ORIGINAL ARTICLE

TRENDS in
Sport Sciences
2024; 31(**1**): 29-36
ISSN 2299-9590
DOI: 10.23829/TSS.2024.31.1-3

Intermittent endurance and biochemical variations during a season in elite professional soccer players

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Abstract
Introduction. **Soccer is a sport with high intensity intervals, heavily relying on aerobic energy mechanisms, leading to various neuromuscular and endocrine responses.** Aim of Study. **The purpose of this study was to examine the changes in intermittent endurance performance, testosterone and cortisol concentrations, and their T/C ratio in elite professional soccer players during a soccer season.** Material and Methods. **Ten elite soccer players from a soccer club of Greek Super League were evaluated for intermittent endurance, using an interval shuttle run test (ISRT), testosterone (T) and cortisol (C) concentrations, and T/C ratio at the beginning of the preseason period (T1), at the beginning of the competitive period (T2), at the start of the second round of the competitive period (T3) and at the end of the competitive season (T4).** Results. **The distance covered at ISRT increased by 26% ($p < 0.001$) after the preseason. T increased by 8.6% after the preseason, while a statistically significant decrease (24.4%, $p < 0.005$) in T was observed between T1 and T3, and between T1 and T4 ($p < 0.005$). C increased significantly by 90% ($p < 0.001$) from T1 to T2, while a significant decrement by 15% ($p < 0.001$) was observed between T4 and T3. T/C decreased by 47% ($p < 0.005$) from T1 to T2, while T2 values of T/C indicated a further 30% ($p < 0.05$) until T3. T/C ratio increased by 13% ($p < 0.005$) from T3 to T4.** Conclusions. **This study shows significant changes in intermittent endurance performance, testosterone, cortisol and T/C ratio over the season. Conclusively, coaches of professional soccer teams shall monitor endurance and hormonal changes over the season so that players successfully improve their aerobic fitness, optimize peak performance, prevent injuries and overreaching.**

KEYWORDS: hormones, football, aerobic endurance.

Received: 23 October 2023
Accepted: 12 March 2024

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Introduction

Soccer is a sport with high-intensity intervals, heavily relying on aerobic energy mechanisms, leading to various neuromuscular and endocrine responses [12, 15, 23]. A full soccer season lasts approximately eleven months and includes a two-month preparation period, an eight-month competitive season, a one-month transition period, and vacation of around another month. The main goal of the preseason is physical adaptation and practicing individual and team tactics and systems that will be used during games. During the competitive period, soccer players are exposed to training and competitive matches with the main purpose of maintaining or even improving their physical performance [33]. Accumulation of fatigue as the season progresses results in a decline in physical performance [38, 41] and changes in hormonal status of players [23]. The absence of sufficient recovery after intensive training periods and match exposure may lead to overreaching and/or overtraining syndrome [24]. Therefore, monitoring a training load as well as other hematological, immunological, hormonal, and physiological markers during a macrocycle seems imperative.

Testosterone and cortisol are the most common reliable hormonal markers related to anabolic and catabolic processes. Testosterone is a steroid hormone with anabolic properties that regulates the development of the male reproductive system and secondary sex characteristics [36]. Cortisol is a corticosteroid hormone which stimulates catabolic reactions that can enhance gluconeogenesis, the breakdown of proteins, and the release of amino acids that can be used in enzyme synthesis and energy production. Both testosterone and cortisol markers are affected by intensity and volume of the training periods [38], a match schedule during the competitive period [37], as well as psychological stress and mood state [11]. Previous studies have shown that salivary testosterone concentration was correlated with a total distance covered in the Yo-Yo intermittent test in elite preadolescent Brazilian soccer players [31]. In another study, testosterone concentration was correlated with a distance covered by elite male soccer players [32]. Conflicting results have been shown for cortisol concentration after a competitive match play in female and male soccer players [18, 29, 30]. The testosterone-cortisol ratio (T/C) is a sensitive and reactive marker, which is used as a general assessment of mechanical stress and fatigue, as well as a marker to identify overtraining syndrome [1]. While a separate estimation of testosterone and cortisol respectively can predict important musculoskeletal adaptations, an assessment of their relative concentrations is a sensitive, reactive, and reliable marker of the anabolic-catabolic balance [42]. Previous studies suggest that athletes with low T/C scores have shown a decrease in performance in physical tests and inadequate adaptation to training [26].

Although numerous studies have examined the variations in physical fitness components (endurance, muscle strength, agility, sprint, body fat) of soccer players during a season [13, 19, 22, 23], little is known about an integrative assessment of physical fitness and hormonal status as the season progresses and fatigue accumulates [23, 30, 39].

Aim of Study

For this reason, the aim of the present study is to examine the changes in intermittent endurance performance using the interval test in parallel with the anabolic and catabolic hormonal markers, such as the testosterone and cortisol concentrations, and their ratio in elite soccer players over the entire soccer season. This approach of the combined monitoring of intermittent endurance and the hormonal concentrations may provide important information to coaches and health professionals who support soccer teams in maximizing performance and preventing soccer players from overtraining throughout the entire season.

Material and Methods

Participants

Ten elite professional soccer players (mean ± SD; aged 23.9 ± 3.24 yrs, weight 77.3 ± 8.3 kg, height 1.73 ± 0.07 m) participated in the study. The participants were informed about the purpose of the study and gave their informed consent in accordance with the Declaration of Helsinki. A local ethics committee approved the research design and procedures of this study. All of the players belonged to the same club and underwent similar soccer training programs. Goalkeepers were not included in the study. The team participated in 30 league matches and 8 cup matches. The participants were evaluated for body fat (%), intermittent endurance using the interval shuttle run test (ISRT), the testosterone (T) and cortisol (C) concentrations, and the T/C ratio in July (at the beginning of the preseason period), September (at the beginning of the competitive period), at the end of January (at the start of the second round of the competitive period), and at the end of May (at the end of the competitive season). Blood sampling was performed at rest, after a day of rest, while body composition and the ISRT were assessed, before and at the beginning of the training, respectively, on the following day.

Protocol

Training program

The preseason lasted 8 weeks. During the preseason, 54 training sessions were conducted with a variety of tactical, aerobic, anaerobic, strength, and flexibility training content with one rest day per week. In addition, six exhibition games were played as a part of the preseason. The average number of training sessions was 6.7 ± 0.4 per week. The weekly training program during the competitive period included six training sessions in five days, an official match (usually on Sunday), and a day off after the match (according to [22]). In the event of two official matches in the week, the content was adjusted accordingly, with the weekly training sessions including only exercises for recovery, tactics, and activation.

Anthropometric characteristics

Height was measured to the nearest 0.1 cm using a stadiometer, and body mass was measured to the nearest 0.1 kg. Skinfold thickness was measured at three sites (chest, abdomen, and thigh) using a Harpenden skinfold caliper. A body fat percentage (%) was calculated according to standard equations [21, 40].

Intermittent endurance

The ISRT consists of 30-second shuttle runs interspersed with 15-second walking recovery periods [2]. In order to fulfill the requirements of the ISRT, the participants run between two lines set 20 meters apart, at a pace dictated by predetermined recorded beeps. During the walking periods, the players had to walk back and forth to the line. The starting test speed was 10 km/h, which increased by 0.5 km/h every 90 seconds. From a value of 13 km/h, the speed increased by 1 km/h. The participants were instructed to complete as many runs as possible. The test was stopped when the participants could not keep the pace (more than 3 meters before the 20-meter lines at two consecutive audio signals) or decided to quit the run. The total number of completed 20-meter runs was recorded as the test score [2].

Blood sampling

Blood samples were obtained in the morning (9 am), after the players had fasted for at least 10 hours. For blood sampling (10 mL), a venipuncture with a butterfly needle was performed from a median cubital vein. The participants laid down in an inclined position. Blood was stored in BD Vacutainer blood collection tubes with an additional coagulation activator. Blood was coagulated at a room temperature and then subjected to centrifugation (1500 g, 4 °C, 15 min) for plasma separation. A serum obtained after centrifugation was used for the measurements. A testosterone and cortisol concentration analysis was performed with a DRG Instruments immunoassay kit, using an ELISA photometer (Elisa Reader Labtech LT-4500). All sample analyses were performed in duplicate. Coefficients of analysis variability between assessor or different assessors for the testosterone and cortisol concentrations remained below 5%.

Statistical analysis

The data were analyzed using the SPSS PC program for Windows. Means ± SD were calculated. Dependent variables in the present study were the distance and stage reached in the ISRT, the serum testosterone and cortisol concentrations, as well as their ratio, with a measurement time point as an independent factor. The repeated measures ANOVA was applied to examine if there were differences among the different time points for each dependent variable. When F ratios were significant, post hoc comparisons of means were performed using the Bonferroni's multiple comparison tests. A relationship between measurements of interest was expressed using the Pearson's correlation coefficient. Statistical significance was accepted at $p < 0.05$.

Results

Body mass and body fat (%)

The repeated measures ANOVA did not reveal a significant time effect on body mass (Wilks' Lambda = 0.934, $F_{3,7} = 0.166$, $p = 0.916$, power = 0.008, $\eta^2 = 0.066$), but a significant time effect on body fat (%) was observed (Wilks' Lambda = 0.083, $F_{3,7} = 25.898$, $p < 0.001$, power = 1, $\eta^2 = 0.917$). Body fat percentage decreased significantly ($p < 0.01$) after the preparation period and remained constant until the end of the season (Table 1).

Intermittent endurance

The repeated measures ANOVA revealed a significant time effect on the distance reached in the ISRT (Wilks' Lambda = 0.091, $F_{3,7} = 23.257$, $p \leq 0.001$, power = 1, $\eta^2 = 0.909$), which was significantly increased ($p < 0.001$) after the preparation period and remained constant until the middle of the season. There was also a significant decrement ($p < 0.001$) at the end of the season in comparison to the middle of the season (Table 1).

Table 1. Body mass, body fat and distance reached in the interval shuttle run test measurement of elite soccer players (n = 10) at different time points during a season

	Start of preseason M ± SD	Start of season M ± SD	Mid-season M ± SD	End of season M ± SD
Body mass (kg)	77.3 ± 8.3	77.4 ± 8.6	77.7 ± 8.2	77,68 ± 8,15
Body fat (%)	6.6 ± 2.2	5.4 ± 1.7*	5.3 ± 1.7*#	5.5 ± 1.64*¥
ISRT distance (m)	1882 ± 340	2378 ± 234*	2456 ± 178*	2282 ± 208*¥

Values are means ± SD.

* p < 0.05 compared with the measurement in the preseason period

\# p < 0.01 compared with the measurement at the start of the season

¥ p < 0.001 compared with the measurement in the middle of the season

Cortisol concentration

The repeated measures ANOVA revealed a significant time effect on the cortisol concentration (Wilks' Lambda =

0.097, $F_{3,7} = 21.671$, $p \leq 0.001$, power = 1, $\eta^2 = 0.903$). The cortisol concentration increased significantly ($p < 0.001$) by 90% from the start to the end of the preseason (Table 2). It increased by another 6% until the middle of the season, but without reaching statistical significance. Thereafter, it decreased significantly ($p < 0.001$) by 15% until the end of the season compared to the middle of the season (Table 2).

Testosterone concentration

The repeated measures ANOVA revealed a significant time effect on the total testosterone concentration (Wilks' Lambda = 0.261, $F_{3,7} = 6.599$, $p < 0.05$, power = 0.810, $\eta^2 = 0.739$). There was an 8.6% increase in the total testosterone value by the end of the preseason, but without reaching statistical significance (Table 2). However, there was a statistically significant decrease (24.4%) in the total testosterone concentration between the start of the preseason and the middle of the season ($p < 0.005$), and between the start of the preseason and the end of the season ($p < 0.005$). The decrease was also observed between the start of the season and the middle of the season ($p < 0.01$), as well as between the start of season and the end of the season ($p \leq 0.001$) (Table 2).

Table 2. Testosterone and cortisol concentration values, and the T/C ratio of elite soccer players (n = 10) in the different periods of a season

	Start of preseason	Start of season	Mid-season	End of season
Cortisol (nmol/L)	230.1 ± 53.1	437 ± 80.8*	463.8 ± 76.2*	394.5 ± 44.1*¥
Total testosterone (nmol/L)	20.7 ± 2.6	22.5 ± 2.7	17 ± 3.8*#	16.7 ± .85*#
T/C ratio	0.094 ± 0.022	0.053 ± 0.015*	0.037 ± 0.01*#	0.042 ± 0.008*¥

Values are means ± SD.

* $p < 0.005$ compared with the measurement in the preseason period

\# $p < 0.05$ compared with the measurement at the start of the season

¥ $p < 0.001$ compared with the measurement in the middle of the season

T/C ratio

The repeated measures ANOVA revealed a significant time effect on the T/C ratio (Wilks' Lambda = 0.112, $F_{3,7} = 18.492$, $p \leq 0.001$, power = 0.998, $\eta^2 = 0.888$), which also changed significantly during the season ($F_{2,18} = 36.817$, $p < 0.0001$, $\eta^2 = 0.804$). The baseline value decreased by 47% from the beginning to the end of the preseason ($p \leq 0.005$). The mid-season T/C ratio values indicated a further 30% decrease from the start of the season ($p < 0.05$). At the end of the season, the T/C ratio increased by 13% ($p \leq 0.005$) compared to the middle season value (Table 2).

Discussion

Monitoring body composition, intermittent endurance, and certain biomarker levels in athletes during a competitive season is crucial for maintaining peak performance, preventing fatigue accumulation, and avoiding overreaching in soccer players. The results of the present study show significant changes in body composition, intermittent endurance performance, the total testosterone and cortisol levels, as well as the variations in the T/C ratio during the soccer season in elite soccer players. The significant decrease in the body fat percentage was observed at the end of the preseason period; however it was not accompanied by the decrease in the body mass, and thereafter it remained relatively constant during the competitive period. The biggest changes in body composition are most often observed in a preseason due to an increased training load aimed at achieving specific performance adaptations after a period of inactivity.

Previous investigators have noticed a significant relationship between a distance covered in the Yo-Yo IR1 test, a field test with similar intermittent activity as the ISRT, and high-speed running distances covered during a match [25]. The present study has shown that the distance reached in the ISRT increased significantly by 26% after the 8-week preseason period, increased by another 3% until the middle of the season without reaching statistical significance, but was still 21% higher at the end of the season than at the beginning of the preseason. The distance reached in the ISRT in the middle of the season was increased by 7% compared to the end of the season, although at this time point the values were significantly higher (21%) than the values obtained at the start of the preseason. Similar variations during a season were reported in previous studies using the Yo-Yo IR1 field test [8, 10, 25]. Castagna et al. [8] have reported a 19.5% increase in a distance covered during the Yo-Yo IR1 field test after an 8-week preseason period in elite soccer players. The same research group [7] has shown a 12.5% improvement in a distance covered during the Yo-Yo IR1 test during a shorter preseason period (6 weeks) in elite soccer players. In addition, Dragijsky et al. [10] have reported a 27.2% improvement in a distance covered during the Yo-Yo IR1 test after a preseason and 9.9% thereafter, until the middle of a season. Casajús [5] has found a decline in an aerobic capacity of soccer players at the end of a season compared to the middle of the season, but this decline was not statistically significant. The decline noticed at the end of the season in this and other studies may be justified by accumulated fatigue, a reduced training load and a number of consecutive matches at the end of the competitive season [5, 14].

Exercises are a form of stress that results in cortisol secretion related to intensity and duration of an exercise [17]. Significant increases in cortisol levels are observed in exercise bouts lasting more than 20 minutes at 60% VO_{2max} or more [6, 9]. In the present study, the largest secretion of cortisol was observed after the end of the preseason, showing an increase of 89%. These results are consistent with a study by Silva et al. [39], in which cortisol levels also peaked at the end of the preseason. However, in contrast to the results of the current study where the cortisol levels continued to rise during the season as well, in the study by Silva et al. [39], the cortisol value decreased during the season, which might be attributable to a protocol and a type of a study design. In a study by Kraemer et al. [23], cortisol levels also peaked in a preseason but remained high in a season, in agreement with the results of the current study. As cortisol is a reliable indicator of catabolic processes, the sustained elevated cortisol concentration suggests that catabolism was prevalent during the season. The same results were observed by Saidi et al. [35], who examined endocrine changes during stressful periods, and concluded that cortisol levels significantly increased when training intensity also increased in a preseason, but not when regular matches were played during a season. Similarly, Huggins et al. [20] have shown an increase in cortisol levels in a preseason, which remained at high concentrations during a season. Filaire et al. [16] have noticed that when a team did not perform well in a competition, cortisol levels rose sharply, leading the researchers to a conclusion that negative emotions, such as anger, tension, fatigue, and psychological stress, also contribute to cortisol responses. This finding was also verified in another study by Filaire et al. [16], who assessed both external and internal load in combination with psychometric questionnaires. In contrast, Michailidis [28] justified increased cortisol levels during a season, indicating that a combination of psychological stress, match intensity, and adverse weather in winter could cause an increase in cortisol during the season.

The present study shows importance of monitoring hormone levels in elite soccer players, as fluctuations in the testosterone and cortisol concentrations can have significant implications for player performance. In relation to the testosterone concentrations, the results of the present study have shown that the total testosterone values increased by 8.6% at the end of the preseason period, whereas the middle of the season measurement showed lower values (24%) than at the start of the preseason. Silva et al. [39] have shown the same pattern during a soccer season. In their study, testosterone concentrations in soccer players increased at the end of a preseason, while a measurement performed in the middle of the season showed that the testosterone concentrations returned to the initial levels [39]. Saidi et al. [35] have examined biochemical changes over 12 weeks, during a stressful season and recorded a decrease in testosterone levels, but with stable cortisol levels. The decrease in testosterone was attributed to accumulated fatigue, and according to the authors, it is a reliable indicator of muscle stress. However, according to Maso [27] it may be an indicator of lack of rest.

The T/C ratio is considered a more reliable indicator in assessing body stress than separate testosterone or cortisol levels. The T/C ratio decreases with increasing training duration and intensity, and during periods of consecutive matches [42]. A 30% decrease in the T/C ratio is considered an indicator of inadequate recovery [43]. In the present study, the T/C ratio appeared to decrease gradually during the season, concurrently with the total testosterone value. In the study by Silva et al. [39], the T/C ratio increased after a preseason and continued to increase during the season, which is justified better by the decrease in cortisol rather than the testosterone changes. However, this study omitted an important period of time (mid-season) during which players are subjected to higher psychological and mechanical stress, and possible decreases in the T/C ratio that may have occurred went unnoticed. Similar results were observed by Kraemer et al. [23], who noticed an increase in the T/C ratio and a concomitant (mild) increase in testosterone. However, as the cortisol concentration remained high throughout the study, the authors speculated that catabolism was predominantly prevalent [23]; this conclusion is similar to the findings by Carli et al. [4]. In a study by Rowell et al. [34], which examined biochemical changes in a professional team over 36 matches, a decrease in the T/C ratio was also observed. This finding was associated with limited neuromuscular recovery, despite recorded increases in the testosterone concentration. The authors indicated that the increase in testosterone was accompanied by positive outcomes of the matches, establishing a relationship between psychological stress and performance. Saidi et al. [35] have examined T/C ratio changes over

12 weeks, during a stressful season. In agreement with the results of the current study, they recorded a decrease in the T/C ratio, testosterone, and stable cortisol levels. The decrease in the T/C ratio in the study by Saidi et al. [35] was attributed to the decrease in testosterone that occurred, which was consistent with increased training load over the season and congested fixtures. The authors noticed that the decrease in the T/C ratio was a factor contributing to reduced physical performance. The results of the present study show 17.8% and 60.7% decreases in testosterone and the T/C ratio, respectively, suggesting that, as per the findings of Saidi et al. [35], the players probably did not rest adequately during the season. In contrast, Michailidis [28] has shown a continuous increase in testosterone and the T/C ratio from a preseason until the end of the season, attributing these results to a good training design, with proper balance of training load and sufficient recovery time. It seems that the T/C ratio is a useful marker of anabolic-catabolic balance in elite soccer players, which can help coaches and trainers monitor player adaptation to training and identify potential performance issues.

Conclusions

The present study shows the significant changes in body composition, intermittent endurance performance, the testosterone and cortisol levels, and the T/C ratio over the course of a season. The present study provides valuable insights into the physiological and biochemical changes in professional soccer players throughout the season, which can have influence on training and performance optimization strategies. According to the present results, there is a need for an integrative assessment of physical fitness and hormonal status as a season progresses and fatigue accumulates, which can help coaches and trainers develop more effective training programs for maximizing players' physical fitness and preventing injuries and overreaching [3]. The testosterone and cortisol levels, as well as the T/C ratio, can reflect athlete's functioning and can be used as predictive guidelines for subsequent changes in a training regimen. The present study is limited by the small number of participants from a single club, which may narrow generalizability of the findings to a broader population of elite soccer players. Also, the study's focus on elite professional soccer players may limit the applicability of the findings to other levels of play or to athletes in different sports. The absence of additional biomarkers (such as creatine kinase, C-reactive protein, growth hormone, sex hormone binding globulin and hematocrit), physiological characteristics (muscle strength, speed, agility, flexibility), and other possible time points during a competitive season or workload variables constitutes limitations for a clearer insight and suggest the need for further research in athletes' biochemical profiling to better understand the physiological and biochemical variations.

Conflict of Interest

The authors declare no conflict of interest.

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