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Original article

β-endorphin level and lactate elimination: effects on the shot and sprint performances in amateur soccer players

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Abstract. *Study Objectives:* This study aimed to examine the effects of lactate elimination and saliva β-endorphin (β-End) levels on the decrease in shot and sprint performances in the last period of a soccer match, and changes in saliva cortisol and testosterone levels during the match and their relationship to performance parameters. *Methods:* Twenty-two trained amateur soccer players performed the 90 minutes soccer match, while saliva β-End, testosterone, cortisol, blood lactate, and glucose measurements were obtained pre-match (M1), after 1st half (M2), and after the 2nd half (M3). Sprint and shot performances were assessed after warm-up and match play and after the Yo-Yo intermittent recovery test level 1 (Yo-Yo IR-1) test. Lactate elimination was evaluated with the Yo-Yo IR-1. *Results:* Soccer match-play elevated some individual β-End levels between M1 and M3 (an increase of %25) but overall differences between M1, M2, and M3 were not significant (%3.34 increase from M1 to M2, %1.12 increase from M1 to M3, and %2.17 decrease from M2 to M3). M3 testosterone levels significantly increased above basal ($p < 0.01$) and M2 levels ($p < 0.001$). Moreover, β-End levels and lactate elimination speeds were not significantly correlated with sprint and shot performance at M3 ($p > 0.05$). *Conclusion:* Our results suggest that β-End and lactate elimination is not effective on the differences in shot and sprint performances after exercise. Therefore, future studies will appropriate for the examination of β-End and other hormone levels to understand the exact mechanisms involved in different levels of soccer players.

Key words: Lactic Acid; Football, Testosterone, Cortisol, Hormone

Introduction

In addition to their frequent use as indicators of psychological stress because of their effect on human behaviour under stress, beta-endorphin (β-End), cortisol, and testosterone hormones also have important roles in regulating energy metabolism during exercise (1-3). β-End also elicits euphoria, reduces feelings of pain, and increases lactic acid (LA) tolerance, which is all-important in the development of fatigue (4). Furthermore, cortisol regulates glucose (GLC) production (gluconeogenesis) from amino acids via protein catabolism (5), and testosterone stimulates glycogen storage, as well as skeletal tissue and inner muscular protein synthesis (6). Also, the testosterone/cortisol (T/C) ratio is also used as an indicator of exercise intensity (7). β-End levels increase when the exercise intensity is >60% of the maximal oxygen consumption (8), cortisol concentrations increase when the exercise intensity is >60% of the aerobic power (9), and testosterone levels increase by %13–185 during treadmill or field running exercises and during high-intensity weight and bicycle ergometer workouts (10,11). Moreover, acute or chronic exercise-related changes are reflected by changes in the levels of these three hormones (12).

Depending on the frequency of high-intensity sprints during a match, peak LA levels of up to 10 mmol/L can be observed during a game, and a match is played at an average LA level of 7 mmol/L (13). The main reason for fatigue during a game of soccer is

probably the emptying of glycogen stores, rather than LA accumulation (14). Due to the decreases in glycogen levels during a match, fatty acid oxidation increases for energy supply, especially during the second half of a game. Thus, this means that the energy supply is slower, and resynthesizes GLC through glycogenesis is an important factor towards the end of the game, especially for a more effective shot and sprint performance. This finding shows that effective lactate elimination is important for performance during the latter parts of a game.

Studies have shown that important soccer performance factors, including shot speed, the possibility of reaching the target, and the amount of high intensity running, decline towards the end of a match. Decreases in these variables are particularly evident during high intensity running in elite players compared to players at lower levels (15,16). However, the relationship between the decrease in glycogen stores and high lactate levels, which are reasons for fatigue towards the end of a match, and the aforementioned hormones have not been determined. Besides, given that the decrease in a maximal exercise work capacity is related to an individual's perception of effort, rather than physical fatigue, endogenous opioid peptides (EOP) like β-End may weaken this perception (1). This has not been previously studied, and this hormone is considered to have positive effects on shot and sprint performances.

For the reasons above, the main purpose of this study was to examine the effects of lactate elimination and salivary β-End levels on the possible decrease in shot and sprint performance during the last period of a game played by 18–25 year-old trained soccer players. Furthermore, changes in salivary cortisol and testosterone levels during a game and their relationships with performance parameters specified were examined.

Materials and Methods

Participants

Twenty-two male players (age 23.7 ± 3.47 years, height 177.43 ± 5.46 cm, body mass 75.2 ± 6.96 kg) who did not smoke or consume alcohol, were not taking any medicine or ergogenic supplement and attended amateur level soccer training (4 days per week, an average of 1.5 h of training, and a 1-day league match) voluntarily participated in the study. After receiving the approval of the Clinical Research Ethics Committee of the Faculty of Medicine, on the testing days, signed written consent was provided by each athlete after informing them of the purpose and benefits of the study and giving a detailed explanation of the tests to be performed on the testing days.

Experimental Design

After a contact meeting with the study participants, height, and body mass (Sinbo SBS-4414, precision: 100 g) were recorded. A week later, a Yo-Yo IR-1 test protocol was performed with soccer equipment on natural grass. Three days after the first test, a lactate elimination test (LET) was performed using multiple sprint tests on the soccer pitch to determine the lactate elimination speed (LES) of each subject. A week after this measurement a soccer match was held on a proper soccer pitch (90 × 120 m). Sprint and shot performance tests were evaluated after warm-up before routine team training was finalized. All testing was carried out at 16:00 ± 1 and at a temperature of 24.5°C ± 2°C. The participants were instructed to avoid changing their diets significantly and were prohibited from consuming alcohol 2 days before the measurements specified. Necessary arrangements were made with the team trainers about the training loads to be given before the tests, and the players were instructed to avoid individual training, except for team training.

Collection of Data

Yo-yo intermittent recovery test: Yo-Yo test procedures were performed according to the recommendations of Krustrup et al (2003) (17). The test consisted of 20-m shuttle runs performed at increasing velocities, with 10 s of active recovery between runs until exhaustion. The increases in running speed during the test were given by signals on a portable CD player (Philips, Az1030 CD player, Eindhoven, Holland) until the athletes finalized the test. The tests were ended when the participants could not reach the front line twice (objective evaluation) within a given signal or when they would not be able to complete another

shuttle run at a specified speed (subjective evaluation). When the test was finalized, the distance covered by the subject was recorded as the test score, and the subject's heart rate (HR) was recorded with a Polar RS 400 HR monitor (Polar, Kempele, Finland).

As soon as the tests were finalized, the athlete's level of perception of the exercise intensity was determined with an oral 10-point Borg Scale (RPE). Subsequently, approximately 5 min after finalizing the test, saliva, and fingertip blood samples were taken for hormone and lactate measurements, and they were termed "after Yo-Yo" (AYY). Yo-Yo IR-1 test results were used to evaluate the individual maximal effort of the participants.

Lactate elimination test: Seven 30-m sprints, with a 25-s turning jog to the start line at the end of each sprint, were performed. The athletes' sprint times were recorded by infrared sensor gates (Newtest Oy, Oulu, Finland) located at the start and 5 m and 30 m of the finish lines. For the participants to reach their maximal LA levels, the test was not finalized after the seventh 30-m sprint but was evaluated after they ran half of the soccer pitch at maximal speed to the test finish point. The amount of lactate in the fingertip blood samples was evaluated at 5 min (maximal lactate; LA_{max}) and 20 min (LA_{20}) after the test. The LES was calculated by dividing the difference between LA_{max} and LA_{20} relative to the time passed (15 min). Besides, to assess the heart rate recovery speed (HR_{rs}), we measured the difference between the maximal HR values taken immediately after exercise (HR_{max}) and those taken 3 min after exercise (HR_3) and divided it by 3.

Soccer match: Five days before the soccer match, the players did not participate in other official matches, and 2 days before the match, they were subjected to training programs without extensive high-intensity work and 1 day of rest. The match was played as a practice match on a proper soccer pitch (90 × 120 m), in which the rules of a 90-min official soccer match were applied. For measuring biochemical and hormonal parameters, saliva and fingertip blood samples were taken approximately 5 min before the match (M1), at halftime (M2), and at the end of the match (M3). The physiological, biochemical, and hormonal parameters measured before the match were accepted as the basal values. During the game, the HRs of all the players were recorded on a computer with an Activio Sport System (Activio AB, Stockholm, Sweden). Fatigue perceptions of each player after the M2 and M3 periods were determined using an RPE. During the last 10 min of the match, 2 players from each team performed 5 × 20-m sprints and shot tests in a specified measurement area, and the percentages of shots on target and shot and sprint speeds under end-of-match conditions were determined.

Sprint and shot performance: Performance tests, including 5 and 20 m sprint times, shot speeds, and shots on target, were evaluated at the end of the match, Yo-Yo IR-1 test, and after warm-up. After the warm-up session testing was performed on the third day following the match, after 1 day of recovery training, and 1 day of aerobic training (60–65% HR_{max}). This test was used as the rest performance of the subjects.

The test procedure included a total of 5 × 20-m sprints and shooting the ball towards the goal after each sprint while standing in the penalty area. Also, 30-s rests were taken after each sprint and shot activity. The athletes' sprint times were recorded using infrared sensor gates (Newtest Oy, Oulu, Finland) located at the start and 5 m and 30 m of the finish lines. The shot speed after the sprints was recorded behind the goal using a Bushnell Speedster II Sports Radar Gun (Bushnell Performance Optics, Kansas, USA), which can take measurements from distances of 27 m and speeds of 16–177 km/s with an accuracy of ±2 km/s. The number of shots hitting the target was evaluated in terms of sending the ball to the target without a goalkeeper, and each shot was recorded as a goal or a miss.

Blood handling and analysis: To perform the LA and GLC analysis, capillary blood samples (10 μL) were taken from the fingertip of each participant. The subject's fingertip was first wiped with alcohol and then dried with a piece of cotton wool. The fingertip was then pierced with a lancet and squeezed gently. LA and GLC concentrations were measured with a Biosen C-Line Sport Analyser (EKF Diagnostics, Magdeburg, Germany).

Salivary handling and analysis: Two to three milliliters of saliva were collected by blowing into high-quality, clean hygienic polypropylene tubes through a 10-cm plastic pipe. The collected samples were stored at 4°C within 30 min. These samples were centrifuged for 20 min at 1000 *g* (NÜVE, NF200, Ankara) within 2 h of collection. Following centrifugation, the top

clear liquid portion was transferred into a clean polypropylene tube, covered with a parafilm, and refrigerated at -20°C (Regal CF 2101, Manisa) until analysis. Samples were thawed before analysis, mixed with a vortex to get rid of mucus and other substances, and were centrifuged again at 1000 g for 20 min. The top clear portion was transferred into polypropylene tubes. β-End, cortisol, and testosterone analyses were carried out with a microplate reader using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The salivary β-End analysis was performed with a human beta-endorphin ELISA Kit (E0806H kit, Uscn Life Science Inc, Wuhan, P.R. China) and testosterone and cortisol analyses with DIAMETRA Saliva Kits (DKO02, Milano, Italy).

Statistical analysis

Simple descriptive statistics are reported as means ± standard deviations (mean ± s). A Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. The data were found to have a normal distribution and parametric analysis techniques were therefore employed. Relationships between the data were evaluated with Pearson r correlation analysis. A repeated-measures ANOVA was used to compare the differences in repeated measures. When a difference was found in the repeated measures, post hoc analysis with a Bonferroni adjustment was performed to determine the time point at which the difference occurred. The data were analysed with SPSS for Windows (version 18.0; SPSS Inc, Chicago). Statistical significance was set at $p < 0.05$.

Results

Data regarding the soccer match

Table 1 shows the M1 (after warm-up as pre-match), M2 (half time), and M3 (after match) physiological, biochemical, and hormonal parameters, as well as the differences between the time points. Although the β-End levels between M1 and M3 increased in some players (an increase of 251% between M1 and M3) overall differences were not significant ($p = 0.83$, 95% CI: -0.97 to 0.80, $p = 0.95$, 95% CI: -1.10 to 1.04, $p = 0.91$, %95 CI: -1.08 to 1.20), between M1, M2, and M3 values (3.34% between M1 and M2, 1.12% increase between M1 and M3, and 2.17% decrease between M2 and M3). Typical errors above 52.6% coefficient of variation (CV) demonstrated an unfavorable level of agreement in β-End levels between M1, M2, and M3. Testosterone levels at M3 showed a significant increase compared to those at M1 ($p < 0.01$) and M2 ($p < 0.001$). Although no significant difference in the testosterone level was observed between M1 and M2 (P= 0.476, 95% CI = -29.50 to 60.38), typical errors were ≥55% CV for these periods. Although cortisol did not show a significant difference in the third period examined (between M1 and M2 95% CI: -4.28 to 3.61, between M1 and M3 95% CI: -3.41 to 3.54, and between M2 and M3 95% CI: -2.17 to 2.97), a bigger typical error (71.5% CV) was found between hormone levels (Table 1).

Correlation analysis of parameters during different parts of the soccer match

The relationships between HR, LA, GLC, β-End, testosterone, cortisol, and T/C ratios during different parts of the match (M1-M2-M3) were analysed. A significant correlation are shown in Table 2. There was no significant correlation (data not shown) between other markers.

Percentage evaluations of the match data in comparison with the data obtained after a maximal effort and the results of the Yo-Yo intermittent recovery test

Comparisons between the data obtained from 3 different parts of the soccer match obtained after the maximal exercise test (Yo-Yo IR-1) are shown in Table 4, and the Yo-Yo IR-1 test results are shown in Table 3. HR values during the match corresponded to a maximal level. On the other hand, LA levels were almost half of the maximal lactate. β-End levels during all parts of the match were close to those at a maximal effort level. M2 testosterone levels corresponded to nearly 68% of the maximal, while they appeared to exceed 100% at M3. M2 cortisol levels were 3 times higher than maximal and returned to the basal values at the end of the

Table 1. Physical, physiological, biochemical measurements and differences between the time points

Variables	M1	M2	M3	Periods	Diff.	*p*-value
LA (mmol. L^{-1})	1.76±0.55	5.21±1.68	5.42±185	M1 – M2 M1 – M3 M2 – M3	-3.4*** -3.6*** -0.2	0.000 0.000 0.698
β-End (pg/mL)	2.67±1.52	2.76±1.76	2.70±1.07	M1 – M2 M1 – M3 M2 – M3	-0.0 -0.0 -0.0	0.837 0.957 0.913
Testosterone (pg/mL)	96.81±46.82	81.37±50.33	140.68±39.30	M1 – M2 M1 – M3 M2 – M3	-15.4 -43.8** -59.3***	0.475 0.010 0.001
Body Mass (kg)	75.21±6.96	74.66±6.80	73.98±6.73	M1 – M2 M1 – M3 M2 – M3	0.5*** 1.2*** 0.6**	0.000 0.000 0.002
HR (beats. min^{-1})	67.56±3.79	154.12±8.42	157.31±5.58	M1 – M2 M1 – M3 M2 – M3	-86.5*** -89.7*** -3.1	0.000 0.000 0.081
GLC (mmol. L^{-1})	3.16±0.66	4.41±1.71	3.26±1.09	M1 – M2 M1 – M3 M2 – M3	-1.2** -0.0 -1.1**	0.008 0.660 0.009
Cortisol (ng/mL)	5.93±4.74	6.26±4.44	5.86±4.37	M1 – M2 M1 – M3 M2 – M3	-0.3 -0.06 -0.4	0.858 0.970 0.745
T/C	25.86±20.15	19.25±15.38	39.59±32.91	M1 – M2 M1 – M3 M2 – M3	-6.6 -13.7 -20.3*	0.259 0.098 0.018

M1= Before match, M2= Half-time, M3= After match, HR= Heart rate, LA= Lactate, GLC= Glucose, β-End= Saliva beta endorphin, T/C= Testosterone-cortisol ratio. *significant at $p < 0.05$, ** significant at $p < 0.01$, ***significant at $p < 0.001$.

match. M2 T/C values were at 25% of the maximal but reached 50% after the match (Table 4).

Lactate elimination test

The lactate elimination test parameters provided in Table 5.

Relationships between the end-of-match sprint and shot performance and β-End, LES, FI, RPE, and other parameters

A negative relationship was found between T/C_3 and the ratio of end-of-match shots on target ($r = -0.613$; $p < 0.01$). Also, a negative relationship was determined between LA_{max} and the ratio of M3 shots on target ($r = -0.775$; $p < 0.001$). No relationships were found between the physiological and biochemical parameters at M3 and those at M3 sprint and shot parameters (Table 6).

Changes in measurements of performance and biochemical parameters

There was a non-significant 9% decrease in the average 20-m sprint running time between M1 and M3 and a 3% decrease between AYY and M3. A significant difference ($p < 0.05$) was found only in the 5-m and 20-m sprint times between M3 and AYY (Figure 1).

Discussion and Conclusion

This is the first study to examine possible relationships between changes in the shot and sprint

Table 2. Relationships between the HR, LA, GLC, β-End, testosterone, cortisol, and T/C ratios during different parts of the match

Variables	Periods	r-value	p-value
β-End$_1$ - β-End$_2$	M1 – M2	0.498	0.05
GLC$_1$ - GLC$_3$	M1 – M3	0.615	0.01
β-End$_1$ - T/C$_2$	M1 – M2	0.533	0.03
Cortisol$_1$ - GLC$_2$	M1 – M2	0.537	0.03
T/C$_1$ and β-End$_3$	M1 – M3	0.489	0.05
T/C$_1$ and Testosterone$_3$	M1 – M3	0.538	0.03
HR$_2$ - HR$_3$	M2 – M3	0.592	0.01
HR$_2$ - GLC$_3$	M2 – M3	0.633	0.01
T/C$_2$ - β-End$_3$	M2 – M3	0.493	0.05
HR$_1$ - HR$_2$	M1 – M2	-0.628	0.01
GLC$_1$ - Testosterone$_2$	M1 – M2	-0.692	0.03
Testosterone$_1$ - Testosterone$_2$	M1 – M2	-0.504	0.04
Testosterone$_1$ - Cortisol$_2$	M1 – M2	-0.486	0.05
Cortisol$_1$ - Testosterone$_3$	M1 – M3	-0.569	0.02
Testosterone$_1$ - GLC$_3$	M1 – M3	-0.628	0.02
T/C$_2$ and testosterone$_3$	M2 – M3	-0.054	0.03

M1= Before match, M2= Half time, M3= After match HR= Heart rate, LA= Lactate, GLC= Glucose, β-End= Beta endorphin, T/C= Testosterone /cortisol ratio

performances observed during the last period of an amateur soccer game and lactate elimination, as well as salivary β-End, cortisol, and testosterone levels. The average HR values at half-time and the end of the match corresponded to 85–87% of the athlete's maximal values, while blood LA values were 5.4 mmol/L and 5.2 mmol/L levels at half-time and the end of the match, respectively. Our match results showed the same effect on HR and LA levels with other training and practice match studies (18-20). The results of our study, which were performed in the form of a training match, generally coincide with those of similar studies, suggesting that the training match had a physiological stress effect on the players similar to the effects of real matches.

β-Endorphin

During a soccer match, the average consumed energy corresponds to approximately %75 of the maximal aerobic power. Due to this high energy demand, a decrease in muscular glycogen levels occurs towards the end of the game, which means that the glycogen content of Type I fibrils is halfway depleted, and Type IIA fibril glycogen is almost depleted (17). This is reflected in a decrease in the work rate (14). Therefore, the depleted muscular glycogen stores may have an important role in the decrease in performance during a game (17). It has also been stated β-End levels increase at anaerobic threshold intensities (21), facilitate working muscle glucose uptake during exercises at this intensity (22), and limit the decrease in blood GLC levels by increasing glucagon synthesis. In our study, no significant relationship was found between β-End and GLC levels at any sampling point. However, positive correlations were found between β-End and testosterone and T/C levels, implying that β-End may only have an indirect anabolic role, parallel to testosterone, in maintaining blood GLC levels during a match.

β-End receptors are excessively located in the neuromuscular intersections of muscles, and production at the neuromuscular junction of β-End by motor nerve terminals may be an important factor in reducing neuromuscular fatigue during the exercise of enough time and intensity (23). In a study by Khan et al. (2005), no significant relationship was found between basal plasma levels (4.8 ± 1.0 pmol/L) and plasma β-End levels after cycle ergometer exercise at work rates of

Table 3. The Yo-Yo intermittent recovery test level-1 results

	LA (mmol. L-1)	GLC (mmol. L-1)	HR (beats. min-1)	Total distance (m)	RPE	Testosterone (pg/ml)	Cortisol (ng/mL)	β-End (pg/ml)	T/C
Mean±SD	11.73±1.94	5.76±0.97	181.75±10.36	813.75±264.19	7.87±0.71	130.18±29.15	2.08±0.75	2.87±1.10	69.87±30.01

HR= Heart rate, LA= Lactate, GLC= Glucose, β-End= Saliva beta-endorphin, T/C= Testosterone-cortisol ratio, RPE= Rate of perceived exertion

Table 4. Percentage ratios of the data obtained from different parts of the soccer match in comparison with the data after the maximal exercise test (Yo-Yo IR-1 test)

Variables	M1 (%)	M1	M2 (%)	M2	M3 (%)	M3
HR (beats. min^{-1})	37.17	67.56±3.79	84.79	154.12±8.42	86.55	157.31±5.58
LA (mmol · L^{-1})	15.00	1.76±0.55	44.41	5.21±1.68	46.20	5.42±1.85
GLC (mmol · L^{-1})	54.86	3.16±0.66	76.56	4.41±1.71	56.59	3.26±1.09
β-End (pg/ml)	93.03	2.67±1.52	96.16	2.76±1.76	94.07	2.70±1.07
Testosterone (pg/ml)	74.36	96.81±46.82	62.50	81.37±50.33	108.06	140.68±39.30
Cortisol (ng/ml)	285.09	5.93±4.74	300.96	6.26±4.44	281.73	5.86±4.37
T/C	37.01	25.86±20.15	27.55	19.25±30.01	56.66	39.59±32.91

M1= Before match, M2= Half time, M3= After match, HR= Heart rate, LA= Lactate, GLC= Glucose, β-End= Saliva beta-endorphin, T/C= Testosterone-cortisol ratio

Table 5. Lactate elimination test results

	5m sprint (s)	30m sprint (s)	LA_B (mmol · L^{-1})	GLC_B (mmol · L^{-1})	LA_5 (mmol · L^{-1})	LA_{20} (mmol · L^{-1})	LES (mM/min)
Mean±SD	0.96±0.06	4.31±0.18	1.63±0.50	4.36±0.42	14.11±2.43	8.43±2.49	0.37±0.08
	HR_{end} (beats · min^{-1})	HR_3 (beats · min^{-1})	HR_{RS} (beats · min^{-1})	GLC_5 (mmol · L^{-1})	GLC_{20} (mmol · L^{-1})	FI (%)	RPE
Mean±SD	184.50±7.44	114.37±12.12	23.37±3.48	5.27±0.87	4.36±0.46	0.28±0.14	9.12±0.71

LA= Lactate, LA_B= Basal lactate, LA_5= Lactate in after 5 min test, LA_{20}= Lactate in after 20 min test, HR_{end}= Lactate in after finish test, HR_3= Heart rate in after 3 min test, HR_{RS}= Hear rate recovery speed, GLC_5: Glucose in after 5 min test, GLC_{20}: Glucose in after 20 min test, FI: Fatigue index, LES: Lactate elimination speed, RPE: Rate of perceived exertion

%40 and %60 VO_{2max} (3.8 ± 0.7 and 6.3 ± 0.9 pmol/L respectively) in 10 healthy men and women (23). However, a significant increase (16.1 ± 4.0 pmol/L) has been shown to occur at high exercise intensities (%80 VO_{2max}) than those performed in the current study (21). When examining the salivary β-End levels in our study, the values obtained at M1 (2.7 pg/mL), M2 (2.8 pg/mL), and M3 (2.7 pg/mL) showed a very small increase, but this was not statistically significant. However, the β-End levels of 2 athletes at M1 were 1.50 pg/mL, whereas those values increased to 5.27 pg/mL at M3. Considering these results, salivary β-End levels may show changes and are useful in place of plasma in these types of studies. Moreover, no significant relationship was found between the RPE and β-End levels after the match. However, in another study, β-End levels were increased 2-fold following maximal treadmill exercise in trained men, and 6 months of endurance training, basal plasma values were decreased by nearly %47, and the feeling of pain after exercise was reduced. The same study also found no significant relationship between blood plasma β-End levels and the feeling of pain (24), which is similar to that reported in our study.

Although the 5-m and 20-m sprint speeds and the shot speeds on target at the end of the match were not significantly different from the after-warm-up values, a %9 decrease in the 20-m sprint times at M1 and M3 was observed. Moreover, the percentage of shots on target was lower, although not significant. In a study carried out with elite players, the end-of-game sprint times did not significantly decrease, like that reported in our study (25). Besides, we found that M3 β-End levels were not significantly related to the shot speed and percentage of shots on target. Therefore, it is not possible to claim that β-End levels may have a role in the end-of-the-match small decreases (%3.2) in the 20-m shot speed and percentage of shots on target.

Table 6. Relationships between the shot and sprint performance data and β-End, LES, FI, RPE, and other parameters at the end of the match

Variables	5 m (s)	20 m (s)	Shot speed (km/h)	Goal percentage (%)
HR_3 (beats · min^{-1})	r=-0.127 p=0.64	r=-0.127 p=0.64	r=-0.168 p=0.53	r=0.336 p=0.20
LA_3 (mmol · L^{-1})	r=-0.002 p=0.99	r=0.079 p=0.77	r=-0.214 p=0.42	r=-0.093 p=0.73
GLC_3 (mmol · L^{-1})	r=-0.174 p=0.51	r=-0.343 p=0.19	r=-0.322 p=0.22	r=-0.054 p=0.84
β-End_3 (pg/mL)	r=0.078 p=0.77	r=-0.041 p=0.87	r=-0.392 p=0.13	r=-0.284 p=0.28
$Testosterone_3$ (pg/mL)	r=-0.463 p=0.07	r=-0.332 p=0.20	r=0.173 p=0.52	r=-0.025 p=0.92
$Cortisol_3$ (ng/mL)	r=-0.233 p=0.38	r=0.055 p=0.84	r=0.369 p=0.16	r=0.345 p=0.19
T/C_3	r=-0.209 p=0.43	r=-0.364 p=0.16	r=-0.314 p=0.23	r=-0.613 p=0.01**
LA_{max} (mmol · L^{-1})	r=0.080 p=0.76	r=-0.202 p=0.45	r=-0.352 p=0.18	r=-0.775 p=0.000***
LES (mmol/min)	r=-0.365 p=0.16	r=-0.147 p=0.58	r=0.049 p=0.85	r=-0.211 p=0.43
HR_{RS} (beats · min^{-1})	r=-0.197 p=0.46	r=0.076 p=0.78	r=0.178 p=0.51	r=0.176 p=0.51
FI (%)	r=-0.159 p=0.55	r=0.119 p=0.65	r=0.447 p=0.08	r=0.435 p=0.09
RPE	r=0.247 p=0.35	r=0.320 p=0.22	r=-0.017 p=0.95	r=-0.103 p=0.70

HR_3= Heart rate in M3, LA_3= Lactate in M3, GLC_3= Glucose in M3, β-End3= Beta endorphin in M3, FI= Fatigue index, T/C_3= Testosterone / cortisol ratio in M3, LES= Lactate elimination speed, HR_{RS}= Heart recovery speed, RPE= Rate of perceived exertion. LA_{max}= Maximal lactate

Lactate elimination

In our study, no significant relationships or differences were found between the LES and shot performances during M1 and M3. In studies examining fatigue and recovery, and the effects of these parameters on leg strength during short-term intensive leg extensions (26,27), 8–9 mmol/L peak LA levels were reached after exercise, which is similar to the values obtained in our study and soccer matches. Furthermore, these authors found a %40–80 decrease in the average leg peak torque following loading periods. Small decreases in LA during 30–60-s recovery periods were found, whereas a much faster recovery (47–85%) was seen in strength levels. Considering the importance of the rectus abdominis and knee extensor muscle strength for effective shot performance, it is possible to 1- 1.5 min rest time before the shot test could have provided enough strength recovery in these muscles to effective shot performance even during M3.

Furthermore, as strength is maintained to a greater extent during maximal voluntary muscle movements, than during concentric movements at large angular speeds (i.e., shot movements) (28), we suggest that the maintenance of leg strength plays a more important role in shot performance efficiency than lactate elimination.

Testosterone and cortisol

Testosterone levels showed biphasic during the match, decreasing in the first half and increasing in the second half. The reason for the decrease during M1 may be due to the suppression of luteinizing hormone production by the pituitary gland (29) and hypothalamic

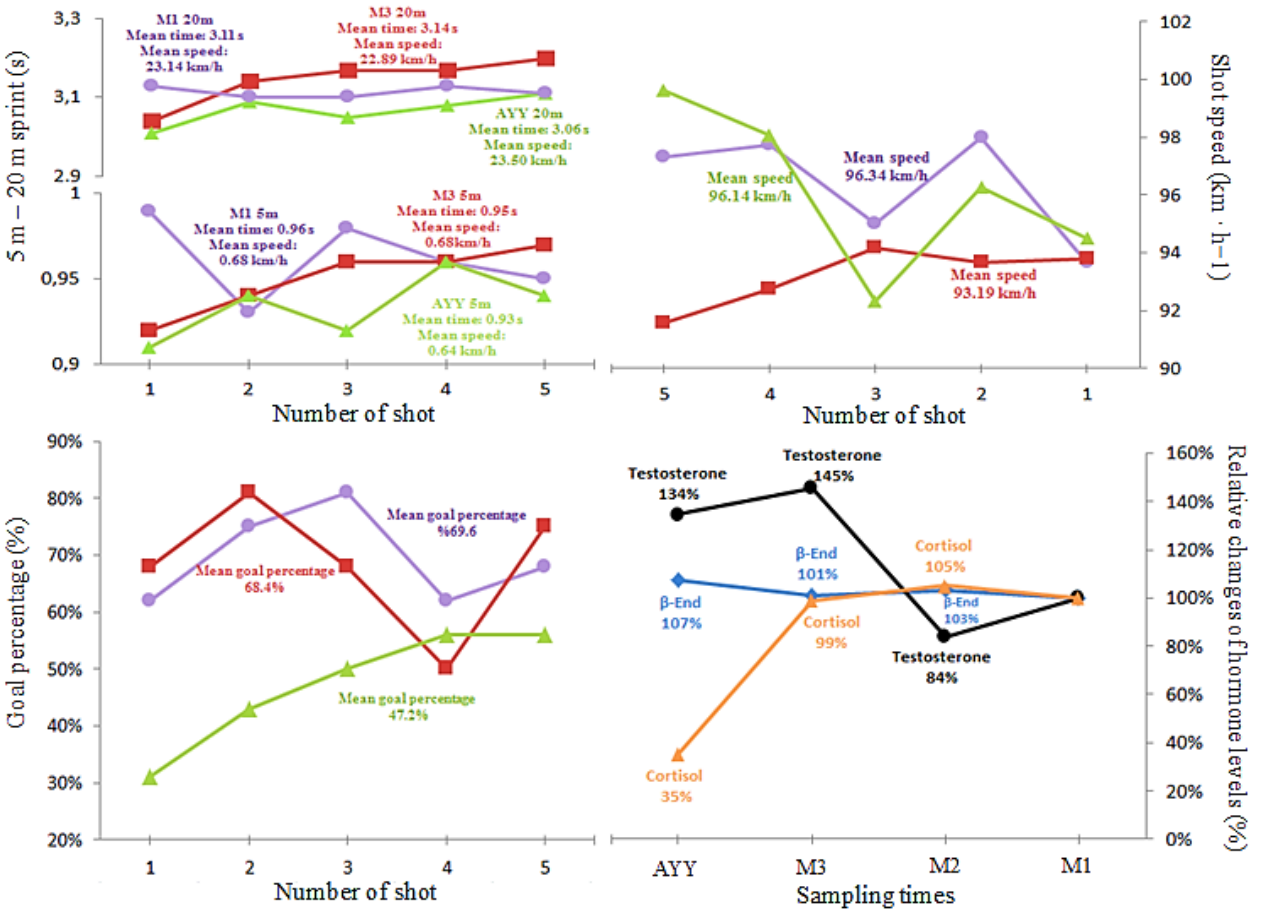

Figure 1. Changes in measurements of performance (5-m sprint, 20-m sprint, goal percentage, and shot speed) at pre-match (M1), post-match (M3), and after Yo-Yo IR-1 test (AYY) and in biochemical parameters (beta-endorphin, testosterone, cortisol levels). The times when the measurements were taken and the average values of these measurements are shown in different colors (Red: before match, purple: after the match, green: after Yo-Yo IR-1 test). Values for the hormones are given at the right down corner (Blue: beta-endorphin, orange: cortisol, black: testosterone).

corticotrophin-releasing hormone (CRH) secretion because of increased cortisol levels. Due to the secretion of gonadotropin-releasing hormone, the corticotropic axis suppresses the gonadotropic axis at the hypothalamic level (29,30) with a direct effect of CRH or via an increase in β-End. This may explain the decrease in testosterone levels during M1, while cortisol levels were high, which was observed at half-time in our study. The reason behind the increase in testosterone during M2 may be related to a decrease in testosterone discharge and inactivation due to a decline in liver blood flow, thus resulting in a temporary increase in blood testosterone levels at the end of the exercise (31). The increase in testosterone levels may also be due to the decrease in glycogen stores towards the end of the soccer match, which employs both anaerobic and aerobic endurance because testosterone is known to have opposite effects to cortisol and also affects the maintenance of glycogen stores (32). Therefore, endogenous hormones may change with an energy metabolism based on fat dominance, rather than GLC, towards the end of the match. In support of this, a study carried out with female soccer players found that parallel with an increased anti-oxidant capacity after a match, blood-free testosterone levels increased while GLC levels decreased to a similar extent as the current study (33). Testosterone is a hormone with positive effects on muscle growth and consequential performance (34). It has been shown that, particularly in studies with male athletes (weight-lifters, soccer players, and handball players), there are low or moderate

level correlations between blood and salivary testosterone and/or cortisol levels and jump height, sprint ability, and maximal strength (35-37). In another study with elite rugby players, significant relationships were found between salivary testosterone and cortisol levels and several measurements of sprint speed, power, and strength (38). In contrast to other studies, we found that the end-of-match performance parameters (sprint, shot speed, and percentage of shots on target) were not significantly different from after warm-up values. The lack of a significant increase in β-End and cortisol levels and an increase in testosterone levels may be due to the non-significant relationships between sprint and shot performance parameters and cortisol and testosterone levels.

Since the present study is the first to examine β-End with salivary samples in a team sport, it is quite difficult to compare these results with those of studies that have used blood hormone levels. This is because, as an indirect determiner of free hormones, saliva allows for an endocrinal measurement that is more sensitive than total hormonal measurements (39). Besides, factors such as the analytic methods and study designs employed, the training state of the subjects, and the type, duration, and intensity of exercises may have played a role in the differences found with other studies. Moreover, the relationships between the performance parameters and the hormones examined in the present study may have been influenced by the positional differences of the players on the field. However, this type of possible relationship could not be studied due to the inadequate numbers of players. This assumption may be addressed in future research.

Conflicts of interest: The authors declare that there is no conflict of interest in this manuscript.

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References

1. Sgherza AL, Axen K, Fain R, et al. Effect of naloxone on perceived exertion and exercise capacity during maximal cycle ergometry. J Appl Physiol. 2002; 93:2023-8.
2. Bhasin S. Regulation of testicular function. In The Encyclopedia Of Sports Medicine an IOC Medical Commission Publication. ed. The Endocrine System in Sports and Exercise, Volume XI. USA: Blackwell Publishing Ltd; 2005:298.
3. Ghayee HK, Auchus RJ. Basic concepts and recent developments in human steroid hormone biosynthesis. Rev Endocr Metab Disord. 2007;8(4):289-300.
4. Cunha GS, Ribeiro JL, Oliveira AR. Levels of beta-endorphin in response to exercise and overtraining. Arq Bras Endocrinol Metabol. 2008;52(4):589-98.
5. Tunn S, Mollmann H, Barth J, et al. Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. Clin Chem; 1992; 38:1491-1494.
6. Viru A, Viru M. Biochemical Monitoring of Sport Training. USA: Human Kinetics Pub; 2001:104-106.
7. Slivka DR, Hailes WS, Cuddy JS, et al. Effects of 21 days of intensified training on markers of overtraining. J Strength Cond R. 2010;24(10):2604-2612.
8. Viru A, Tendzegolskis Z. Plasma endorphin species during dynamic exercise in humans. Clin Physiol. 1995; 15:73-9.
9. Gozansky WS, Lynn JS, Laudenslager ML et al. Salivary cortisol determined by enzyme immunoassay is preferable to serum total cortisol for assessment of dynamic hypothalamic-pituitary-adrenal axis activity. Clin Endocrinol. 2005; 63:336-341.
10. Crewther BT, Lowe TE, Ingram J, et al. Validating the salivary testosterone and cortisol concentration measures in response to short high-intensity exercise. J Sports Med Phys Fitness. 2010; 50:85-92.
11. Wilkerson JE, Horvath SM, Gutin B. Plasma testosterone during treadmill exercise. J Appl Physiol. 1980; 49:249-253.
12. Cumming DC. Hormones and Athletic Performance. In Endocrinology and Metabolism. New York: Mc Graw Hill; 1995:1837-1886.
13. Christensen PM, Krustrup P, Gunnarsson TP, et al. VO_2 kinetics and performance in soccer players after intense training and inactivity. Med Sci Sports Exerc. 2011;43(9):1716-1724.
14. Reilly T. Energetics of high intensity exercise (soccer) with particular reference to fatigue. J Sports Sci. 1997;15(3):257-63.
15. Mohr M, Krustrup P, Bangsbo J. Match performance of high-standard soccer players with special reference to development of fatigue. J Sports Sci. 2003; 21:439-449.
16. Rienzi E, Drust B, Reilly T, et al. Investigation of anthropometric and work-rate profiles of elite south american international soccer players. J Sports Med Phy Fitness. 1998; 40:162-169.
17. Krustrup P, Mohr M, Amstrup T, et al. The yo-yo intermittent recovery test: physiological response, reliability, and validity. Med Sci Sports Exerc. 2003; 35:697-705.
18. Bangsbo J. The physiology of soccer - with special reference to intense intermittent exercise. Acta Physiol Scand. 1994; 619(Suppl 1): S155.
19. Mohr M, Krustrup P, Nybo L, et al. Muscle temperature and sprint performance during soccer matches – beneficial effect of re-warm-up at half time. Scand J Med Sci Sports. 2004; 14:156–162.
20. Krustrup P, Mohr M, Steensberg A, et al. Muscle and blood metabolites during a soccer game: implications for sprint performance. Med. Sci. Sports Exerc. 2006;38(6): 1165 – 1174.

21. McMurray RG, Sheps DS, Guinan DM. Effects of naloxone on maximal stress testing in females. J Appl Physiol. 1984; 56:436-40.
22. Angelopoulos TJ. Beta-endorphin immunoreactivity during high-intensity exercise with and without opiate blockade. Eur J Appl Physiol. 2001; 86:92-96.
23. Khan S, Evans AA, Hughes S, et al. Beta-endorphin decreases fatigue and increases glucose uptake independently in normal and dystrophic mice. Muscle Nerve. 2005;31(4):481-6.
24. Øktedalen O, Solberg1 EE, Haugen AH, et al. The influence of physical and mental training on plasma beta-endorphin level and pain perception after intensive physical exercise. Stress and Health. 2001; 17:121-127.
25. Ispirlidis I, Fatouros IG, Jamurtas AZ et al. Time-course of changes in inflammatory and performance responses following a soccer game. Clin J Sport Med. 2008;18(5): 423-31.
26. Tesch PA, Wright JE. Recovery from short term intense exercise: its relation to capillary supply and blood lactate concentration. Eur J Appl Physiol. 1983; 52:98-103.
27. Jansson E, Dudley GA, Norman B, et al. Relationship of recovery from intense exercise to the oxidative potential of skeletal muscle. Acta Physiol Scand. 1990;139(1):147-52.
28. Tesch PA, Dudley GA, Duvoisin MR, et al.): Force and EMG signal patterns during repeated bouts of concentric or eccentric muscle actions. Acta Physiol Scand; 1990;138(3):263-71.
29. Cumming DC, Quigley ME, Yen SSC. Acute suppression of circulating testosterone levels by cortisol in men. J Clin Endocrinol Metab. 1983; 57:671-673.
30. Barbarino A, De Marinis L, Tofani A, et al. Corticotropin-releasing hormone inhibition of gonadotropin release and the effect of opioid blockade. J Clin Endocrinol Metab. 1989;68(3):523-528.
31. Cadoux-Hudson TA, Few JD, Imms FJ. The effect of exercise on the production and clearance of testosterone in well trained young men. Eur J Appl Physiol. 1985;54: 321-5.
32. Guezennec Y, Leger L, Lhoste F, et al. Hormone and metabolite response to weight-lifting training sessions. Int J Sports Med. 1986;07(2):100-105.
33. Gravina L, Ruiz F, Lekue JA, et al. Metabolic impact of a soccer match on female players. J Sports Sci. 2011;29(12):1345-52.
34. Viru A, Viru M. Resistance Exercise and Testosterone. In the Encyclopaedia of Sports Medicine an IOC Medical Commission Publication. ed. The Endocrine System in Sports and Exercise, Volume XI. USA: Blackwell Publishing Ltd; 2005:321-322.
35. Cardinale M, Stone MH. Is testosterone influencing explosive performance? J Strength Cond Res. 2006; 20:103-107.
36. Kraemer WJ, French DN, Paxton NJ, et al. Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. J Strength Cond Res. 2004; 18:121-128.
37. Passelergue P, Robert A, Lac G. Salivary cortisol and testosterone variations during an official and a simulated weight-lifting competition. Int J Sport Med. 1995; 16:298-303.
38. Crewther BT, Lowe TE, Weatherby RP, et al. Neuromuscular performance of elite rugby union players and relationships with salivary hormones. J Strength Cond Res. 2009;23(7):2046-53.
39. Gotshalk LA, Loebel CC, Nindl BC, et al. Hormonal responses of multiset versus single-set heavy-resistance exercise protocols. Can J Appl Physiol. 1997; 22:244-255.

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