BIOLOGICAL ASPECTS OF SALIVARY HORMONES IN MALE HALF-MARATHON PERFORMANCE

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Abstract: Physical effort is known to alter the blood levels of many hormones, but there are only a few studies about the biological changes of salivary hormones. The aim of this work was to determine whether salivary testosterone and salivary cortisol levels, measured two weeks before a half-marathon race, relate to running performance in male recreational athletes. A group of eleven male recreational athletes preparing for a half-marathon was included in the study. Saliva for testosterone and cortisol determinations was collected before and immediately after a 15-km training run, two weeks before the half-marathon. Individual official half-marathon times, expressed in hours, were used as a measure of performance. Mean testosterone concentrations were 1.07±0.33 nmol/l before the run and 0.88±0.35 nmol/l after the run ($p<0.05$). Mean cortisol concentrations were 12.28±8.46 nmol/l before the run and 38.08±19.63 nmol/l after the run ($p<0.05$). The pre-run salivary testosterone levels marginally correlated with the corresponding half-marathon running times ($p=0.068$, 95% bootstrap CI for slope -0.40 to -0.06). However, post-run salivary testosterone levels significantly correlated with the corresponding half-marathon running times ($p=0.011$, 95% bootstrap CI for slope -0.41 to -0.16), even considering correlations with the runners' age. Salivary cortisol levels, either pre- or post-run, did not correlate with the corresponding half-marathon running times. The results of this study suggest that post-exercise salivary testosterone levels could have the potential to predict performance in endurance running, at least in recreational athletes.

Key words: salivary hormones; testosterone; recreational athlete; running; age

INTRODUCTION

The levels of testosterone and cortisol are acutely elevated following intensive physical efforts, including most physical competitions [1]. The rise in levels of endogenous hormones, testosterone and cortisol, improves muscle performance and energy mobilization, respectively. An additional spike in testosterone levels can usually be seen in winners immediately after competitive situations, while data about cortisol changes are inconsistent [2]. Intensive endurance exercise induces changes in the hypothalamic-pituitary axis, both during training and after the event, during rest [3]. In males, intensive exercise usually causes decreased testosterone and increased cortisol levels, although other patterns have also been found [4-7].

Both testosterone and cortisol concentrations correlate with performance in a variety of sports, but data are conflicting. In elite rugby players, salivary testosterone and cortisol concentrations correlate with speed, power and strength [8]. In females, after exercise salivary cortisol levels correlate with the volume of load lifted [9]. During fifteen weeks of mixed aerobic and weight training in junior wrestlers, testosterone concentrations increased, while the testosterone/

cortisol ratio decreased [10]. There was also a significant positive correlation between power increase and testosterone and a negative correlation with cortisol [3]. On the other hand, in a professional triathlon competition, performance of the athletes correlated with morning salivary cortisol concentrations, but not with salivary testosterone concentrations [11]. In male Olympic weightlifters, however, salivary testosterone concentrations significantly correlated with the weight-lifting performance [12]. It should be noted that overtraining causes hypothalamic dysfunction that may be accompanied by decreased cortisol concentrations [3]. On the other hand, the decrease in cortisol levels may be a sign of adaptation to adverse environment [13].

Hormonal environment influences sports performance. On the other hand, physical stress, such as exercise, modulates the biological effects of hormones and their concentrations in the body [14]. Therefore, both basal and stimulated hormone concentrations measured before a competition could be used to predict physical fitness and performance during the event.

The aim of this study was to assess whether salivary testosterone and cortisol concentrations, basal and running-induced levels, measured two weeks before a race, relate to running performance in male recreational athletes. Because of the well-known correlation between age and testosterone, as well as age and running performance, age was also included in the analyses [15,16].

MATERIALS AND METHODS

Participants and testing conditions

This study was approved by the Ethical Committee of the Faculty of Medicine, University of Belgrade (Ethics Approval No 29/VII-21). A group of eleven male recreational athletes preparing for a half-marathon was included in the study. All subjects were physically active throughout the previous year, in good health, and drug and injury free. None of them worked in shifts. Median age was 32 years (range 16 to 49 years). Due to the small quantity of saliva, testosterone was determined in 8 subjects before the trial run and in 7 subjects after the run. Cortisol was determined in all subjects before the run and in 10 subjects after the run, since one subject did not produce enough saliva. Preparation for the half-marathon started six weeks before the study and the half-marathon was held two weeks after the study. On the day of the study, each subject completed a 15-km trial run at his own pace. The run started at 08:00. The track was flat and the track surface was hard. Two weeks later, all the subjects completed the half-marathon and their individual official half-marathon times, expressed in hours, were used as a performance measure. Unstimulated saliva samples were collected at 07:45 on the running track, in a fasted state, before the participants' warm-up, and immediately after the run.

Testing procedures

Collection of saliva was performed by trained personnel and eye sponges (BD Visispear, Beaver-Visitec International, USA) were used as the saliva-collecting device [17]. By holding the plastic shaft of the eye sponge, a sponge was introduced into the mouth, placed under the tongue and moved around in the mouth and lip area for about 1 min. The swollen eye sponges were placed into 5-mL polypropylene tubes with screw caps and transported immediately. On the same day, saliva was extracted from the sponges by centrifuging the tubes in a swing-out rotor for 20 min at 1500 *g*. Testosterone and cortisol concentrations in the saliva were measured by the electrochemiluminescence immunoassay (ECLIA) on an Elecsys 2010 analyzer (Roche Diagnostics, Mannheim, Germany). The detection limits of the assay for testosterone and cortisol concentrations were 0.087 nmol/l and 0.500 nmol/l, respectively. The within-assay and between-assay imprecision for salivary testosterone concentrations of 0.588 nmol/l and 0.998 nmol/l were 5.3% and 4.1%, and 8.4% and 5.8%, respectively. The within-assay and between-assay imprecision for salivary cortisol concentrations of 4.84 nmol/l and 15.08 nmol/l were 8.3% and 4.8%, and 19.2% and 6.4%, respectively.

Statistical analysis

Multivariate linear regression was used to analyze data. Because of the collinearity between age and testosterone concentrations, data were centered before the analysis. To obtain a parameter confidence interval, the bootstrapping procedure was used with 10000 bootstrap replicates. Significance was set at an alpha level of p<0.05. Data are presented as mean±95% confidence interval (CI). Because of the different number of samples obtained before and after the run, a mixed linear model was used to compare testosterone and cortisol before and after the run.

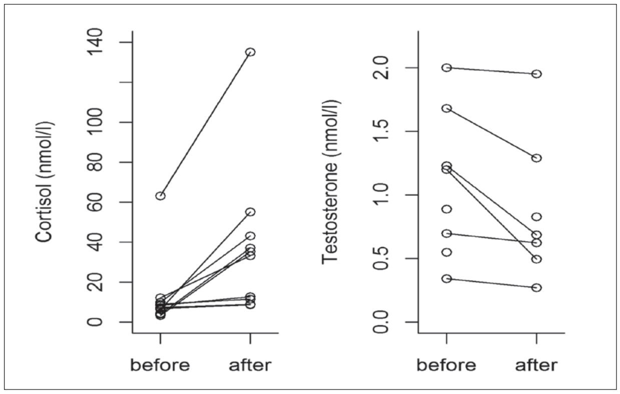

Fig. 1. Salivary cortisol and testosterone concentrations before and after the 15-km run.

RESULTS AND DISCUSSION

The method of determining salivary hormones using eye sponges is more recent, and has proved to be reliable, quick, comfortable, requiring minimal amounts of samples. The applied statistical methodology allowed the comparison of a different number of samples obtained before and after the run (mixed linear model) as well as consideration of the impact of age, in addition to testosterone levels, on the half-marathon times (multivariate linear regression).

Exercise training produces changes in the concentration of several biologically active molecules, including major anabolic (testosterone) and catabolic (cortisol) hormones. Our results suggest that endurance running significantly but differently affects the levels of salivary testosterone and cortisol (Fig. 1). While basal testosterone levels were significantly decreased to around 82%, basal cortisol levels were significantly higher, by as much as 310%, after the trial run. Mean testosterone concentrations were 1.07±0.33 nmol/l before the run and 0.88±0.35 nmol/l after the run (p<0.05) (Fig. 1). Mean cortisol concentrations were 12.28±8.46 nmol/l before the run and 38.08±19.63 nmol/l after the run (p<0.05) (Fig. 1).

Some subjects exhibited small changes in testosterone concentrations after the run, while others showed major decreases in testosterone concentrations, which is in agreement with other publications [7,18,19]. Vaamonde et al. [20], however, found that in males, endurance exercise does not cause changes in testosterone levels. Also, formal exercise training was found to increase salivary testosterone concentrations in previously sedentary men [18].

A relevant question was whether performance can be predicted. We hypothesized that testosterone and cortisol levels could relate to the running performance, especially after stress of the same type as the event stress (endurance running). Therefore, the study was designed to assess correlations between basal and stress-induced hormone concentrations measured after a 15-km run two weeks before a half-marathon, and half-marathon times.

The obtained results indicate that, whether before or after the run, the salivary cortisol concentrations did not correlate with the corresponding half-marathon running times (Fig. 2). Contrary to this, post-run salivary testosterone concentrations significantly inversely correlated with the corresponding half-marathon running times (p=0.011, 95% bootstrap CI for slope -0.41 to -0.16) and before the run salivary testosterone concentrations also negatively but marginally correlated with those times (p=0.068, 95% bootstrap CI for slope -0.40 to -0.06) (Fig. 2). Consequently, an assumption could be made that a male runner with a higher level of salivary testosterone after a trial run, will after two weeks be faster in the half-marathon and vice versa.

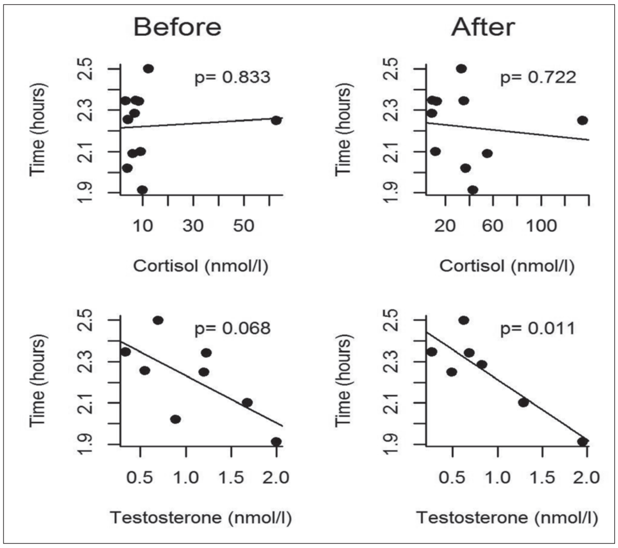

Fig. 2. Relationships between salivary cortisol and testosterone concentrations (before and after the 15-km run) and at half-marathon running time.

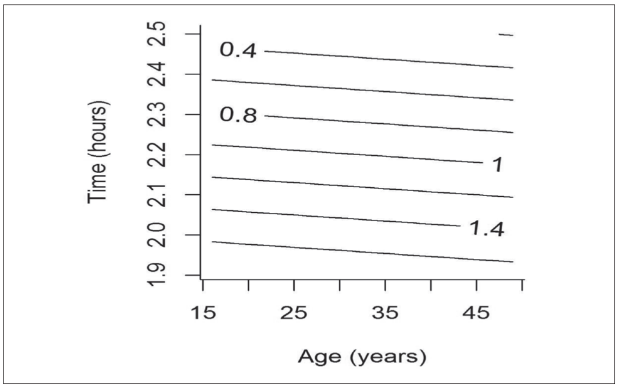

Fig. 3. Relationships between half-marathon running time, salivary testosterone concentrations and age. Contours represent salivary testosterone concentrations in nmol/l.

The results of previous studies do not fully agree [8,10-12]. Crawther et al. [21] found that pre-exercise testosterone levels were a good predictor of performance in good squatters, but not in average ones. In addition, the impact on the outcome of a competition of social behavior and testosterone changes that occur naturally after losing a competition should be carefully considered [19].

Discrepancies between the studies may be partially explained by the different types of responses among the subjects. In addition, we assessed the acute effect of exercise on salivary testosterone concentrations, while other studies assessed the prolonged effect of an exercise program on testosterone concentrations [5,20].

Additional studies are necessary to determine whether the decrease in basal testosterone levels, after exercise stress, is more pronounced in less fit subjects. It could, therefore, be speculated that post-exercise salivary testosterone levels could be used to assess physical fitness and to predict results in endurance running, at least in recreational athletes. This is the most significant result.

There are multiple explanations of why the salivary testosterone concentrations correlated with athletic performance in our group. In the mitochondria, testosterone increases the expression of genes that encode carnitine palmitoyltransferase-1β and pyruvate dehydrogenase kinase 4, improving fatty acid oxidation in the mitochondria, which enhances mitochondrial function and energy metabolism [22]. In skeletal muscle cells, testosterone concentrations correlate with the oxidative phosphorylation gene expression and increase the activity of phosphofructokinase, hexokinase and glycogen synthase [23-25]. In addition, testosterone concentrations significantly correlate with the measure of the maximum volume of oxygen that an athlete can use (VO_{2max}) and power output [18,23], stimulate muscle mass and increase motivation for competition [18,23,26,27].

Local cortisol concentrations are regulated by the 11β-hydroxysteroid-dehydrogenase (11β-HSD) system. Endurance training increases 11β-HSD type 1 and decreases 11β-HSD type 2 activity, influencing cortisol concentrations independently of the hypothalamo-pituitary-adrenal axis [5].

Age is a very important parameter that should be taken into consideration when estimating testosterone levels for running performance [15,16]. As the participants were of different age (range 16-49 years) and having in mind the well-known correlations between age, testosterone concentration and running performance, we performed multivariate linear regression using both testosterone concentrations after the run and age as predictors of half-marathon running times.

Again, testosterone concentrations were significantly inversely correlated with the performance measures (Fig. 3). This has prompted us to form the following, bold conjectures: with the same level of testosterone, a man is faster (shorter half-marathon running time) if he is older; to achieve the same half-marathon time, an older man needs a smaller concentration of post-run testosterone; during the aging process, the body in many ways adapts to age-related changes.

Due to the limitations of this study, the next similar study should be scheduled closer to the day of the half-marathon and include an increased number of participants involving professional athletes with consideration and measurement of additional parameters, especially stress-related ones.

The present work represents a pilot study undertaken to assess the ability of exercise-induced salivary testosterone and cortisol measures to predict future performance in a half-marathon in recreational male athletes. The main contribution of this paper is the finding that salivary testosterone concentrations, determined after a trial run, significantly inversely correlated with the corresponding half-marathon running times two weeks later. Even the age of runners did not disturb this significant correlation. Based on these preliminary results, it could be concluded that salivary measures, especially salivary post-run testosterone levels, may have the potential for predicting performance.

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Authors' contributions: Branimir B. Radosavljević wrote the manuscript, interpreted the data and critically revised the work); Miloš P. Žarković designed the study, performed the statistical analysis, interpreted the data and participated in writing of the manuscript; Svetlana D. Ignjatović designed and implemented the applied methodology, interpreted the data and co-wrote the manuscript; Marijana M. Dajak implemented the methodology; Neda Lj. Milinković co-wrote the manuscript and approved the final version.

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