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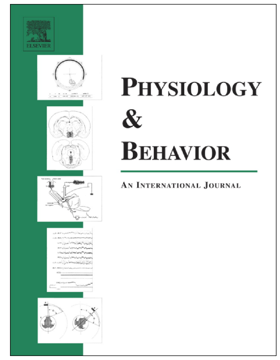

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Monitoring training load and fatigue in soccer players with physiological markers.

Léo Djaoui [1]*, Monoem Haddad [2], Karim Chamari [3$], Alexandre Dellal [1,4$]

1. Inter-university Laboratory of Human Movement and Biology (LIBM), University Claude Bernard Lyon1, University of Lyon, Villeurbanne, France; leo.djaoui@gmail.com
2. Sport Science Program, College of Arts & Sciences, Qatar University, Doha, Qatar; mhaddad@qu.edu.qa
3. Athletic Health and Performance Research Centre, Aspetar, Qatar Orthopaedic and Sports Medicine Hospital, Doha, Qatar; karim.chamari@aspetar.com
4. FIFA Medical Centre of Excellence, Centre Orthopédique Santy, Sport Science and Research Department, Lyon, France; alexandredellal@gmail.com

(**$.** These authors have contributed equally to this work.)

**Corresponding author*:
Leo Djaoui
Inter-university Laboratory of Human Movement and Biology, University of Claude Bernard Lyon 1, Bâtiment Raphaël Dubois, 27-29 boulevard du 11 Novembre 1918, Campus de la Doua, 69622 Villeurbanne Cedex, France
Tel.: +33(0) 472 432 848
Fax: +33(0) 472 432 846
E-mail: leo.djaoui@gmail.com

Abstract

The quantification and monitoring of training load (TL) has been the topic of many scientific works in the last fifteen years. TL monitoring helps coaches to individually prescribe, follow-up, analyse, adjust and programme training sessions. In particular, the aim of the present review was to provide a critical literature report regarding different physiological markers of TL monitoring, particularly in soccer, as the load is specific to individual sports. Therefore, the interests and limitations of heart rate (HR), HR variability (HRV) and biochemical variables (blood, urinary and hormonal variations) were analysed, with a special focus on daily measures (before, during and after training) and monitoring throughout a whole season. It appears that the most relevant markers were the resting HR before training, HR reserve during training, HRV during rest days, blood lactate, and blood and salivary immunological status in follow-ups throughout the season. Urinary markers indicative of the players' hydration status also deserve attention. However, these objective markers should be considered with a subjective marker of TL such as the rating of perceived exertion to give a more precise quantification of TL and its perception. Future research could be directed towards urinary marker analysis and the analysis of specific markers of TL, which could be related to injury occurrence and to performance during competition.

Key words: recovery; performance; overtraining; blood sample; heart rate variability

Abbreviations:

A = Adrenaline
ANS = Autonomic nervous system
C = Cortisol
CK = Creatine kinase
Cn = Cortisone
HDL = High-density lipoprotein
HF = High frequency
HIT = High-intensity training exercise
HR = Heart rate
HR_{max} = Maximal heart rate
HR_R = Recovery heart rate
HR_{res} = Reserve heart rate
HR_{rest} = Resting heart rate
HRV = Heart rate variability
IgA = Immunoglobulin A
LDL = Low-density lipoprotein
LF = Low frequency
NA = Noradrenaline
rMSSD = The square root of the mean of the sum of the squares of differences between adjacent normal R-R intervals
RPE = Rating of perceived exertion
SD = Standard deviation
sIgA = Salivary IgA
SSG = Small-sided games
T = Testosterone
TC = Total cholesterol
TL = Training load
TRIMP = Training impulse
URTI = Upper respiratory tract infection

VO_2 = Oxygen uptake

1. Introduction

Soccer is characterized by high neuromuscular demands with accelerations, decelerations, changes in direction, jumps and tackles [1]. In elite soccer, one to three matches can be played in a 7-day period. Thus, elite soccer programmes are designed by coaches and staff to prepare the players to repeat these high neuromuscular demand efforts several times a week during a whole season (usually 9 to 11 months of continuous training and competition [1]). While designing training programmes appears to be the first step of training management, monitoring the impact of the sessions on players appears be the second important step towards being successful in the training process. Indeed, both training and matches induce physiological changes that are important to assess. Thus, monitoring these changes would give coaches an indication of the individual internal training load (TL) so that adjustments could be made to the training regimen [2,3]; therefore, overtraining could be avoided, fitness and performance could be optimized [2], and the occurrence of injury and illness could be reduced [4,5]. Additionally, a player's turnover could be managed when necessary.

Subjective measures such as the rating of perceived exertion (RPE) [6], for which players are asked to grade their own perceived load, could be used to quantify fatigue and TL. The latter parameter could also be assessed based on objective measures using both external (e.g., distance covered at different speeds, number of sprints, and accelerations) [7] and internal (e.g., heart rate, oxygen uptake, and blood, urinary and salivary markers) [8] indicators of training intensity. Within the present review, we specifically aimed to present the different physiological indicators. Therefore, heart rate measures at different times before, during and after soccer efforts/sessions will be introduced and criticised. Following this discussion, different markers obtained from blood, salivary and urinary sampling will be described to present their contributions and limitations in a training monitoring process in soccer players.

2. Heart rate

Heart rate (HR) can be used to monitor TL as follows: (i) during exercise with exercise HR (HR_{ex}), percentage of maximal HR ($\%HR_{max}$), and percentage of reserve HR ($\%HR_{res}$); (ii) just after exercise with recovery HR (HR_R); and (iii) to monitor TL and the state of fatigue at rest by the means of HR variability (HRV) and resting HR (HR_{rest}) [9,10]. HR is the most common physiological parameter used in soccer as it has been validated as an indicator of workload in different types of exercises and sessions in soccer [11,12,13]. In general, while making individual comparisons, a lower HR correlates with a better fitness level in the player tested [2]. Indeed, during exercise it would mean that for a given intensity or at rest, the heart would not need to beat as fast due to better efficiency of heart pumping mechanisms such as an increase in stroke volume, cardiac muscle hypertrophy and/or an improvement in oxygen transport mechanisms [15,16,17]. In the same way, during post-exercise, it would mean that the player recovers faster from a given exercise intensity. Within the HR measurement, changes in HRV have also been examined and reviewed by several authors as a relevant and practical tool to monitor TL and fatigue in athletes [1,18,19,20]. HRV can be measured from the variation in the R-R intervals on an electrocardiogram. For instance, HRV is represented in a time domain in milliseconds, or in a frequency domain, which is the frequency at which the length of the R-R interval changes [9,20]. In this frequency domain, the contribution of parasympathetic activity prevails in high frequency (HF) power peaks (0.15-0.40 Hz) [11], and both sympathetic and parasympathetic systems contribute to low frequency (LF) power peaks (0.04-0.15 Hz) [21]. The ratio LF:HF is another index to measure HRV to reflect autonomic reactivity, with high values of this ratio reflecting sympathetic dominance [22]. The square root of the mean of the sum of the squares of differences between adjacent normal R-R intervals (rMSSD) is the usual cardiac parasympathetic activity-related index as it is not affected by breathing [23]. Another usual index calculated as a vagal-related HRV index is the standard deviation (SD) of instantaneous beat-to-beat R-R interval variability [24], which can be measured from Poincaré plots (SD1) [25].

Physiologists and medical and technical personnel can use HR monitoring before, during and after training. It is possible to perform the monitoring every day including during the athletes' rest days. The following sections provide information about contributions and limitations when using HR and HRV and all their related markers in the monitoring process.

2.1 HR before training

2.1.1 Resting heart rate (HR_{rest})

HR_{rest} is defined as the lowest measure of HR taken from a 10-min lying position or immediately upon awakening [10]. Sleeping HR is defined as the lowest measure recorded during an entire night of sleep monitoring [9]. Measured in a seated or lying position, a decrease in HR_{rest} is suggested to be associated with an increase in the predominance of parasympathetic control [26]. It is well known that a decrease in HR_{rest} is a common response to endurance training; however, changes in HR_{rest} might also be influenced by environmental factors, thus its use as a marker of endurance TL or training adaptation is limited [2]. HR_{rest} is known to be a valid tool to detect short-term fatigue [27] even in soccer [28,29], but it is still questionable as a tool to detect a state of overtraining. Indeed, similar HR measures between normal and overtrained states were found in several studies, and some others reported an increase in HR_{rest} in overtrained individuals [9]. HR_{rest} might be the easiest marker to assess because it does not require sophisticated monitoring equipment and any further analysis. However, its use might be limited to the estimation of general fatigue without reporting day-to-day variations in TLs.

2.1.2 Resting heart rate variability (HRV)

Resting HRV is usually measured in a seated position for 5 minutes immediately after awakening in the morning to reduce external confounding factors (noise, light, and temperature). It is measured using a single marker such as Ln rMSSD (please see list of abbreviations), which is the most reliable HRV variable [1,30]. High Ln rMSSD has been associated with a high perception of fatigue and low fitness [31] and correlated with physical activity [32]. With respect to the timeline of a full season, lower values were observed during pre-season [33,34] and at the end of the season [34], which are periods where the fatigue is reported to be higher. More recently, it was observed that LF measures of HRV that were measured from a standing position were significantly altered at the end of a professional pre-season period [29]. Resting HRV as an indicator of cardiac parasympathetic activity could thus be used to monitor both acute and chronic training adaptations.

However, in the day-to-day monitoring process, resting HRV has some limitations; it can help identify a global state of fatigue, but it cannot measure different levels of fatigue [31]. Interesting results of swimmers highlighted the influence of pre-competitive stress on rMSSD, SD1 and HF, the measures of which were significantly lower in comparison to those associated with standardized training sessions [35]. Cognitive activity and the emotional state might therefore influence HRV indicators. Furthermore, a recent study of professional players in the English Premier League reported that no changes in resting HRV could be detected over a period of a week [36], which confirms that this tool remains of limited interest to date for practitioners on the field in training settings.

2.2 HR during exercise

2.2.1 *Heart rate during exercise (HR_{ex}, %HRmax, %HRres)*

HR has been well studied, and it has been demonstrated that HR is correlated to exercise VO_2 and to metabolic thresholds [9,10,20]. HR could be monitored easily during all types of training including high-intensity training exercises (HIT) or during small-sided games (SSG) [10]. To monitor HR during exercise, several markers should to be considered as follows:

- HR_{ex}: exercise HR, expressed in beats per minute, is the marker of the cardiac implication at a given time;
- Maximal heart rate (HR_{max}): the highest individual measure observed for the player when exercising;
- HR_{rest}: the lowest measure observed for the player at rest;
- Reserve heart rate (HR_{res}): calculated using the following formula: $HR_{res} = HR_{max} - HR_{rest}$ [37], i.e., the difference between HR_{max} and HR_{rest}.

To indicate the relative individual cardiac implication, HR_{ex} can be expressed in $\%HR_{max}$ using the following formula: $(HR_{ex} / HR_{max}) \times 100$. HR_{ex} can also be expressed in $\%HR_{res}$ using the following formula: $[(\text{mean } HR_{ex} - HR_{rest}) / (HR_{max} - HR_{rest})] \times 100]$. When calculating HR_{res}, the biorhythm variations are considered and therefore allow for inter-individual comparisons within a group of soccer players for different types of training [10].

HR_{ex}, expressed as $\%HR_{max}$ or $\%HR_{res}$, is a good marker for individual relative exercise intensity; a low HR correlates with a more physically fit player for a given standard sub-maximal exercise bout [14]. However, the increase in HR during exercise should not be systematically interpreted as a decline in fitness or a marker of fatigue [38]. It is important to know that during HIT, HR responses are influenced by the duration and the nature of recovery (active vs. passive), the presence or absence of directional changes, the coaches' required intensity and/or encouragement, and by the duration of the runs [39,40].

Indeed, active recovery maintains a high level of HR, while passive recovery decreases the HR if the duration is long enough. Furthermore, HIT with directional changes consistently increases HR because of the impact of the repeated decelerations and re-accelerations. Finally, exercise of short intermittent duration allows the achievement of elevated HR levels: a 15-15 second (15 seconds of running and 15 seconds of recovery) at 120% of maximal aerobic speed allows the achievement of 91-92% of HR_{max}; 5-20, 10-10, 15-15 and 30-30 second exercises allows the achievement of 77 to 86% of HR_{max} [39,40].

During SSG, HR responses were demonstrated to be similar or even higher than during HIT [39], most likely due to the accumulation of the technical components of the exercise, the repetitions of directional changes and the motivational factors with the use of the ball and the coaches' commands [41]. Different variables may influence the intensity of SSG and therefore the HR responses and have to be considered in the monitoring analyses. Thus, the number of players involved, pitch size, presence of goalkeepers, goal size, scoring rules (goals vs. stop-ball), number and duration of bout periods, duration and nature of recovery, availability of soccer ball replacement, and balanced/unbalanced opposition and coaches' demands and encouragements [10] have an impact on the players' activity and their physiological responses and adaptations.

HR_{ex} is an effective tool to monitor the individual aerobic intensity of an exercise and individual training adaptations [20]. Buchheit et al. [42] found a relationship between an increase of > 4% of HR_{ex} in response to an increase in TL the previous day and increased sickness probability the following day. HR-based methods such as Bannister's training impulse (TRIMP) [43] and Edwards method [44] were also reliable and associated with fitness indicators [45,46] and RPE [47] in soccer players. Many parameters influence HR responses and they need to be considered for a proper and relevant analysis. The period of the season, the moment of the day, the age, the schedule, the workload, the interactions during training, the emotional status, the sleep quality and other parameters directly affect HR responses during exercise; therefore, it is essential to be careful in interpreting the HR in a team-sport such as soccer. In this context, HR is presented as a very reliable tool to detect fatigue and the general physical fitness level of the soccer players [20].

2.2.2 Heart rate variability (HRV) during exercise

As it has been already described and reviewed by several authors, HRV measurements during exercise might present several limitations because they would be intensity-dependent, environment-dependant and non-exclusively related to the autonomic nervous system (ANS) [20,48]. Indeed, respiratory fluctuations determine HRV from the respiratory compensation point, and vagal activity has a predominant influence on HRV below the first ventilatory threshold [49]. Even in a team sport such as soccer, it would mean that all players would have to run at individually calibrated running speeds to not exceed the first ventilatory threshold [20]. However, during specific exercises such as SSG, the neuromuscular and metabolic involvements might not be sensitively assessed with changes in HRV [20]. Furthermore, the recording of HRV during exercise presents some limitations with the technical problems related to the non-stationary signals [48] and the "noise" in the recordings caused by the belt movement [20].

During exercise, HRV monitoring presents too many limitations to be a relevant tool for practitioners on the field. More studies are needed in this particular field before using a proper and confident HRV measures to monitor TL; however, it represents a useful tool to monitor fatigue when measured at rest.

2.3 Post-exercise HR

2.3.1 Post-exercise recovery heart rate (HR_R)

HR_R is considered the decline in HR (beats per minute) immediately after exercise [50]. In the ANS, an increase in sympathetic activity combined with a decrease in parasympathetic activity causes an increase in HR, whereas HR_R is regulated by a decrease in sympathetic activity and parasympathetic reactivation [51,52] and may provide a measure of the disturbance in autonomic control in response to endurance training [2]. HR_R can be calculated over different time frames, classically between 30 sec and 3 min post-exercise, by subtracting the HR value obtained after the chosen time frame from the final HR_{ex} [53]. Although no differences were observed between the reliability of HR_R measured after one and two minutes [27], the coefficient of variation of HR_R measured after two minutes was shown to be higher [54]. Recently, Daanen et al. [52] conducted a systematic review on HR_R and suggested measuring HR_R after one minute to better detect meaningful differences over time. They also suggested the importance of finding a relative way to express HR_R in beats/min, as it is generally expressed in absolute terms [52]. However, they concluded that the last findings on the topic suggested that HR_R would be a valuable indicator used to monitor the TL and also to monitor the accumulation of fatigue [52]. These conclusions were confirmed by Dellal et al. [53] who stated that HR_R was a relevant tool after specific soccer training such as SSG, repeated sprint ability (RSA) and HIT, while some other authors found no correlation between HR_R and performance changes or physical activity in elite soccer players [32,38].

It is important to be aware that HR_R is influenced by several factors such as the intensity of exercise, the type and duration of exercise, the rules or the instructions of exercise and environmental factors. Indeed, high intensity will induce a high HR that is achieved by the end of exercise, and therefore will most likely create a larger decrease in HR [55]. Via long term-induced adaptation, intermittent exercise training leads to a faster HR_R in comparison to continuous exercise training [55]. Furthermore, it was observed that HR responses were higher in intermittent exercise when changes in direction were included, or when, for instance, goalkeepers were included in SSG [53]. Finally, it was observed that practising in the heat increases vasodilatation and consequently causes an increase in HR. Therefore, HR_R was found to be slower in high ambient temperature than in moderate conditions [56]. All these elements have to be considered in HR_R analyses to avoid misunderstanding, and the data processing would most likely be time-consuming, which might limit its usefulness with an entire soccer team.

2.3.2 Post-exercise heart rate variability

Post-exercise HRV is the consequence of parasympathetic reactivation and a decrease in sympathetic activity. It must thus be assessed immediately after the end of exercise (two to five minutes). Post-exercise HRV is influenced by post-exercise plasma epinephrine levels, blood lactate concentrations, and blood acidosis through the accumulation of stress metabolites in the blood and skeletal muscle and arterial oxygenation [57]. Arterial baroreflex activity, regulation of blood pressure, vasodilatation of blood vessels and the stimulation of the metaboreflex, which optimizes the oxygen transport to muscles [20,57], all determine the level of parasympathetic reactivation, and thus post-exercise HRV. Furthermore, cardiac parasympathetic activity is known to be influenced by duration and intensity of exercise, and age, gender, baseline physical fitness, training status, psychological status, central fatigue and fluid intake [57]. In soccer, post-exercise Ln rMSSD and the maximal aerobic speed were correlated in highly trained young players with specific changes in cardiorespiratory and neuromuscular variables related to performance throughout a season [58]. Correlations were found between Log SD1 (defined above) and changes in plasma volume; however, no relationship was observed in changes of RPE or plasma creatine kinase (CK) in well-trained players [18]. In young soccer players, different HRV temporal indicators such as the R-R interval and pNN50, which is the proportion of interval differences of successive N-N intervals greater than 50 ms, were significantly altered by competition in comparison to the resting day [59]. However, as post-exercise HRV is influenced by the intensity of exercise and is mostly related to intensity when intensity is too high, it becomes redundant with HR_{ex} [20]. Furthermore, post-exercise Ln rMSSD and Ln LF measures are influenced by both the time of the day and players' chronotype [60]. In conclusion, post-exercise HRV may not contribute much information beyond the $HR_{ex,}$ and it is influenced by too many factors to be a relevant, efficient and valuable monitoring tool for soccer players.

2.4 HR and HRV during the rest days

During rest days (days without any training or competition), a focus on the players' follow-up is still important. Indeed, even if players are not practising, the cardiac ANS and the neuromuscular complexes are still stimulated during the recovery process, especially according to the super-compensation theory [61]. After exercise, in the short-term perspective (0-90 min), metaboreflex stimulation predominates, while long-term (1-48 h), the baroreflex stimulation influences parasympathetic reactivation [57]. According to the report of Stanley et al. [57], both reflexes overlap for approximately 30 minutes. In comparison to a standard training programme, it was observed in healthy men that the day-to-day adjustment of TL according to the day-to-day individual changes in HRV measurements allowed for better improvements in VO_{2max} and in maximal running velocity [62,63]. Further research is needed in soccer players and especially in elite players, as HRV is a marker of cardiac ANS [64]. However, its use may help coaches have an individual perspective of the training impact and thus help them to individually adjust and optimize the TL in response to these individual variations, as it has been well advised and reviewed [3].

Moreover, no association between HRV and the recovery of muscle complexes (observed with perceived exertion and blood creatine kinase) was found in soccer players [18] or weightlifters [65]. These observations specifically confirmed that HRV only reflects the cardiac ANS. In a recent study, it was observed after soccer matches that the HR_{rest} of professional soccer players increased significantly the day after the match and returned to baseline two days later, while the HR_{rest} did not change at all after a youth elite soccer match [28]. Its use may thus provide information on the recovery process during rest days. Furthermore, the sleeping HR marker was observed to increase when individuals are overtrained [9]; thus, it might present more reliable observations as it is less likely to be influenced by cofounding variables [9]. More research on soccer players is needed to confirm its application.

3. Biochemical Monitoring

During a competitive season, a soccer team trains differently according to different periods. During the pre-season, players have to train very hard with a high TL, whereas during the competitive periods, the TL is reduced. In this context, few authors analysed biochemical indicators from soccer players across a competitive season to assess changes in blood, salivary and urinary parameters. The section below relates the markers observed, their applications and their limitations in the process of TL monitoring.

3.1 Blood samples

3.1.1 Blood lactate concentration

To reflect the training status [66], blood lactate concentration is a useful tool for evaluating variations in aerobic endurance using lactate thresholds [67]. Lactate threshold improves with VO_{2max} [68]; therefore, it could be speculated that the higher the lactate threshold, the higher the average intensity a player could maintain during a match without accumulating lactate. It was reported that the post-exercise peak of blood lactate was reached approximately 3 min after the end of exercise when no active recovery was performed [69]. During a soccer match, measures of blood lactate concentration were observed between 1.0 and 15.5 $mmol.l^{-1}$ [1]. These measures could be collected from an intravenous blood sample and analysed in the laboratory or from the fingertip and analysed using a portable analyser, which produces validated measures directly on the field [70].

Depending on the velocity in a standardized running test, the level of lactate increased from the start of the pre-season to the early stage of the competitive season and remained stable to the end of the competitive season [71]. In the same way, no changes during the season were observed in lactate threshold velocity and running velocity at a lactate concentration of 4 $mmol.l^{-1}$ in soccer players. There were only significant differences in pre- versus in-season data, as players were relatively untrained at the beginning of the pre-season [71]. Blood lactate concentration is well correlated to other markers of exercise intensity such as session RPE and HR measures [72]. Blood lactate is easy to collect from the tip of a finger, and the measuring tools are of relatively low cost and very reliable [73]. The collection of lactate would be a valuable tool to monitor TL; however, its concentration is extremely dependent on the activity performed in the five minutes preceding blood sampling [74]. Furthermore, unlike HR or RPE measures, the collection of blood lactate is an invasive method, and its daily use would remain very inconvenient because only qualified staff are able to perform the collection. Therefore, blood lactate concentration sampling presents limiting factors making its use less desirable than RPE or HR, especially in elite players. It is also difficult to perform the collection simultaneously in all players.

3.1.2 Creatine Kinase (CK)

CK is a marker of skeletal muscle fibre damage [75]. A reference study with a large sample (n = 400) of elite players showed that CK values could increase throughout the season

[76]. The study showed significant differences between the samples from the first day of training in July (~183 U.l^{-1}) and samples that were collected in February/March (~331 U.l^{-1}). These data were confirmed in professional players with similar values [77]. Another study that was conducted a few years later showed additional information as follows: CK mostly increased during the pre-season conditioning period because of the increase in the TL [78]. Values increased from ~300 U.l^{-1} to ~500 U.l^{-1} during the pre-season period and returned to ~300 U.l^{-1} during the whole competitive season [78]. However, it is important to notice that CK values are highly dependent on several variables. It is well known that measurements differ from one individual to another and are highly affected by the activity performed during the previous days. Therefore, the individual TL has to be considered and analysed along with the CK values for better interpretation. Furthermore, the interpretation should take into account that as in any professional sport, professional soccer players would have elevated CK values most of the time because playing soccer requires high levels of muscular involvement [79].

3.1.3 Urea

Urea can be used as a marker of glyconeogenesis and is normally elevated during prolonged physical effort or an increase in TL [80]. This marker would thus increase during the conditioning periods in comparison with the end of break periods and would remain stable throughout the competitive periods [76,80]. The values increased from 300 pmol.l^{-1} at the start of the pre-season to 400 pmol.l^{-1} throughout the season [78]. Other studies related that urea increases independent of the age of the players during the conditioning period, and even more during the competitive period [81]. They suggested that the levels of urea indicated an index of time to perform as an adaptation of a subsequent training programme [81]. As urea does not vary during the season and even after a soccer match [82], from a day-to-day perspective, monitoring this marker would only be of value in the case of kidney disease or recurrent dehydration. It is not necessary to measure urea in a recurrent monitoring process [76].

3.1.4 Creatinine

Creatinine is a marker of creatine degradation during energy metabolism. The higher the muscle mass, the higher blood creatinine concentrations [83]. Nevertheless, creatine intake could influence the measures [84] without affecting any health markers [87]. However, no significant changes in creatinine concentrations (from 96 to 99 µmol.l^{-1}) [80] or only small changes that were not sufficient to be discussed [76] were observed in professional soccer players throughout a season. Even if creatinine concentration was increased after a soccer match [82] due to the high intensity of the performance during the match, creatinine would only be of minor interest for frequent blood monitoring in elite soccer players.

3.1.5 Haematocrit

Haematocrit is known to increase with physical capacity improvement [76,86]. It remains stable throughout the season (0.45) and slightly increases (0.47) at the end of the season [78]. This final increase, accompanied by a decrease in haemoglobin values [78,87], helps to detect any indication of the overtraining condition. Haematocrit rose after a 6-week soccer-specific training programme where the TL was increased [77], which indicates its reactivity to TL variations. Furthermore, after a soccer match, values of haematocrit were higher than those before the match as a consequence of a decrease in plasma volume [82]. It has also been reported that haematocrit was significantly correlated to the general development of VO_{2max} and to the degree of maturation in young soccer players [88]. It has been suggested that haematocrit could be monitored in parallel with haemoglobin measurements to objectify the beginning of an over-fatigued state. More research is needed to clarify its real value to the day-to-day monitoring process.

3.1.6 Immunological status

As physical activity affects immunological status, its monitoring could be used to detect overtraining [89]. Research on immune parameters indicated that lymphocytes decreased throughout a season [78,90] and leucocytes decreased during the conditioning and the ending periods of the season [78], whereas white blood cells and C-reactive protein did not change throughout the season [76]. Moreover, during the conditioning period, a variation in the changes of immunoglobulin IgA, IgG and IgM occurred in players according to the literature; they decreased for some French professional players [87] and increased for some Danish players [78]. Differences between studies might be explained by external factors such as the diet of the players, training intensity, training type and environmental characteristics such as climate. It has even been reported that the recovery period between two seasons was not long enough to allow players to fully recover, especially regarding their immunological status [91]. The monitoring of immunological status might be relevant, as inflammatory markers are influenced by TL and training periods. Nevertheless, investigating immunological status should be approached through salivary measures rather than invasive blood sampling.

3.1.7 The lipid profile

It is commonly mentioned that the metabolism of lipoproteins such as low- (LDL) and high-density lipoproteins (HDL) and triglycerides is related with the amount of physical activity [92]. However, there is no evidence showing that levels of these three markers are connected with physical performance. No differences were found in the lipid profile of professional players after a 3-week resting period and one month later after an intensive conditioning period [93]. Throughout a soccer season, most values of the lipid profile remained stable except at the end of the season, where total cholesterol (TC) and LDL decreased significantly [78]. These decreases were most likely reflective of a prolonged period of training and match exposure, which induced an improvement in fat metabolism [78]. Conflicting observations were obtained in other studies where decreases in triglycerides and LDL and an increase in HDL were reported between baseline values and the competitive period [81]. Similar variations were observed in recreational soccer players [94] or after a competitive soccer match [95]. Furthermore, it was found that when adding HIT to a classical soccer training programme during 12 weeks, the lipid profile was slightly but not significantly improved with a reduction in TC and LDL and an increase in HDL [96]. All these findings combined indicate that TL may affect TC, triglycerides, LDL and HDL, but changes in these markers would only significantly occur when the variation in the TL was significant. Therefore, monitoring lipids during the first phases of the season might be interesting. However, the use of the lipoprotein profile would not be relevant for monitoring TL during a whole season. A regular monitoring process of lipids and lipoproteins might be recommended to reveal any potential health [79] issues or to optimize health status, which could have an indirect effect on players' performance [81].

3.1.8 Iron status

Iron deficiency is known to affect performance [97]. According to Fallon [98], physical performance could be impaired if serum ferritin levels were < 20 $\mu g.l^{-1}$. It is well known that iron deficiency is common in elite women soccer players [99,100], mainly related to menstruation. Across different soccer seasons, no significant changes in ferritin, serum iron and transferrin saturations were observed [76,100]. However, male soccer players could also have a small decrease in ferritin throughout the competitive period of their season [78]. Values above 20 $\mu g.l^{-1}$ may be observed without any additional signs of anaemia, and thus, this reduction in ferritin could not be considered problematic from a performance or a health perspective. It has been reported that iron status markers were only transiently affected by a soccer game [101], whereas some authors showed that some players were iron deficient at the end of a professional season and that even the recovery period between two seasons was not sufficient to return ferritin to baseline levels in some players [102]. Thus, the screening of iron status markers appears to be relevant, but more from a health perspective than performance or fatigue-related aspects.

3.1.9 Procedures and conclusions for blood sampling

Important highlights have been raised regarding the standardization of the blood sampling process, especially concerning the posture of the subjects (lying, sitting or standing), the collection (venous vs. capillary blood), the storage (placed on ice at a temperature of -20°C to -80°C until later analysis) and analytical assays [103]. A recent investigation also highlighted the influence of circannual rhythm of blood markers such as vitamin D, testosterone (T) and cortisol (C), which significantly vary between winter and summer in professional soccer players [104]. There is still a lack of studies in the literature on the relationship of specific blood parameters to performance and injury incidence or that discuss the precise diagnostic role in detecting overtraining in soccer. Furthermore, blood sampling is invasive, stressful and requires funds and qualified staff. Therefore, frequent blood sampling is not recommended at this time. The use of intra-individual profiles with individual baseline levels is a better recommendation [79].

3.2. Salivary samples

The collection procedure of saliva has different advantages compared to other biological fluids as follows: the procedure is easy to apply; it can be used in almost every competitive environment; and it is non-invasive and stress free [105]. This last advantage is important because it is well known that physical stressors induce hormonal, biochemical and immunological changes in saliva as described by several authors [106].

Salivary IgA (sIgA) is one of the most investigated markers of immunological involvement in physical activity as it might be related to the risk of upper respiratory tract infection (URTI) [107], which is well known to negatively affect training performance [108]. Different authors observed a correlation between a reduction in TL and a reduction in sIgA and URTI symptoms [109]. Other studies demonstrated that sIgA decreased three days before URTI symptoms appeared [110]. Moreover, a decline in sIgA of approximately 28% occurred three weeks before the onset of an URTI [111]. Recently, it was shown that sIgA significantly decreased just after high-intensity soccer training sessions, and it was totally dependent on volume and intensity of the training sessions performed just before sampling [112]. All these observations pointed to the value of the use of routine monitoring of this particular marker to monitor the individual TL and to avoid URTI.

T and C are frequently used in order to observe variations in protein anabolism and catabolism. The T / C ratio might be used to represent the level of wellness/tiredness and therefore help to prevent the development of the overtraining syndrome [113]. A decrease in the T/C ratio could be associated with a reduction in fitness test performance during a soccer season [114], but it would not automatically lead to a reduction in the physical activity during a match [115]. Silva et al. [77] observed a significant increase in the T / C ratio at the end of a soccer season, which revealed an elevated fatigue status at this time but without any correlation with performance. In other sports, both positive and negative relationships were found between these hormonal parameters and performance in cycling or rowing [116,117]. Moreover, data analyses differed from one report to another. Some authors noted that the T / C ratio decrease was related to a fatigue state [118], and others suggested that it reflected an optimal fitness level before the point of exhaustion [115]. This variation in analysis represents a limitation in the use of saliva hormones to monitor soccer players, as special care is needed to adapt results with the environment and the context. For example, C could also be related to the level of anxiety before a match [119]. Furthermore, when collecting T and C hormones from saliva, fundamental care should be taken regarding sampling procedures, especially regarding environment, nutrition, stress, sleep, physical activity [104], and circadian rhythms [120]. Collection must be performed from unstimulated saliva and after the subjects have rinsed out their mouths with clear water to clean the oral cavity. Measurements of salivary biomarkers require correction for flow rate as well detailed in Lindsay & Costello's review [121]. For the different indicators that could be collected from both blood and salivary procedures, it has been suggested that blood and salivary levels mirror each other in their changes with correlations of $r = 0.7$ to 0.8 [103]. It also important to highlight that saliva is limited to free steroid hormonal concentrations and that in soccer players, saliva cortisol concentrations have been reported to be influenced by the time of the day and the chronotype of the players (salivary cortisol morning levels are much more elevated than those observed later in the day) [103, 122].

3.3. Urinary samples

Urinary samples are often used for anti-doping controls [123]; however, they might also be used to monitor players. To the best of our knowledge, no study has deeply analysed the use of urinary samples in soccer players; however, results of their use in other sports may bring valuable information regarding its potential use and related limitations in the monitoring of the TL and fatigue. Indeed, several authors observed significant correlations between the increase in fatigue and changes in urinary hormones and catecholamines. Liederbach et al. [124] observed an increase in urinary norepinephrine (or noradrenaline, NA), urinary epinephrine (or adrenaline, A) and RPE values in professional ballet dancers from the beginning to the end of a season. Atlaoui et al. [125] observed an increase in urinary cortisone (Cn) and a decrease in the urinary C / Cn ratio after a period of intense training in elite swimmers. They also observed interesting correlations between performance that was measured in official competitions and both C / Cn ($r = 0.52$, $p < 0.01$) and A / NA ($r = 0.59$, $p < 0.01$) ratios. Rouveix et al. [126] confirmed these results in tennis players in which increases in the urinary C/Cn ratio and decreases in the urinary A/NA ratio in relation to alterations in mood state and performance were observed. Monitoring urinary samples might also offer some value to the hydration status of the players; assessing urine colour, specific gravity or osmolality would provide some information about hydration in athletes [113]. A urine osmolality > 900 $mOsm.kg^{-1}$ is consistent with an athlete who is experiencing a hydration deficit of more than 2% of body mass [113], which has been reported to be correlated with a reduction in physical performance in soccer matches [127]. Furthermore, a urine osmolality ≤ 700 $mOsm.kg^{-1}$ or a urine specific gravity < 1020 $g.ml^{-1}$ could be used to diagnose dehydration, also called hypohydration [113,128], which has been reported to negatively affect physical performance [129]. Finally, the urine colour collected at the first urination in the morning or immediately before training or competition [130] was positively related to both osmolality and specific gravity [131] and could be assessed to estimate hydration status using a scale of eight colours developed by Armstrong [132]. Nevertheless, if this last tool remains less precise than the two aforementioned ones, it is worth using on the field to estimate hydration status when no other more sophisticated tool is available.

Urine markers offer information related to a general state of fatigue, represented by a change in mood, decrease in performance, increase in training volume and intensity and perceived exertion but also information related to the hydration status [133]. Furthermore, it is non-invasive, and high-level athletes are familiar with the simple testing protocol for medical and doping concerns. However, as widely suggested by Hackney and Viru [103], urine analysis is limited to steroid-based hormones and when they must be collected over a 24 h-period, the collection procedure tends to be a more tedious and demanding process. Furthermore, as urine can sit in the bladder for several hours, urine measurements may not systematically reflect real-time hormonal status [103]. Additionally, it is important to highlight that urine analysis requires a volume correction as detailed by Lindsay and Costello [121]. Nevertheless, both urine and saliva are considered "the future" of objective monitoring tools in sports and exercise medicine [121]. The challenges for the practice of collecting urine and saliva biomarkers are oriented towards assessments of psychophysiological indicators of stress and workload in response to training and matches.

4. Conclusions

Many objective internal markers of TL and fatigue could be used to monitor soccer players from a day-to-day perspective and/or across a whole season. However, from a large non-exhaustive list of markers, some markers are highlighted because they have been observed to be more relevant than others, especially HR_{rest}, HR_{res}, HRV during rest days, blood lactate, blood immunological status and salivary IgA. Among this reduced list, HR markers might even be mostly considered because their use would not require a significant financial burden such as would pertain to blood or salivary samples. Thus, from a day-to-day monitoring perspective, the use of (i) HR_{rest} in the morning before breakfast, taken from a 10-min lying position; (ii) resting HRV (Ln rMSSD), especially during rest days, taken during 5 minutes immediately after awakening; and (iii) HR_{res} during training sessions, might be valuable in the individual monitoring of a soccer player's TL. These objective markers have to be associated with subjective markers such as RPE as suggested in a recent review of the literature [134]. Further studies are needed regarding urinary samples, hormones and catecholamines to reveal the potential interest of using them in soccer players. Moreover, further studies should analyse the specific area relating markers of TL to injury occurrence or the specific decrease in performance during competition, especially regarding congested periods that are part of the elite soccer season. Therefore, the focus of future studies should include the analysis of which physiological marker is affected by the congested areas of the schedule, and which day is of particular value to the medical and technical staff of the elite soccer team.

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Title: Monitoring training load and fatigue in soccer players with physiological markers.

Highlights:

- At rest, HR_{rest} would be the most valuable physiological marker to monitor fatigue,
- Resting Ln rMSSD would be a valuable marker to monitor fatigue during rest days,
- HR_{res} might be valuable in the individual monitoring of a soccer player's TL,
- Further research is needed regarding urinary samples, hormones and catecholamines,
- Further research is needed to relate physiological markers to injury occurence and performance