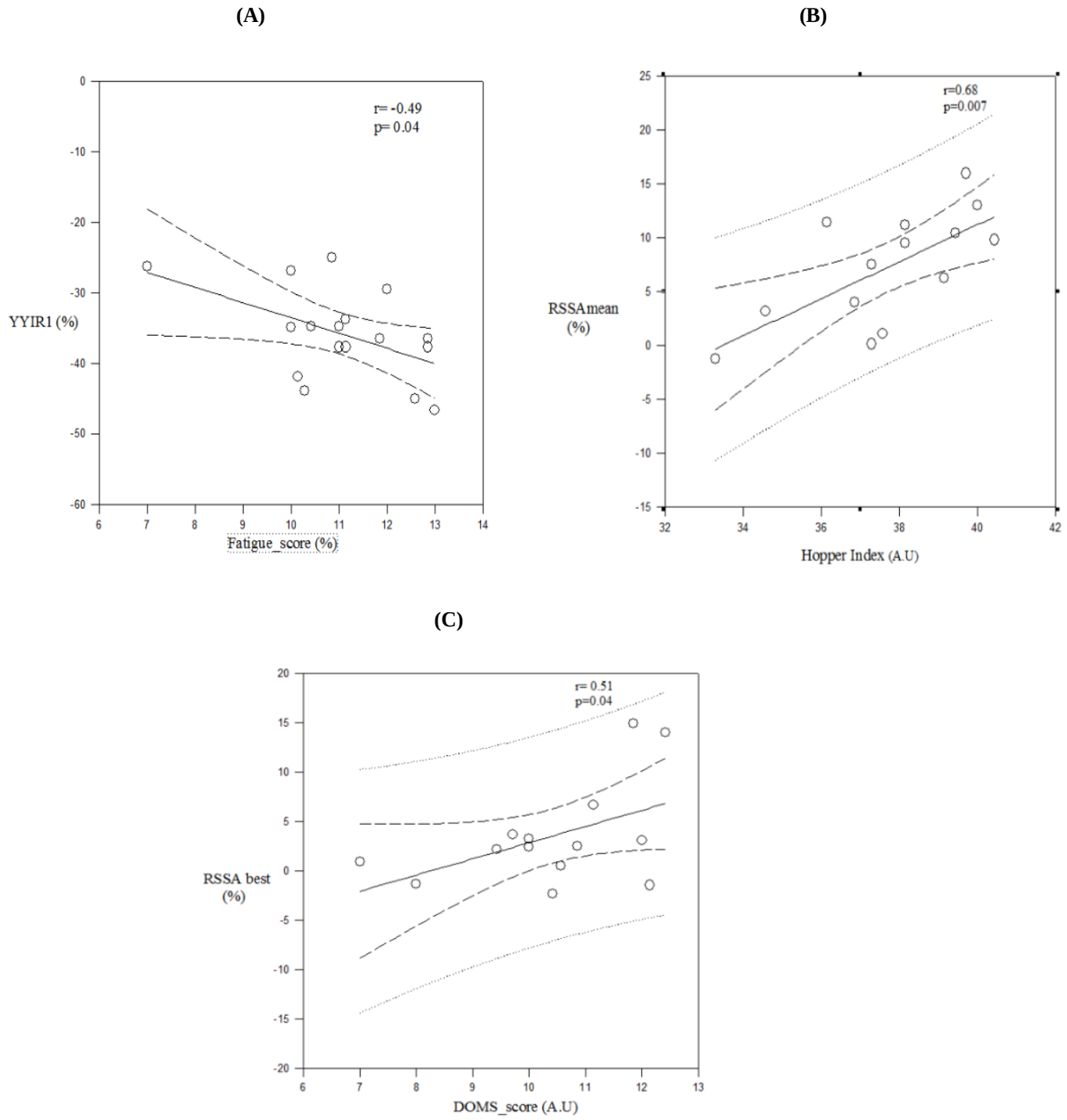


Figure 3. (A) Correlation between Δ%YYIR1 and fatigue score. (B) Correlation between Δ% $RSSA_{mean}$ and Hopper Index. (C) Correlation between Δ% $RSSA_{mean}$ and DOMS.

YYIR1: Yo-Yo intermittent recovery test level 1. **DOMS**: delayed onset muscle soreness. **RSSA**: repeated shuttle sprint ability.

Relationships between percentage of biochemical parameters, physical fitness, Hopper Index and workload parameters

Table 9 summarizes relationships between percentage changes of biochemical parameters, physical fitness, wellness score and workload parameters during the study.

We observed that the Hopper Index were related to workload parameters during the non-congested period (TRIMP: r= 0.56, p=0.03; monotony: r=0.54, p= 0.04; strain: r= 0.56, p=0.03) and the congested period of match play (TRIMP: r=0.66, p=0.009; strain: r=0.69, p=0.006). The DOMS score was related to workload parameters during the congested period of match play (TRIMP: r= 0.70, p=0.004, strain: r= 0.71, p=0.004).

In addition, the increase in CK level was related to monotony (r=0.69, p= 0.005) during the congested period. The increase in CRP was correlated to workload parameters (TRIMP: r= 0.52, p= 0.04, monotony: r= 0.50, p=0.04; strain: r= 0.51, p=0.03). Also, the change in $RSSA_{mean}$ were related to workload parameters during the congested period (monotony: r= 0.63, p=0.01; strain: r= 0.65, p=0.01) and the change of YYIR1were related to strain (r= 0.52, p=0.04).

Table 9. Relationships between percentage of biochemical parameters, physical fitness, wellness and workload parameters

		From T1 to T2 Regular period			From T2 to T3 Congested Period		
		TRIMP	**Monotony**	**Strain**	**TRIMP**	**Monotony**	**Strain**
C-Reactive Protein (mg/L)	r	0.30	0.38	0.40	0.52	0.50	0.51
	p	0.29	0.17	0.15	0.04*	0.04*	0.03*
Creatine Kinase (UI/L)	r	-0.47	-0.5	-0.45	0.35	0.69	0.30
	p	0.08	0.06	0.79	0.22	0.005*	0.29
Creatinine (mg/L)	r	-0.49	0.30	-0.35	-0.25	-0.15	0.16
	p	0.07	0.22	0.51	0.21	0.59	0.72
sleep	r	0.06	0.17	0.13	0.43	0.03	0.36
	p	0.81	0.55	0.63	0.11	0.91	0.20
stress	r	-0.32	-0.31	-0.36	-0.09	0.40	-0.08
	p	0.25	0.28	0.19	0.74	0.15	0.77
fatigue	r	0.40	0.40	0.41	-0.15	-0.14	-0.04
	p	0.15	0.14	0.14	0.59	0.63	0.88
DOMS	r	0.40	0.37	0.42	0.70	0.30	0.71
	p	0.15	0.19	0.12	0.004*	0.28	0.004*
Hopper Index	r	0.56	0.54	0.56	0.66	0.33	0.69
	p	0.03*	0.04*	0.03*	0.009*	0.18	0.006*
YYIR1 (m)	r	0.20	0.19	0.20	0.10	0.12	0.52
	p	0.48	0.49	0.47	0.72	0.67	0.04*
$RSSA_{mean}$(s)	r	-0.08	0.63	0.65	-0.11	-0.17	-0.19
	p	0.77	0.01*	0.01*	0.68	0.54	0.50
$RSSA_{best}$ (s)	r	-0.12	0.09	0.02	0.29	-0.10	0.24
	p	0.67	0.73	0.93	0.31	0.72	0.39
SJ (cm)	r	-0.04	0.05	0.01	-0.04	0.13	-0.01
	p	0.88	0.84	0.96	0.88	0.63	0.95
CMJ (cm)	r	0.01	-0.003	-0.0009	-0.25	0.29	-0.11
	p	0.95	0.99	0.97	0.37	0.31	0.68

RSSA : repeated sprint shuttle ability. **SJ:** squat jump. **CMJ**: countermovement jump.*significant correlation between parameters

Discussion

The aim of this study was to examine fluctuations in inflammatory and muscle damage markers, wellness status and physical fitness during a competitive soccer season, which included a congested period of match play. In addition, the inter-relations among these parameters were assessed. Our findings suggest a significant increasein inflammatory and muscle damage markers as well as wellness measures (stress, fatigue, DOMS, and Hopper Index) after the congested period of matchplay. Additionally, we observed a significant decrease in physical fitness performance.The change in wellness status was related to physical fitness performance decline **(Figure 4).**

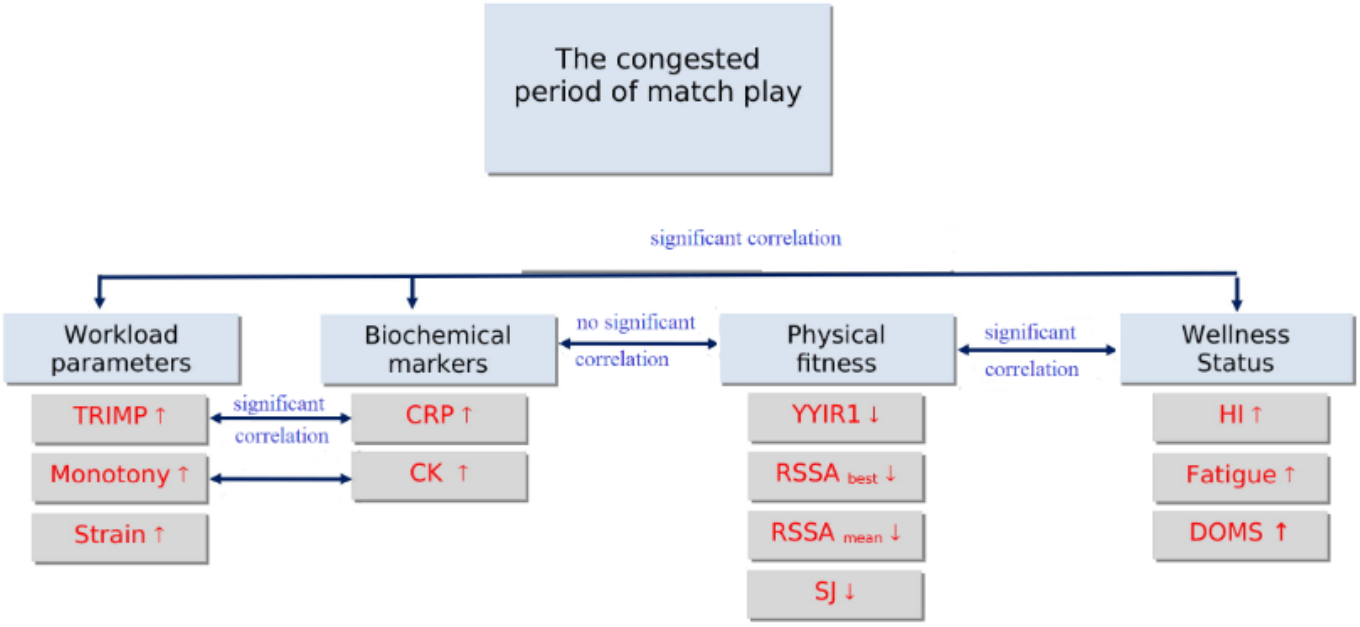


Figure 4. Summary of the main findings of this study.

TRIMP: training impulse. **CRP**: C-reactive protein. **CK:** créatine kinase. **YYIR1**: Yo-Yo intermittent recovery test level 1. **SJ**: squat jump. **TMD**: total mood disturbance.

Biochemical data

In the present study, we showed an increase in CRP and CK after the congested period of match play. These results arein agreement with data reported by Walker et al. (2019) who showed a significant increase in CK concentrations after 16 competitive weeks in female professional soccer players. The authors of this latter study demonstrated that the increase of CK could be caused by theelevated training load. In addition, Pimenta et al. (2015) showed an increase for CK concentration after the pre-season period when the players were exposed to higher volume and intensity of training. Therefore, investigators have speculated that

intensified training or match exposure could influence the increase of inflammation and muscle damage markers (Nédélec et al., 2012; Souglis et al., 2018). Activity during soccer matches and soccer training include high amounts of jumping, sprinting, and eccentric muscle actions (Mohr et al., 2003). These type of muscle activities will result in micro tear of structural and contractile components in the muscle fibers (Brancaccio et al., 2007) leading to increased serum CK levels (Hartmann et Mester., 2000).

Additionally, the current results suggest that the higher number of matches played in the last 6 weeks (10 matches) potentially affect resting CRP concentration. Conversely, the current results disagree with the results of Coppalle et al. (2019) who showed anon-significant changein CRP and CK levels after the pre-season period. Differences in study methodology between studies could partially explain these different outcomes. For example, the study of Coppalle et al. (2019) and the current study analyzed the soccer season at different time points (e.g., pre-period and 12 weeks competitive periods). Moreover, Coppalle et al. (2019), for technical reasons, analyzed theirblood samples one week after the end of the pre-season which may be sufficient time to allow the recovery of players. Of note, we did not observe alterations in the creatinine concentrations.This result is an agreement with Heisterberg et al., (2013) who showed a non-significant change for creatinine level during a six-month period of soccer training and competition. Thus, creatinine would only be of minor interest for bloodmonitoring of elite soccer players (Djaoui et al., 2017).

Wellness measures

Concerning the wellness status, we noted that the Hopper Index, stress, fatigue, and DOMSscores were higher in the congested period as compared to the regular period of match play. Moreover, we also noticed that Hopper index increases were strongly related to workload parameters.This resultagrees with data reported by Fessi et al., (2016) who showed significant associations betweentraining load parameters and wellness measures (sleep, stress, fatigue, and muscle soreness) in professional soccer players. Accordingly, our data corroborate the outcomes presented by Buchheit et al. (2013) and Thorpe et al. (2015) who demonstratedthat greater daily training loads affect wellness measures the following day in Australian rulesfootball and elite soccer players, respectively. It was hypothesized that fatigue and DOMS scores were affected by the greatertraining load applied along with the higher number of matchesplayed during the period of congested match play. Further, the increase in stress scores can be explainedbecause thisperiod included the most important matches, that is, the play-offs. In fact, the stress perception seems to requireinformation about the impact of

somatic and cognitive state anxiety of a pre-match situation (Madrigal et al., 2017). However, this possible increase in anxiety did not influence the quality of sleep, which is in line with other studies (Moalla et al., 2016; Thorpe et al., 2015). These findings might be indicative ofsleep perception notbeing sensitive to the workload. Indeed, for elite soccer, it was suggested that in a competitive period (with different training programs and level of competition), the perception of sleep quality may only provide information on potential recovery status rather than any association with workload (Clemente et al., 2017).

Relation between biochemical data and physical fitness performance

In this study, no significant correlations were observed between biochemical parameters and players' performance in the YYIR1, RSSA, SJ and CMJ tests. Thus, we observed that the decline in physical fitness performance during the congested period was associated with increases in CRP and CK concentrations. These findings agree with previous reports showing negative associations between changes in biochemical markers and performance declines with maintenance of higher CRP and CK values (Silva et al., 2014). In the current study, the decline in physical fitness after the congested period of match play may be reflected in the observed increase in inflammation (CRP) and muscular damage (CK). Hence, Walker et al. (2019) showed that the increased CK level was associated with the decline in vertical jump performance. It can reasonably be supposed that the alteration in CK concentration might affect explosive-type performance such as jumping. Moreover, increases in CK level without impairments in muscle performance have been reported (Nedelec et al., 2012; Silva et al., 2013*a*). Therefore, some caution should be taken when trying to predict muscle recovery and (or) impairments from changes in serum CK concentrations. Our results further suggest that the increase in CRP and CK noted in T3 after the congested period of match play may reflect tiredness or a state of fatigue caused by inflammation and muscular damage. To determine more precisely the influence of fatigue on these biological variations, it is necessary to address these two variables. To do this, we examined potential correlations between the wellness scores and biochemical data.

Relation between wellness and biochemical markers and physical fitness performances

In our study, the CRP and CK concentrations were significantly correlated with wellness scores (Hopper Index) during the congested period suggesting the involvement of wellness changes on CRP and CK responsiveness. Moreover, we found a significant positive correlation between the CRP and the fatigue score. This suggests that it is useful to follow

variations in CRP and CK level to determine the degree of tiredness and change in wellness or vice versa. In addition, there appears a negative relation between physical fitness and wellness measures during a congested period of match play. Thus, the increase in wellness score was associated with physical fitness declines. More precisely, we noticed that the Δ% of fatigue score correlated with Δ% YYIR1. In addition, the Δ% of DOMS score correlated with Δ% $RSSA_{best}$and we observed a positive correlation between Δ% $RSSA_{mean}$ and Δ% Hooper Index.These results cannot, however, be used to indicate whether changes in physical fitness are affected by changes in wellness scores (fatigue, DOMS, and stress) increased or vice-versa. Our results confirm that physical performance depends simultaneously on physical and psychometric status. Thus, it seems that the participation in a high number of matches during the congested period induced psychological stress on thesoccer players. The present results agree with previous findings showing a large correlation between changes in wellness scores and high-intensity running performance (Buchheit etal. 2013), which confirms the potential of these simple, non-invasive and non-fatiguing measures for monitoring training responses and physical performance in highly-trained teamsport players (Buchheit et al. 2012).

In this study, we showed a positive correlation between monotony and CK during the congested period of match play. In addition, changes in CRP level showed significant relations with workload parameters. These results confirm that some biochemical markers are sensitive to competitive periods and soccer training which is in agreement with the study of Walker et al. (2019) who showed that the CK increases are associated with training load increases and higher number of practices during 16 weeks of soccer training and competition. Thus, these biochemical levels can be used as a potential marker for variation in intensity of training and/or competition. A few studies exist that assessed inflammation, and muscular damage variations, wellness status and performance data in athletes (Moalla et al., 2016; Walkers et al., 2019). Our study additionally examined the associations between these factors. Findings from this study suggest that the observed changes in inflammatory and muscle damage parameters during the congested period of match play were related to wellness status (Hopper Index, fatigue, and DOMS scores) but not physical fitness parameters. We acknowledge that the use of the Hopper questionnaire is limited in team sports. Different motivational levels of the team players may have affected the outcomes of the Hopper questionnaire. Internal training load was only measured by session-RPE and this may have constrained the real quantification of the physiological impact of the training. External load was not used in this study, nevertheless the physical quantification of training impact may be an interesting set of variables to justify the perception of DOMS and fatigue.

This study has a number of limitations that warrant discussion. First, the study cohort was composed of a singleteam only which is why findings are specific to the examinedcohort and environment. Caution is needed when transferringour findings to female soccer or male soccer of different expertiselevel. Second, the sample size is rather small and the number of measurements and an assessment time point is limited. Obviously, this means our statistical interpretations may be under powered, and must be viewed with caution.

Conclusions and practical applications

The results of this study showed significant changes in selected biochemical markers, wellness status and physical fitness parameters during the final competitive period of the season (congested match play). The observed changes in biochemical parameters were related to declines in wellness scores (Hopper Index, Fatigue, and DOMS). Moreover, the physical fitness performance was related to wellness status.

The results of this study confirm the usefulness of wellness measures to provide information about the impact of training and competition on elite soccer players. In addition, our findings support the use of the Hopper questionnaire as a simple monitoring tool for identifying performance decrements in players exposed to highly competitive loads during the congested period.

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6 DISCUSSION GENERALE

L'objectif principal de cette thèse est de mieux comprendre l'impact du processus d'entraînement et de compétition intense (période congestive) sur les réponses biologiques (hématologiques, hormonales, inflammatoires), psychologiques et physiques chez des footballeurs élites. Ce travail de thèse nous permettra ainsi d'identifier les marqueurs et les indicateurs les plus pertinents qui aideraient à la gestion de la charge d'entrainement et à la prévention d'un état de fatigue ou de surentrainement durant la période compétitive congestive. Ce projet de thèse devra permettre d'améliorer les connaissances relatives à la fatigue engendrée par la congestion des matchs pendant la période compétitive et permettre de palier à cela.

L'objectif de la première étude était double ; 1) étudier les effets de six semaines d'entrainement et de compétition intense (période congestive) sur le volume plasmatique, les paramètres hématologiques, la charge d'entrainement et la performance physique chez des footballeurs élites ; et (2) examiner la relation entre ses différents paramètres. Nous avons émis l'hypothèse que des matchs successifs disputés pendant une courte période pourraient affecter le volume plasmatique et les paramètres hématologiques qui pourraient à son tour impacter la performance physique des footballeurs élites. Nos résultats ont montré qu'à l'exception du saut en contremouvement jump, tous les paramètres de performance évalués pendant les testes physiques (YYIRTL1, RSSA, et SJ) ont été affectés après la période congestive. En outre, le volume plasmatique et certains paramètres hématologiques ont été altérés (diminution du volume plasmatique et augmentation de l'hémoglobine et de l'hématocrite). Le déclin de la performance physique des joueurs pourrait s'expliquer principalement par la fatigue survenue après une période d'entrainement et de compétition intense (10 matchs joués en six semaines). Néanmoins, la baisse de la performance observée ainsi que l'altération des marqueurs hématologiques pourraient s'expliquer par les effets aigus de la dernière semaine de compétition. En effet, la monotonie et la contrainte de la dernière semaine étaient plus élevés que celles des semaines précédentes.

L'intérêt pratique de cette étude est qu'il peut fournir des informations utiles aux entraîneurs et au personnel médical sur la charge d'entrainement appliquée et la variation de la performance physique après la période congestive. Nos résultats peuvent aider à la gestion de la charge d'entrainement pour éviter le surentraînement et réduire le risque des blessures chez les joueurs de football. Les résultats trouvés dans la première étude illustrent clairement la nécessité des périodes de récupération suffisamment longues à la fin de la saison. Ceci est particulièrement important pour les joueurs qui sont nominés par leurs équipes nationales et

exécutent des tournois internationales après la saison régulière. De plus, nos résultats suggèrent que la période de récupérationa près la fin de la saison devrait être individualisée en prenant en compte le taux de participation de chaque joueur pendant les entrainements et les matchs. Enfin, les résultats la première étude soulèvent des inquiétudes sur les pratiques actuelles dans les clubs de football. Parmi celles-ci, les stratégies de rotation de l'équipe appliquées par les entraineurs afin de réduire le risque de blessures. Si l'objectif d'un entraineur est de développer la performance physique des footballeurs toute au long de la saison, il est recommandé de surveiller les paramètres de performance physique et les exigences physiologiques pendant la période congestive.

L'objectif de la deuxième étude était d'examiner les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs hormonaux, psychologiques et la performance physique et d'essayer de montrer s'il y a des relations entre tous ses paramètres. Nous avons émis l'hypothèse que les paramètres hormonaux et psychologiques pourraient être modifiés après la période congestive qui, à son tour, pourrait entraîner des changements négatifs de la performance physique des joueurs. Nous avons montré qu'il ya une altération significative des marqueurs hormonaux (diminution de la testostérone et du ratio T/C), psychologiques (augmentation du score total de perturbation de l'humeur [TMD]), et des paramètres de la performance physique après la période congestive. De plus la baisse de la performance physique est liée aux changements des paramètres psychologiques. Aucun lien n'est établi avec les changements des paramètres biologiques**.** En effet, le changement des états d'humeur reflètent la baisse de la performance physique des footballeurs pendant la période congestive. Ainsi, la surveillance des états d'humeur pourrait être un outil utile pour les entraîneurs et le personnel médical pour mieux gérer la charge d'entrainement et prévenir les blessures. De plus, la diminution de la concentration de testostérone et du ratio T/C était en relation avec de le score total de la perturbation de l'humeur (TMD), ce qui suggère que l'hormone anabolique (la testostérone) reflète mieux la perturbation de l'humeur que l'hormone catabolique (le cortisol). Cette deuxième étude complète la première. Elle nous a permet d'identifier, autre que les marqueurs biologiques (homonaux), des marqueurs psychologiques (le changement des états d'humeur évalués par le questionnaire du POMS) qui peuvent être un outil de surveillance simple et non invasive pour identifier la diminution de la performance chez des joueurs exposés à une charge compétitive élevée pendant la période congestive. Cette recommandation peut fournir des informations utiles pour les coachs et le personnel médical pour un meilleur suivi de la charge d'entrainement et de la performance physique afin de mieux gérer le ratio entrainement-récupération et réduire le risque des blessures.

L’objectif de la troisième étude était d’analyser les effets de la période compétitive (non congestive vs.congestive) dommages musculaires), les états du bien être (la qualité du sommeil, la fatigue, le stress et les douleurs musculaires (DOMS) et la performance physique chez des footballeurs élites. Nous avons montré une augmentation de la concentration de CRP et de CK après la période congestive. On peut supposer qu'un entraînement intense et/ou une période congestive pourrait influencer l'augmentation des marqueurs d'inflammation et de dommage musculaires (Nédélec et al., 2012; Souglis et coll., 2018). Les mouvements et l’activité d’un footballeur pendant les matchs et les entraînements de football comprennent de grandes quantités de sauts, de sprint et de contractions musculaires excentriques (Mohr et al., 2003). Ce type d'activité musculaire entraînera des microlésions dans les composants structurels et contractiles des fibres musculaires (Brancaccio et al., 2007) et par conséquent une augmentation de la concentration de CK dans le sang (Hartmann et Mester., 2000). De plus, les résultats actuels suggèrent que le nombre trés élevé des matchs joués au cours des six dernières semaines (10 matchs) a potentiellement affecté la concentration de CRP au repos. En outre, les scores du stress, de fatigue, les douleurs musculaires (DOMS) et l’indice Hopper étaient plus élevés pendant la période congestive par apport à la période non congestive. On a remarqué que l’augmentation de l'indice Hopper était fortement liée aux paramètres de la charge d’entrainement. Ce résultat est en accord avec les données rapportées by Fessi et al., (2016) qui ont montré une relation significative entre les paramètres de la charge d'entraînement et les indicateurs du bien être (sommeil, stress, fatigue et douleurs musculaires) chez des footballeurs professionnels. L’importance des matchs disputés pendant cette période de compétition peut expliquer l'augmentation du stress, car cette période comprend les matchs les plus importants et décisifs de la saison. Les changements des paramètres biochimiques n'étaient pas liés au déclin de performance physique, Par contre, les changements des états de bien être (Fatigue et DOMS) étaient en relation avec le déclin de la performance. Les résultats de cette étude confirment l'utilité de la prise en compte des états de bien être pour surveiller la performance physique des footballeurs élites. De plus, l'utilisation du questionnaire de Hopper comme simple outil de monitoring nous permet d’identifier la baisse de la performance des joueurs exposés à une charge compétitive élevée pendant la période congestive.

7 CONCLUSION

L’objectif principal de cette thèse est de mieux comprendre l'impact du processus d'entraînement et de compétition intense (période congestive) sur les réponses biologiques (hématologiques, hormonales, inflammatoires), psychologiques et physiques chez des footballeurs élites. Ce travail de thèse nous permettra ainsi d’identifier les marqueurs et les indicateurs (biologiques et psychologiques) les plus pertinents qui aideraient à la gestion de la charge d'entrainement et à la prévention d’un état de fatigue ou de surentrainement durant la période compétitive congestive.

La présente thèse comprenait trois études originales ayant pour objectifs de :

1) Etudier les effets de six semaines d’entrainement et de compétition intense (période congestive) sur le volume plasmatique, les paramètres hématologiques, la charge d’entrainement et la performance physique chez des footballeurs élites.

2) Etudier les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs hormonaux, psychologiques et la performance physique.

3) Etudier les effets de la période compétitive (non congestive vs.congestive) sur les marqueurs inflammatoires, les dommages musculaires, les états de bien être et la performance physique chez des footballeurs élites.
La relation possible entre tous ses paramètres a été explorée afin de fournir aux entraîneurs des outils utiles pour contrôler les stimuli d'entraînement et prévenir un état de surentraînement pendant la période congestive.

Nos résultats suggèrent que la période congestive modifie les divers paramètres biologiques, psychologiques et de performance physique chez des footballeurs élites. De plus la baisse de la performance physique est liée aux changements des paramètres psychologiques. Aucun lien n’est établi avec les changements des paramètres biologiques**.** Nos résultats nous amènent à suggérer que l'utilisation des questionnaires (POMS et Hopper) comme des outils de surveillances simples et non invasives nous permettent identifier le déclin de la performance chez des footballeurs exposés à une charge compétitive élevée pendant la période congestive.

Cette recommandation peut fournir des informations utiles pour les entraîneurs et le staff médical afin de mieux gérer la charge d’entrainement. L’objectif principal est d’optimiser la

performance physique des footballeurs et de prévenir un état de surentraînement chez des footballeurs élites.

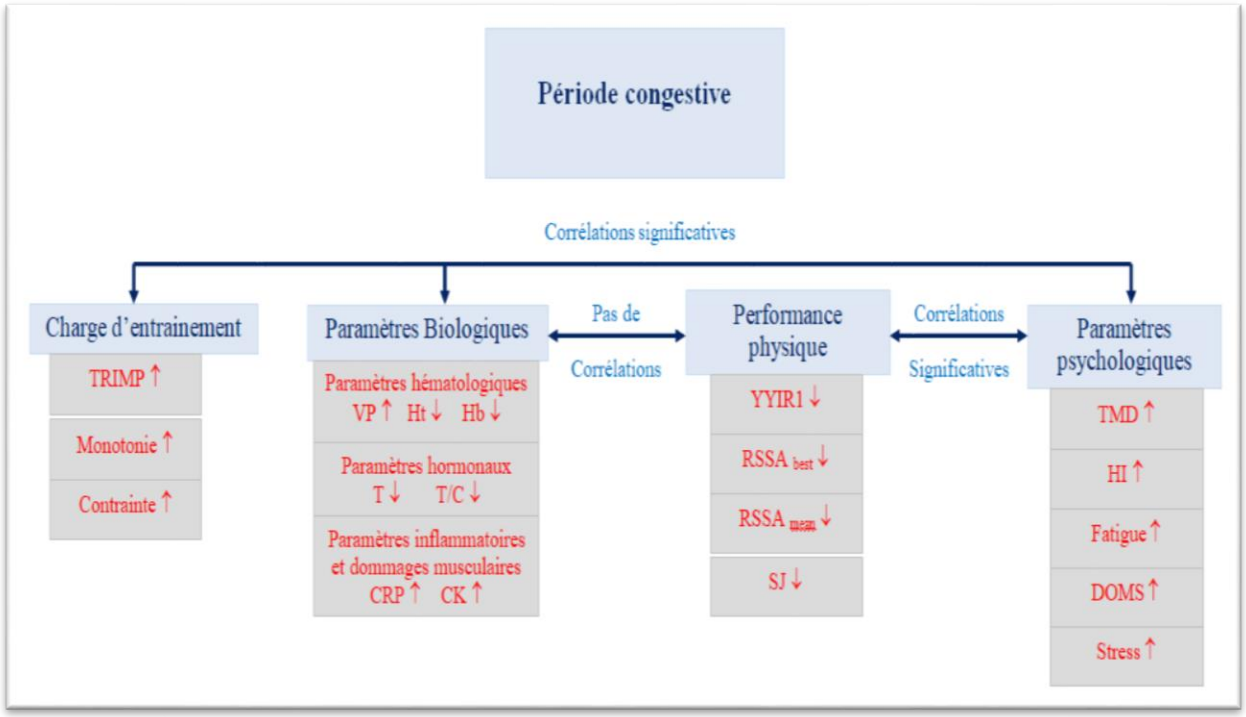


Figure 1. Résumé des principaux résultats de la thèse

VP: volume plasmatique. **Ht**: hématocrite. **Hb:**hémoglobine. **T**: testostérone. **T/C :** Testostérone /Cortisol ratio**. CK:** créatine kinase**. CRP:** C-réactive protein (proteine C reactive)**. YYIR1**: Yo-Yo intermittent recovery test level 1. **RSSA**: Repeated Shuttle Sprint Ability. **SJ**: Squat Jump. **CMJ**: countermovement Jump.**HI:** Hopper Index (Indice Hopper). **DOMS**: Delayed onset muscle soreness (Douleurs musculaires). **TRIMP**: training impulse**. TMD**: Total Mood Disturbance(score total de perturbation de l'humeur).

8 Perspectives

Au terme de ce travail de thèse, différentes perspectives peuvent être envisagées. En effet, ce travail permet de répondre à certaines questions relatives à l'influence des différents périodes compétitives (non congestive vs. congestive) sur les marqueurs biologiques (hématologiques, hormonales, inflammatoires), psychologiques, et la performance physiques chez des footballeurs élites. Toutefois, la période congestive n'a durée que six semaines ce qui est insuffisant pour bien comprendre et maîtriser les effets à long terme de la congestion des matchs durant la période compétitive.

Dans nos différentes études le suivi nutritionnel n'a pas été intégré. Il serait souhaitable, dans les futures études d'incorporer ces paramètres dans l'analyse de l'effet des différents périodes d'entrainement et de compétition (non congestive vs.congestive). De plus, d'autres paramètres endocriniens, inflammatoires (IL-6, TNF-α…) ainsi que d'autres paramètres physiques (qualités d'agilité, de coordination etc.), physiologiques (mesures directes en laboratoire etc.) devraient faire partie de ce genre d'études.

Dans nos différentes études, les prélèvements sanguins ont été réalisés au repos uniquement ce qui ne permet pas de rendre compte des réponses et des adaptations à l'exercice. Il serait intéressant de réaliser des prélèvements sanguins après les testes physiques (exemple., YYIRTL1, RSSA) et à la fin du match afin de bien préciser les différentes réponses hématologiques, hormonales, inflammatoires physiques et psychologiques à ce genre d'épreuve.

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10 ANNEXES

PLOS ONE

RESEARCH ARTICLE

Effects of a six-week period of congested match play on plasma volume variations, hematological parameters, training workload and physical fitness in elite soccer players

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Abstract

Objectives

The aims of this study were to investigate the effects of a six-week in-season period of soccer training and games (congested period) on plasma volume variations (PV), hematological parameters, and physical fitness in elite players. In addition, we analyzed relationships between training load, hematological parameters and players' physical fitness.

Methods

Eighteen elite players were evaluated before (T1) and after (T2) a six-week in-season period interspersed with 10 soccer matches. At T1 and T2, players performed the Yo-Yo intermittent recovery test level 1 (YYIR1), the repeated shuttle sprint ability test (RSSA), the countermovement jump test (CMJ), and the squat jump test (SJ). In addition, PV and hematological parameters (erythrocytes [M/mm^3], hematocrit [%], hemoglobin [g/dl], mean corpuscular volume [fl], mean corpuscular hemoglobin content [pg], and mean hemoglobin concentration [%]) were assessed. Daily ratings of perceived exertion (RPE) were monitored in order to quantify the internal training load.

Results

From T1 to T2, significant performance declines were found for the YYIR1 ($p<0.001$, effect size [ES] = 0.5), RSSA ($p<0.01$, ES = 0.6) and SJ tests ($p< 0.046$, ES = 0.7). However, no significant changes were found for the CMJ ($p = 0.86$, ES = 0.1). Post-exercise, RSSA blood lactate ($p<0.012$, ES = 0.2) and PV ($p<0.01$, ES = 0.7) increased significantly from T1 to T2.

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A significant decrease was found from T1 to T2 for the erythrocyte value ($p<0.002$, ES = 0.5) and the hemoglobin concentration ($p<0.018$, ES = 0.8). The hematocrit percentage rate was also significantly lower ($p<0.001$, ES = 0.6) at T2. The mean corpuscular volume, mean corpuscular hemoglobin content and the mean hemoglobin content values were not statistically different from T1 to T2. No significant relationships were detected between training load parameters and percentage changes of hematological parameters. However, a significant relationship was observed between training load and changes in RSSA performance ($r = -0.60$; $p<0.003$).

Conclusions

An intensive period of "congested match play" over 6 weeks significantly compromised players' physical fitness. These changes were not related to hematological parameters, even though significant alterations were detected for selected measures.

Introduction

In contemporary professional soccer, teams are confronted with a large number of matches throughout their competitive season, including league, cup, and international matches [1]. Tight timetables frequently demand that teams play consecutive matches with less than three to four days of recovery in-between competitions. It has been recommended that the recovery period between matches should be at least 72 h to avoid injuries and excessive residual fatigue [2]. Previous studies examined the effects of such a "congested period" of match play on physical and physiological performances in elite soccer players [3] and demonstrated that running activity wasnegatively affected during such periods. Of note, skill-related performance can also be affected by a decline in soccer players running capacity [4,5]. These studies provide important insight into the demands of congested match play, but they are limited in scope and few in number. For the safety of players, more research is warranted to better examine the negative effects of different congested periods on physical fitness, technical and tactical performance in elite soccer players. Furthermore, little is known on how an intensified in-season soccer period affects critical physiological markers such as hematological parameters and how these parameters may affect and explain the impairment of physical performance during congested periods of match play in soccer players.

Excessive physical exercise may influence body homeostasis and may result in impaired immune function and changes in hematological variables. This could lead to a state of overreaching which ultimately affects physical performance. It is mandatory [6] to monitor players' physical fitness together with physiological markers during different periods of a soccer season to improve training quality and to promote health for instance through lowering the risk of sustaining injuries. To this end, blood samples could be taken to monitor changes in hematological variables (e.g., plasma volume, hematocrit, hemoglobin) [6].

It is well known that most common hematological measurements (e.g., plasma volume [PV], hemoglobin [Hb], hematocrit [Ht], and red blood cells [RBC] count) are linked to the development of aerobic capacity [7]. Previous research suggested that intensive physical stress affects PV and hematological parameters, which may negatively influence players' physical performance [8,9]. Variation in PV and the parameters associated with it is considered an important form of body fluid adaptation in response to different training loads [10] and

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exercise intensity [11]. For example, Hb values determine the oxygen transport and consumption [12], which is linked to physical performance through aerobic capacity [13]. Ht levels are usually associated with enhancements in oxygen transport capacity [14]. The main function of RBC is oxygen transport and consumption. Thus, the longitudinal monitoring of Hb, Ht and RBC over 6 months revealed increases in hematological parameters at the end of the pre-season period in professional football [6]. According to Silva et al. [15], Ht was significantly increased after 3 months of training while it was decreased during the more intense periods of training in Brazilian soccer players. Moreover, Heisterberg et al. [16] monitored these hematological parameters in players from the best Danish league, and found a decrease in Hb and Ht as the competitive season progressed. In addition, the highest value of Ht and the lowest value of Hb were obtained after a period characterized by frequent matches [16]. Hence, these Blood parameter variations are related to the amount of aerobic and anaerobic training, strength training, and the number of matches per week [16]. Given the importance of these measurements for physical fitness, it is imperative to better understand the concurrent effects of a congested period of matches on physical and hematological parameters for a better management of training and competitive workloads.

To our knowledge, no studies have examined PV and hematological parameters of elite soccer players from the same team, before and after a congested period of match play and training. In addition, there are no studies available that quantified the relationships between training load parameters, hematological parameters and physical fitness. Thus, the aims of this study were twofold: i) to investigate the effects of a congested fixture period on PV, hematological parameters, and physical fitness in elite soccer players; and ii) to examine the relationships between training load, hematological parameters and physical fitness of elite soccer players. It was hypothesized that successive matches played during a short time period could negatively affect PV, hematological parameters which may have an impact on players' physical fitness [1, 16].

Materials and methods

Participants

Eighteen elite male Tunisian soccer players with a mean age of 20.1±0.4 years from the same first division Tunisian football team volunteered to participate in this study. Participants included three central backs, four fullbacks, seven midfielders, and four attackers. All players had been engaged in systematic training programs and in national competitions for at least 10 years. The included players were those with the longest match time (i.e., players who were most often in the starting line-up). Thirteen players performed all matches. The other 5 players trained with the first team and played their matches with the reserve team. The first and the reserve team had the same match schedule. None of the participants reported recent injuries that could interfere with study participation. Each player provided written informed consent to participate in this study after being fully informed about all experimental procedures. The study was carried out in accordance with the guidelines contained in the Declaration of Helsinki and was approved by the Ethics Committee of the medical unit of the included soccer club (Jeunesse Sportive Kairouanaise, JSK) and the Ethics Committee of the Scientific Council of the University of Rennes 2, France.

Procedures

All players were evaluated before and after a period of congested match play during the competitive season's play-off period. The first test session (T1) took place in the middle of April, while the second test session (T2) was performed at the end of the season (late May until early

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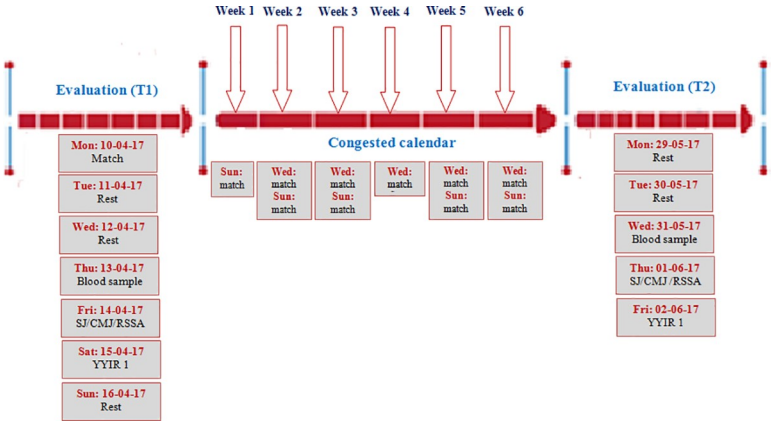


Fig 1. Experimental design. Evaluation T1: Before the period of match congestion Evaluation T2: After the period of match congestion Congested calendar: A six-week period of congested match play Mon: Monday, Tue: Tuesday, Wed: Wednesday, Thu: Thursday, Fri: Friday, Sat: Saturday, Sun: Sunday, Rest: a day of recovery RSSA: Repeated sprint shuttle ability SJ: Squat jump CMJ: countermovement jump YYIR: Yoyo intermittent recovery.

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June), after 6 weeks of competition. Evaluation of hematological parameters and physical fitness were performed over three-days. On the first day, a blood sample was collected to determine resting PV and hematological parameters. On the second day, participants performed three physical fitness tests in the following sequence: squat jump test (SJ), countermovement jump test (CMJ), and repeated shuttle sprint ability test (RSSA). On the third day, participants performed the Yo-Yo intermittent recovery test level 1 (YYIR1) (see **Fig 1** for experimental design). All tests were performed in the afternoon, 3 hours after having taken a light standardized meal. In general, the applied physical fitness tests chosen were regularly employed to monitor and assess players' fitness over the course of the season. To minimize diet-related changes in performance, players were asked to standardize and follow the same nutritional plan 24 hours before each testing session. Daily ratings of perceived exertion (RPE; Borg scale) were assessed in order to quantify the internal training load [17].

During the 6-weeks period, 10 matches were played. The number and duration of training sessions were the same for all players. The overall number of sessions was 26 (Table 1). The duration of each training session was 1 h 30 min for all players. Ten games were played over the 6-weeks study period. Training sessions were performed during the morning hours.

The 1st week and the 4th week were scheduled with 5 sessions per week, which were conducted on Tuesday, Wednesday, Thursday, Friday, and Saturday. On the 2nd, 3rd, 5th, and 6th week, four training sessions per week were executed on Monday, Tuesday, Thursday, and Friday. In addition, two matches were played per week (Wednesday and Sunday). Full recovery (i.e., day off) was provided on Saturday (see Table 1). The duration of each training session was 1h 30 min for all players. The weekly training load was evaluated from Monday to Sunday. The training program consisted of low/moderate intensity continuous running (i.e., 50–70% of maximum heart rate [HRmax]), high-intensity interval running (i.e., 90–100% of HRmax), specific soccer training drills according to the respective playing position, speed 'sprint' training over 10-30-m (short) and 30-60-m (long) with and without a ball, tactical training, technical training (attack against defense in small fields), small sided games, and strength training.

The strength training during the congested period was different with regards to duration and intensity from the pre-season or competitive season. The applied strength training sessions (25 min each week) consisted of circuit training to primarily improve muscle power. Plyometric exercises together with high velocity strength exercises were performed during

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Table 1. Training program during the congested period.

Day	Weekly program when playing one match per week (weeks 1 and 4)	Weekly program when playing two matches per week (weeks 2, 3, 5, and 6)
Monday	Recovery	Warm up, 15 min Low-to-moderate intensity aerobic training Tactical training, 30 min Technical training, 35 min
Tuesday	Warm up, 15 min Technical training, 30 min Low-to-moderate intensity aerobic training, 25 min Small sided games, 15 min	Warm up, 15 min Tactical training, 30 min High-intensity aerobic training, 10 min Small sided games, 15 min
Wednesday	Warm up, 15 min Strength training, 30 min Technical tactical training, 20 min Reduced game, 15 min	Match
Thursday	Warm up, 15 min Tactical training, 35 min Speed training, (short distances), 20 min Small sided games, 15 min	Low-to-moderate intensity aerobic training, 40 min Soccer specific training, 30 min
Friday	Warm up, 15 min Technical training, 30 min Speed training (long), 20 min Soccer specific training, 20 min	Warm up/ technical, 25 min Speed training (short), 15 min Small sided games, 25 min
Saturday	Warm up /technical, 25 min Speed training (short), 20 min Soccer specific training, 30 min	Recovery
Sunday	Match	Match

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circuit training. After strength training, players conducted shots to the goal. All training sessions were preceded by a 5–15 min standardized warm-up. All players completed all training sessions and completed all matches. However, only 3 substitute players had less match time than the rest of the cohort. Therefore, training load of the substitution players was increased during the day after the match to reach a similar overall level as the players who were active during the match. Table 1 summarizes the training characteristics during the congested period of match play.

Anthropometric testing

Body mass (kg) was measured to the nearest 0.1 (kg), with the participant in light clothing and without shoes, using a mechanical scale (M-i525, medical robe, France). Body height (m) was determined to the nearest 0.5 cm using a measuring tape fixed to a wall. The body mass index (BMI) was subsequently calculated (kg/m^2). Percent body fat (%BF) was estimated using four skin folds (biceps, triceps, sub-scapular, and suprailiac) measurements and assessed with a skin fold caliper (CZ-store, France) [18]. Lean body mass was calculated using the equation of Forsyth et al. [19], LBM (kg) = mass—(BF [%] × weight / 100).

Blood analyses

For the hematological parameters, 15 ml of venous blood were collected in the morning between 08:00–10:00 am. Blood samples were collected following an overnight fast, at least 12 h without exercise, and after the participants had remained seated for 30 min. Blood samples were drawn via the median ante brachial vein into vacutainer tubes (Vacuette, Greiner bioOne, France) without anti-coagulant and with EDTA. After being centrifuged at 2,500 (rpm) for 10 (min), the serum was stored at -70˚ C for determination of hematological parameters;

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erythrocytes (M/mm^3), hematocrit (%), hemoglobin (g/dl), mean corpuscular volume (fl), mean corpuscular hemoglobin content (pg), and mean hemoglobin concentration (%), in a flow cytometry (CelltacES, Japan).

The measured Ht and Hb were utilized in order to calculate plasma volume changes (ΔPV) and expressed as % ΔPV [20].

$$\%\Delta PV = 100 \times \left[\frac{Hb_B}{Hb_A} \times \frac{(1 - Ht_A \times 10^{-2})}{(1 - Ht_B \times 10^{-2})}\right] - 100$$

Where B refers to resting values (before the period of match congestion) for Ht and Hb, and A refers to the subsequent values for Ht and Hb after the period of match congestion.

Yo-Yo intermittent recovery test level 1 (YYIRTL1)

The YYIRT1 was used to assess players' endurance performance and the ability to repeat high-intensity exercise. Cones were placed on 2 lines 20 m apart and 5 m behind the starting line. Players ran two 20 m shuttles on a beep signal, separated by a 10 s recovery. The beep increased in stages and players continued the test until they were unable to maintain the required pace for 2 successive beeps. Players were required to place 1 foot either on or behind the 20-m line at the end of each shuttle. If a player did not reach the line in time, he was instructed that he must do so on the following run. The final stage (km/h) achieved and total distances covered (m) were recorded.

Repeated shuttle sprint ability

Repeated shuttle sprint ability (RSSA) was used to assess players' ability to cope with the intermittent demands of soccer. This test includes rapid changes of direction, which represents sport-specific movement patterns in soccer.

After a 15 min warm up, players completed 6 × 40 m maximal sprints interspersed with 20 s of passive recovery. Sprint times were recorded with a chronometer. The best sprint time was recorded for further analysis ($RSSA_{best}$). In addition, dependent variables comprised mean time obtained over six sprints, and the decrement in performance ($RSSA_{decrement}$ = ([RSA_{mean}] / [RSA_{best}] × 100)– 100) [21]. Blood lactate concentration was determined at the end of the test using a portable lactate analyzer (Lactate Pro2, Matsport, France).

Squat jump (SJ) and countermovement jump (CMJ)

The squat jump (SJ) started from a static semi-squatting position with knees at approximately ~90˚, and, after a short rest (1 s), the player jumped at maximal effort as high as possible. For the countermovement jump (CMJ), players started in a standing position, and performed an explosive jump using a slow stretch-shortening cycle (SSC) at ~90˚ knee flexion. Players were asked to jump at maximal effort and as high as possible. All players performed their vertical jumps in the same order (i.e., SJ followed by CMJ) with hands akimbo. All players performed three trials for each jump test, with a 60 s interval between each trial. The mean of the best two trials was taken for further analysis [22].

Quantification of training load

The total weekly training load (TWTL) was quantified on a daily basis by means of the session rating of perceived exertion (session-RPE) using an adapted Borg's 0–10 scale [17]. To ensure that the rating of perceived exertion was reflective of the entire session, data was collected 15–20 min following each training session. Monotony was calculated as the weekly average load

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divided by the standard deviation of the load. Strain was also calculated as the training load multiplied by monotony [23]. It has previously been suggested that these indices are associated with signs of overreaching and/or overtraining.

Statistical analyses

Data were expressed as mean values and standard deviations (SD). All statistical tests were conducted using Sigma Stat 3.5 software (Systat, Inc, USA). Normal data distribution was tested using the Kolmogorov-Smirnov test and confirmed. Paired t-tests for dependent samples were used to compare all parameters measured before and after the period of match congestion (T1 vs. T2). A one-way analysis of variance with repeated measures (ANOVA) was computed to determine the difference between the weekly training loads during the period of match congestion. Relationships between parameters were assessed using Pearson's product-moment correlation coefficients (r). The magnitude of the correlations was defined according to Hopkins's scale: r<0.1, trivial; 0.1–0.3, small; >0.3–0.5, moderate; >0.5–0.7, large; >0.7–0.9, very large; >0.9, nearly perfect; and 1, perfect. Statistical significance for all analyses was set at $p < 0.05$. The 95% confidence intervals (CI) and effects sizes (ES) were calculated to compare differences in mean values for all analyzed parameters before and after the six-weeks in-season period. When calculating ES, pooled standard deviations (SD) were used as no control group was available (Cohen's d = [M1-M2] / SD pooled). ES with values of 0.2, 0.5, and 0.8 were considered small, medium, and large, respectively [24].

Results

Anthropometrics

Changes in anthropometrics are illustrated in Table 2. No significant pre-to-post (T1 vs. T2) differences were found for the following measures: body height (p = 0.2; ES = 0.3), body mass (p = 0.1; ES = 0.3), BMI (p = 0.06; ES = 0.4), BF (p = 0.1; ES = 0.1), LBM (p = 0.4; ES = 0.1).

Physical fitness

In general, physical fitness declined over the 6-weeks period (Table 3). More specifically, significant decreases were found for the YYIR1 ($p \leq 0.05$; ES = 0.5), the RSSA ($p \leq 0.05$; ES = 0.6) and the SJ ($p \leq 0.05$; ES = 0.5). In contrast, CMJ performance did not change significantly (p = 0.86). Post-exercise blood lactate after the RSSA test increased significantly from T1 to T2 (p = 0.04; ES = 0.5).

Training load

Total weekly training load (TWTL), the calculated training monotony as well as the strain over the six training weeks are illustrated in Fig 2A, 2B and 2C. The TWTL of the 6th week was

Table 2. Effects of a congested period of match play on soccer players' body height, body mass index (BMI), body fat percentage (BF%), and lean body mass (LBM).

Anthropometric measures	Pre testing (T1)	Post testing (T2)	p-Value	Effect size (ES)
Body height (m)	1.78±0.04	1.78±0.05	0.3	0.2
Body mass (kg)	72.6± 5.1	72.2± ±5.1	0.1	0.3
BMI (kg/m^2)	22.7±1.19	22.6±1.14	0.06	0.4
BF (%)	8.2±2.9	8.5±2.4	0.1	0.1
LBM (kg)	66.6 ±4.3	66.1± 4.7	0.4	0.1

BMI: Body mass index, BF: Body fat, LBM: Lean body mass.

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Table 3. Changes in performance in physical fitness tests after the period of match congestion.

Fitness test	Pre testing (T1)	Post testing (T2)	p-value	Percentage change	Effect size (ES)
YYIR1 (m)	2520±362.6	1640±326.6	<0.001*	34.9%	5
RSSA mean (s)	8.07±0.3	8.28±0.3	0.01*	2.6%	0.6
RSSA best (s)	7.4±0.4	7.7±0.4	0.001*	3.8%	0.7
RSSA decrement (%)	7.8±4.7	6.6±3.1	0.3	0.06%	0.2
$[Lac]_{RSSA}$ (mM/L)	18.2±3.2	20.3±2.3	0.04*	11.5%	0.5
SJ (cm)	36.5±3.8	35.5±3.9	0.01*	2.5%	0.5
CMJ (cm)	39.0±3.6	39.2±2.4	0.8	0.4%	0.1

YYIR1: yoyo intermittent recovery test Level 1, Lac: Blood lactate concentrations; RSSA: repeated sprint shuttle ability, SJ: squat jump, CMJ: countermovement jump.
* = Significant difference between T1 (before the period of match congestion) and T2 (after the period of match congestion).

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higher than the TWTL during the first 4 weeks ($p \leq 0.01$; see Fig 2A for total weekly training load [TWTL] over the six training weeks of congested match play).

The monotony level of the 6th week was higher than that of the 1st week, the 3rd week, and the 5th week ($p \leq 0.01$). The monotony level during the 3rd week, the 4th week, and the 5th week were higher than that during the 1st week ($p \leq 0.01$) (see Fig 2B for monotony over the six training weeks of congested match play).

The strain experienced by the players during the 6th week was higher than that during the 1st, 2nd, 3rd, 4th, and 5th week ($p \leq 0.01$). The strain experienced during the 3rd, 4th, and 5th week were higher than that during the 1st week ($p \leq 0.01$; see Fig 2C for strain over the six training weeks of congested match play).

Hematological and plasma volume variation

Table 4 summarizes data of all analyzed hematological parameters. A significant decrease was found from T1 to T2 for erythrocyte count ($p = 0.018$; ES = 0.5), Hb ($p = 0.002$; ES = 0.8), and Ht percentage ($p = 0.006$; ES = 0.6). Conversely, MCV, MCHC, and MHC values did not change significantly over the experimental period, while PV increased significantly from T1 to T2 (6.1± 8.7%; $p = 0.01$; ES = 0.5).

Relationships between PV, hematological parameters, physical fitness, and training load parameters

Table 5 shows that there were no significant correlations between PV, Δ% of hematological parameters, and Δ% of physical fitness. However, there were significant positive correlations between Δ% of SJ and Δ% of Ht ($r = 0.54$; $p = 0.02$), MCV ($r = 0.51$; $p = 0.01$), and MCHC ($r = 0.62$; $p = 0.001$). In addition, there was a significant negative correlation between percentage change of SJ and PV ($r = -0.43$; $p = 0.03$).

Table 6 illustrates that there was no significant relationship between training load parameters and Δ% of hematological parameters (Hb [$r = 0.12$; $p = 0.6$], Ht [$r = -0.02$; $p = 0.9$], PV [$r = 0.12$; $p = 0.6$], erythrocytes [$r = -0.22$; $p = 0.2$], MCV [$r = -0.05$; $p = 0.9$], MCHC [$r = 0.22$; $p = 0.2$], MHC [$r = 0.41$; $p = 0.08$]). There were no significant relationships between monotony and Δ% of hematological parameters (Hb [$r = 0.3$; $p = 0.1$], Ht [$r = -0.12$; $p = 0.6$], PV [$r = -0.14$; $p = 0.6$], erythrocytes [$r = -0.34$; $p = 0.1$], MCV [$r = -0.12$; $p = 0.6$], MCHC [$r = 0.3$; $p = 0.1$], MHC [$r = 0.32$; $p = 0.1$]).

Moreover, there were no significant relationships between strain experienced and Δ% of hematological parameters (Hb [$r = 0.14$; $p = 0.5$], Ht [$r = -0.05$; $p = 0.8$], PV [$r = 0.18$; $p = 0.6$],

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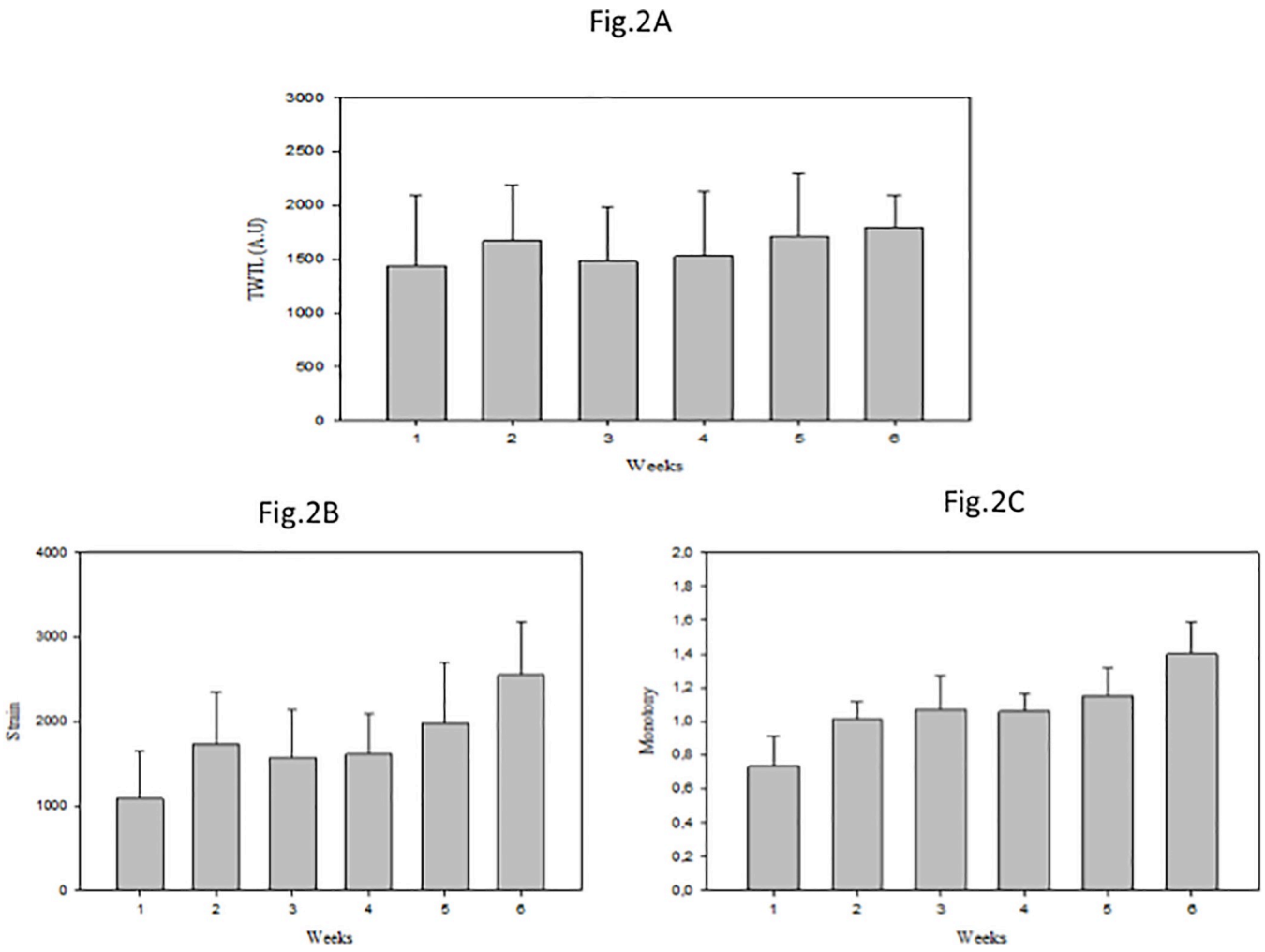


Fig 2. Total weekly training load (TWTL) (Fig 2A), the monotony (Fig 2B) and the strain (Fig 2C) of the six weeks training during the match congestion period. ** = Significant differences compared to week 1, week 3, and week 4 ($p \leq 0.01$). TWTL = Total weekly training load; A.U. = arbitrary units, monotony and strain.

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Table 4. Effects of the period of match congestion on soccer players' hematological parameters.

Hematological parameters	Pre-testing (T1)	Post-testing (T2)	p-value	Effect size (ES)
Erythrocytes (M/mm^3)	5.3 ± 0.4	5.1 ± 0.3	0.018*	0.5
Hemoglobin (g/dl)	14.9 ± 1.04	14.3 ± 0.7	0.002*	0.8
Hematocrit (%)	43.9 ± 2.7	42.5 ± 2.07	0.0006*	0.6
MCV (fl)	83.1 ± 4.5	82.9 ± 4.5	0.1	0.3
MCHC (pg)	28.05 ± 1.7	28.09 ± 1.6	0.4	0.1
MHC (%)	33.9 ± 0.7	33.7 ± 0.6	0.1	0.3
PV (T1-T2) (%)	6.1±8.7%		0.01*	0.7

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin content, MHC: Mean hemoglobin concentration, PV (T1-T2): Plasma volume variation between T1 and T2; YYIR1: yoyo intermittent recovery test Level 1, RSSA: repeated sprint shuttle ability, SJ: squat jump, CMJ: countermovement jump.

* = Significant difference between T1 (before the period of match congestion) and T2 (after the period of match congestion).

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Table 5. Relationships (Pearson correlation coefficient) between PV, percentage changes of hematological parameters and percentage change of physical fitness.

Hematological parameters / physical fitness	YYIR1	RSSA mean	SJ
Hemoglobin (g/dl)	p = 0.12; r = 0.21	p = 0.08; r = 0.15	p = 0.07; r = -0.4
Hematocrit (%)	p = 0.11; r = 0.19	p = 0.15; r = 0.21	p = 0.02*; r = 0.54
PV (%)	p = 0.9; r = -0.01	p = 0.4; r = 0.20	p = 0.03* ; r = -0.42
Erythrocytes (M/mm^3)	p = 0.8; r = 0.04	p = 0.4; r = -0.12	r = 0.4; p = 0.06
MCV (fl)	p = 0.6; r = 0.15	p = 0.3; r = -0.22	p = 0.01*; r = 0.51
MCHC (pg)	p = 0.5; r = -0.12	p = 0.3; r = -0.20	p = 0.001*; r = 0.64
MHC (%)	p = 0.6; r = -0.14	p = 0.2; r = -0.25	p = 0.1; r = 0.32

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin content, MHC: Mean hemoglobin concentration, PV (T1-T2): Plasma volume variation between T1 and T2. YYIR1: yoyo intermittent recovery test Level 1, RSSA: repeated sprint shuttle ability, SJ: squat jump, CMJ: countermovement jump.
(*) Significant relationship between parameters.

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erythrocytes [r = -0.34;p = 0.1], MCV [r = -0.08; p = 0.7], MCHC [r = 0.30; p = 0.2], MHC [r = 0.42; p = 0.08]).

There was no significant relationship between performance in the YYIR1 and training load parameters (r = 0.14; p = 0.5), monotony (r = -0.01; p = 0.9), and strain (r = 0.12; p = 0.6). There was, however, a significant relationship between training load and strain, with RSSA (r = -0.64; p = 0.003; r = -0.63; p = 0.005, respectively).

Discussion

The main purpose of this study was to analyze the effects of a 6-week period of congested match play on PV, hematological parameters, training load and physical fitness in elite soccer players. In accordance with our research hypothesis, the main results of this study were significant modulations in soccer players' PV, selected hematological parameters, and physical fitness after an intense 6-weeks match congested period. However, the observed changes were not related to declines in physical fitness other than SJ performance.

In this study, Hb and Ht values decreased after the 6-weeks congested period. It is well established that training may alter homeostasis, including hematological parameters. Hb is a

Table 6. Relationships (Pearson correlation coefficient) between PV, percentage changes of hematological parameters, physical fitness, and workload parameters.

Hematological parameters and training load	TWTL	Monotony	Strain
Hemoglobin	p = 0.6; r = 0.12	r = 0.30; p = 0.1	p = 0.5; r = 0.14
Hematocrit	p = 0.9; r = -0.02	p = 0.6; r = -0.12	p = 0.8; r = -0.05
PV	p = 0.6; r = 0.12	p = 0.6; r = -0.14	p = 0.6; r = 0.18
Erythrocytes	p = 0.2; r = -0.22	p = 0.1; r = 0.34	p = 0.1; r = -0.34
MCV	p = 0.9; r = -0.05	p = 0.6; r = -0.12	p = 0.7; r = -0.08
MCHC	p = 0.2; r = 0.22	p = 0.1; r = 0.30	p = 0.2; r = 0.30
MHC	p = 0.08; r = 0.41	p = 0.1; r = 0.32	p = 0.08; r = 0.42
YYIR1	p = 0.5; r = 0.14	p = 0.9; r = -0.01	p = 0.6; r = 0.12
RSSA-mean	p = 0.003*; r = -0.60	p = 0.2; r = -0.22	p = 0.005*; r = -0.63
SJ	p = 0.8; r = -0.54	p = 0.5; r = 0.14	p = 0.8; r = -0.03

MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin content, MHC: Mean hemoglobin concentration, PV (T1-T2): Plasma volume variation between T1 and T2. YYIR1: yoyo intermittent recovery test Level 1, RSSA: repeated sprint shuttle ability, SJ: squat jump, CMJ: countermovement jump, TWTL: Total weekly training load.
(*) Significant relationship between parameters.

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key determinate of oxygen transport and consumption [25], which is related to physical performance, primarily aerobic capacity [26,27]. Elevated Hb is generally associated with an increase in blood oxygen transport capacity and aerobic performance, while an increase in Ht increases blood viscosity [28]. The kinetics of variation of these hematological parameters may partly explain players' aerobic performance. Thus, it seems beneficial to monitor soccer players' Hb and Ht parameters.

Several studies showed decreases in Hb and Ht values after periods of intense training or competition [6,7,26]. These declines are known as an adaptation to training and called sports pseudo-anemia [29,6]. In the concept of sports pseudo-anemia, stimulation of the erythrocytosis during exercise induces an increase in the absolute concentration of Hb [6]. This mechanism is masked by a rise in PV [15,30,31]. Sports anemia can be treated as a first sign of overtraining or regular training that can lead to altered aerobic performance [31]. To date, only a few studies have evaluated Hb and Ht variations during a competitive season in soccer players [29, 15]. For example, in a previous study by Silva et al. [15], it was observed that Hb and Ht of Brazilian soccer players were significantly increased after 12 weeks of soccer training. These authors postulated that these alterations occurred due to plasma volume changes (i.e., hemoconcentration). In fact, in this study [15], PV decreased by 11.2% after the soccer-training program and was significantly correlated to the alteration percentage of Hb and Ht changes. In contrast, Heisterberg et al. [16] recorded no significant changes in Hb and Ht levels over a 6-months period in which the training and match load varied considerably. These discrepancies in results may be mainly explained by differences in the experimental protocols involved, and factors such as training intensity, duration, frequency, and players' expertise level.

In the present study, we hypothesized that the period of congested match play would negatively affect plasma volume and hematological parameters, which, in turn, would lead to negative changes in players' physical fitness. The current results showed a significant decrease after the period of match congestion with respect to the values of erythrocytes. In agreement with our results, many studies suggest that the number of erythrocytes is decreased at the end of a competitive period in soccer [6]. In general, erythrocytes, Hb, and Ht decrease after endurance training [32]. This is mainly caused by PV expansion [33]. In contrast, Silva and al. [15] showed training-induced increases in erythrocytes after 12 weeks of training in Brazilian soccer players. A possible explanation for this increase might be plasma volume changes [15,26]. In fact, Silva et al. [15] were able to show that the altered percentage of erythrocytes significantly correlated with plasma volume change (i.e., reductions) during the 12-weeks soccer-training program. As to MCV, MHC and MCHC response, our data showed no significant changes after the period of match congestion. These results are not in accordance with findings from Meister et al. [34] who observed a decrease in MCH and MCHC values and an increase in MCV values during a competitive period. These differences in findings can primarily be explained by psychological factors, players' diet [9,15], and/or differences between players' effective match time.

In our study, PV measured after a 6-week period of match congestion was significantly higher (+ 6.1%) in T2. In contrast, Silva et al. [15] demonstrated a decrease in PV after 12 weeks of specific soccer training. The specifics of the soccer-training program and/or participants' expertise level could explain the contradictory findings. For example, our mean weekly training volume was lower (= 9 h/week) than that of a previous study conducted by Silva and colleagues with Brazilian soccer players (14.7–16.3 h/week) [15]. Additionally, our study period lasted 6 weeks which is less than the study of Silva et al. [15] (12 weeks). This might also be a reason for the different outcomes in the two studies.

Previous studies showed that improved endurance performance was accompanied by increases in PV and decreases in Ht [35]. This finding can be explained by auto-hemodilution,

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which is manifested by the decrease in blood viscosity [36,37]. This decrease in Ht together with the increase in PV improves physical fitness performance by decreasing peripheral vascular resistance and ventricular post load [37]. Thus, the beneficial effects of training were not a decrease in PV and an increase in Ht, but rather the opposite.

Our findings with regards to the observed changes in hematological parameters and how they impacted on performance are contradictory to the results of previous studies [15,35]. In fact, the decline in physical fitness performance of elite soccer player after the period of match congestion was accompanied by an increase in PV and a decrease in the values of Hb and Ht. Thus, our results confirm that the soccer players enrolled in this study did not show signs of sport anemia as was reported for endurance athletes [31]. A likely explanation for the different outcomes could be that training volume of endurance athletes is much higher compared with that of soccer players. The training program of soccer players is characterized by a large variety of training contents (e.g., tactical/technical drills, physical fitness [endurance, strength, speed, power]). Therefore, the training of one specific physical quality is less intense in soccer compared with an endurance related sport that demands high levels of aerobic capacity during training. This may additionally explain the observed discrepancy in outcomes between our study and previous studies. Consequently, the type of training associated with soccer may be the main reason for the observed changes in erythrocytes, Hb, Ht, and PV variations [15,16].

Interestingly, we found no significant correlations between PV, hematological parameters, and players' performance in the YYIR1 and RSSA tests. In contrast, several studies conducted with endurance athletes and soccer players showed significant negative correlations between Ht and measures of physical fitness, suggesting increased performance with a decreasing Ht value [6,27]. In addition, there is evidence that Ht correlates well with the development of VO_{2max} in young soccer players [38]. Moreover, Abdderrahman et al. [35] showed a significant positive correlation between PV and VO_{2max} in physical education students.

Notably, Silva et al. [38] showed that Ht increased with increasing training volume over a 6-week soccer-training program. This is indicative of the reactivity of hematological parameters to variations in training volume. In addition, Colombini et al. [39] reported that Ht values were higher after a soccer match due to a decrease in PV. In the present study, the changes observed in hematological parameters and training load were not related to declines in physical fitness other than SJ performance. This difference in findings among the studies may in part be explained by experimental factors such as the level of participants' expertise, the respective training period, the training protocol, and the utilized testing methodology.

The observed decline in players' physical fitness in this study could mainly be explained by fatigue which occurred after the application of the heavy training load and the large number of matches (10 matches during 6 weeks). Nevertheless, the observed performance decline together with altered hematological markers could also be explained by the acute effects of the last week of competition as opposed to an aggregated effect. In fact, the monotony and strain experienced by our players during the last week were higher compared with that during the previous weeks.

Interestingly, Meister et al. [34] assessed the effects of a 3-week period of high match exposure (>270 min during 3 weeks) compared with a low (<270 min during 3 weeks) match exposure period in German male elite soccer players on hematological markers. In the study of Meister et al. [34], no relevant alterations were observed in the analyzed parameters. It might be argued that the periods of high match exposure were too low to induce alterations in physical fitness. Thus, it seems plausible that the training volume was intentionally reduced during this period to avoid excessive overload [34]. However, the average match exposure in our

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study was 1.3 matches per week which equals 900 min of match time over the 6 weeks intervention period. This indicates that the high match exposures time of elite soccer players could partly explain the observed performance decline. In contrast to the procedures reported by Meister et al. [34] on training volume, the participating coaches of this study rated training volume as highly important performance criteria which is why similar training volumes were scheduled in weeks with one or two matches.

In this study, physical fitness significantly declined over the 6-weeks experimental period. It therefore seems justified to suggest that physiological signs of fatigue or maybe overreaching were present after the 6-week period with 10 matches. However, the analysis of training load clearly proved that the highest loads were applied during the last week compared with the previous weeks. These acute effects of the last week of competition could partly explain the observed physiological signs of fatigue at T2. This is supported by the distance covered during the YYIR1 that was reduced by 34.9%. A possible explanation for this decline could be impaired cell contractile properties. In fact, structural damage has been suggested to interfere with optimal muscle glycogen store replenishment which is required if the player performs prolonged high-intensity intermittent exercise [38]. The parameters of the repeated sprint schedule ability test (RSSA-test); $RSSA_{mean}$ and $RSSA_{best}$, increased by 2.6% and 3.8%, respectively. This performance enhancement may be explained by the observed 15.8% increase in lactate level measured at the end of the test. The increase in lactate can be explained by at least two hypotheses. First, it could be caused by an increase in energy production through the lactic anaerobic system and/or a decrease in energy production through the anaerobic alactic system. Accordingly, soccer players may exhibit decreases in stored phosphagen (ATP-CP) in the muscles at the end of an intense training period [40]. The second assumption is the decrease in the players' ability to eliminate lactate as a result of the performance decline in the aerobic system [41]. We speculate that the decrease in performance in the first sprint during the RSSA-test is a result of the degradation of the efficacy of the alactic anaerobic system. The observed increase in time to complete the first sprint could be related to a depletion of the ATP-CP energy reserves [41]. This however is speculative and needs to be verified in future studies. Further, the examined decline in RSSA and YIYR1 performances may result from mechanical (e.g., force generation) and metabolic (e.g., glycogen depletion) impairments [42].

Soccer players' SJ performance decreased by 2.5% while CMJ performance was unaffected. A potential explanation for this finding could be that alterations in peripheral neuromuscular function occurred which were induced by the reiteration of damaged skeletal muscle fibers [43]. These results confirm the fatigue state of elite soccer players after the congested period (10 matches over 6 weeks). However, it is important to note that in the current study, the assessment of physical fitness occurred 72 hours after termination of the congested period. Therefore, it appears plausible that playing multiple games over a prolonged period (6 weeks) together with an increased training load during the last week could have further reduced the time required to restore neuromuscular system properties. It is possible that changes in physical fitness were due to inadequate recovery during the congested match period, particularly during the last week of the intervention period. This may have caused the observed declines in protein synthesis rate, glycogen resynthesize, and the ability to generate force within the musculature [44], which would relate to muscular performance measures such as speed and power ($RSSA_{mean}$, $RSSA_{best}$, and SJ).

Similarly, Bangsbo et al. [45] suggested that the alteration of soccer players' physical performance during the season might be due to the lack of in-week physical training caused by the mid-week matches. In this regard, Impelizzeri et al. [46] reported that soccer players' ability to jump, sprint and perform repeated high intensity exercises was impaired after 6-weeks of training when playing two matches per week. Alternatively, reduced performance could be

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explained by increases in muscle soreness [47]. In general, muscle soreness peaks 24 to 48 hours after physical exercise. This exercise-induced phenomenon is called delayed onset muscle soreness (DOMS) [48].Furthermore, the deterioration of physical fitness may also be related to other psychological factors caused by the negative results of the team that won only 2 out of 10 games that were played during this period.

The present study does have a number of limitations that warrant discussion. First, we examined only one soccer team in this study. Hence, our sample size and the training regime of the subjects are limited. Second, only a small number of physiological and physical fitness tests were applied. Thus, our findings are specific to these outcomes only. Future studies should examine additional biochemical, immunological and psychological markers to further elucidate this research topic and provide more insight.

Conclusions and practical implications

This study examined the effects of a congested match period on PV, hematological parameters and physical fitness in elite soccer players. The results of this study showed significant changes in various hematological parameters and physical fitness, which suggests potentially negative effects of the training load and match play during the experimental period. Specifically, the congested period of match play resulted in declines in physical fitness that seemed related to TWTL.

This research may provide useful information for coaches and medical staff with regards to TWTL and physical fitness performances during an intensive period of congested match play. Our findings may aid to better manage strain during exercise to avoid overreaching and overtraining, while reducing the risk of sustaining injuries in players. Our findings clearly illustrate the need for sufficiently long recovery periods at the end of the season. This is of particular importance for players who are nominated for their national teams and perform international tournaments after the regular season. Moreover, our results suggest that the recovery period after the end of the season should be individualized to take each player's exposition in terms of match and training time into account. Finally, results from this study raise some concerns with regards to current practices in soccer clubs. Among the key questions are the application of team rotation and recovery strategies to reduce the risk of injuries. If the goal is to develop soccer players' physical fitness over the course of a season, it is recommended to monitor physical and physiological demands during congested periods of match play.

Supporting information

S1 Data. Individual data of anthropometrics, hematological values and performances of the participating soccer players.
(XLSX)

S2 Data. Individual data of plasma volume variations of the participating soccer players.
(XLSX)

S3 Data. Individual data of training load, monotony and strain of the participating soccer players.
(XLSX)

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DOCTORAT / EDUCATION
BRETAGNE / LANGAGES, INTERACTIONS
LOIRE / COGNITION, CLINIQUE

UNIVERSITÉ RENNES 2

Titre : **Réponses biologiques et psychologiques chez les footballeurs élites : Effets de la congestion des matchs**

Mots clés : état d'humeur, état de bien être, performance physique, récupération, biologie, fatigue

Résumé : Les joueurs de football sont parfois obligés de jouer des matchs successifs avec seulement 2-3 jours de récupération. La période durant laquelle les joueurs jouent plusieurs matchs successifs est appelée « période congestive ». L'objectif principal de cette thèse est de mieux comprendre l'impact du processus d'entrainement et de compétition intense (période congestive) sur les réponses biologiques, psychologiques et physiques chez des footballeurs élites. Ce travail de thèse nous permettra ainsi d'identifier les marqueurs et les indicateurs les plus pertinents qui aideraient à la gestion de la charge d'entrainement et à la prévention d'un état de fatigue ou de surentrainement durant la période congestive.

La présente thèse comprend trois études ayant pour objectifs (1) d'étudier les effets de la période compétitive (non congestive vs. congestive) sur les marqueurs biologiques (volume plasmatique, paramètres hématologiques, hormonaux, inflammatoires, et les dommages musculaires), psychologiques et la performance physique chez des footballeurs élites ; (2) d'examiner la relation entre les marqueurs biologiques, la charge d'entrainement, les paramètres psychologiques et la performance physique chez des footballeurs élites.

Nos résultats ont montré que la période congestive modifie les divers paramètres biologiques, psychologiques et de performance physique. De plus la baisse de la performance physique est liée aux changements des paramètres psychologiques. Aucun lien n'est établi avec les changements des paramètres biologiques**.**

Nos résultats nous amènent à suggérer que l'utilisation des paramètres psychologiques comme des outils de surveillances simples et non invasives nous permettent d'identifier le déclin de la performance chez des footballeurs exposés à une charge compétitive élevée pendant la période congestive.

Cette recommandation peut fournir des informations utiles qui aideraient les entraîneurs et le staff médical à la gestion de la charge d'entrainement afin d'optimiser la performance et de prévenir un état de surentraînement.

Title: **Biological and psychological responses in elite soccer players: Effects of match congestion.**

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Keywords: Mood states, wellness status, physical performance, recovery, biology, congested calendar

Abstract : Soccer players are often play consecutive matches with only 2-3 days of recovery in between. This period of intense competition is called « a congested period » can lead to a state of overreaching which ultimately affects physical performance. The main objective of this thesis is to better understand the impact of training process and intense competition (congested period) on biological, psychological and physical responses in elite's soccer players. This thesis work will allow as identifying the most relevant markers and indicators that would help in the management and the prevention of fatigue or overtraining during the congested period of match play.

Three experimental studies were conducted to investigate (1) the effects of the competition period (uncongested vs. congested) on biological markers (plasma volume, hematological, hormonal, inflammatory and muscle damage markers), psychological parameters and physical fitness; (2) to examine the relationships between biological markers, training load, psychological parameters and physical fitness in elite soccer players.

Results showed a significant alteration in biological, psychological and physical fitness parameters after the congested period of match play. However, while the changes observed in biological parameters (hormonal, inflammatory and muscle damage markers) were not related to physical performance fitness, the changes in psychological parameters were related to performance decline in physical fitness. Our findings lead us to suggest that the use of psychological parameters as an appropriate and simple monitoring tool for identifying performance decrements in players exposed to highly competitive loads during the congested period.

This recommendation may provide useful information for coaches and medical staff for better monitoring workload and physical fitnees to manage players' work-strain ratio to optimize performance and to overreaching - overtraining.

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