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Variation of Daily Plasma Testosterone Levels in Tunisian Male Football Players in Relation to the Time-of-Day of the Strength Training

Sarah Chéour[1], Chouaieb Chéour[1], Tommy Gendreau[2], Majdi Bouazizi[3], Nejah Kacem[4], Aymen Guemri[4], Foued Chéour[5]

[1]High Institute of Sport and Education of Sfax, Tunisia; [2]Laval University, Québec, Canada; [3]Faculty of Sciences of Bizerte, Tunisia; [1]High Institute of Sport and Education of Gafsa, Tunisia; [4]High Institute of Applied Biology of Médenine, Tunisia.

Corresponding author: Dr Sarah Chéour
High institute of Sport and Education of Sfax, Tunisia
E-mail: sarah.cheour@yahoo.com

ABSTRACT

BACKGROUND: *The aim of this study was to assess the change in the plasma testosterone (T) levels in male football players in relation to the time-of-day-strength training*
METHODS: *Forty-four Tunisian male football players (mean ± SD, age: 22.34±0.62 years; height: 1.74±0.41 m; weight: 72.31±1.89 kg) participated in this study. They were randomly assigned into three groups: morning strength training group (MSTG, 07:00-08:00 hours, n=14), afternoon strength training group (ASTG, 16:00-17:00 hours, n=15) and morning and afternoon strength training group (MASTG, 07:00-08:00 and 16:00-17:00 hours, n=15) and they were subjected to strength training of the knee extensor and flexor muscles. The level of T for each football player was collected in all three conditions in the morning, noon and afternoon, before and after 8-weeks of strength training. The effects of group, time of the day and pre- to post-training were verified by a 3-way analysis of variance with repeated measures.*
RESULTS: *Our results indicate that strength training induces an increase of plasma T in both groups of players who have always trained for only one session per day mainly in the afternoon compared with the MASTG (P< 0.01). However, a reduction of the plasma T was observed in the players who have always trained successively in the morning and in the afternoon (P< 0.01). Plasma T exhibit circadian rhythmicity in all groups showing a high level in the morning, whereas in the afternoon a decreased value was more noted (P< 0.01).* ***CONCLUSIONS:*** *From an applied perspective, this study suggests that male football players should train at the afternoon to maximize their performance gain.*
Key words: *strength training, testosterone, diurnal variation, time-of-day, football players.*

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I. Introduction

The effects of physical exercise on T levels have been widely documented, and more particularly during endurance exercise with higher energetic cost (Lac & Berthon, 2000). T has many physiological roles within the body, all of which can be placed into two categories: androgenic and anabolic (Bouazizi *et al.*, 2019; Izquierdo *et al.*, 2001; Slimani *et al.* 2017). T has a potential anabolic effect on muscle-skeletal system, including an increase in lean body mass, dose related to hypertrophy of muscle fibers, and increase of muscle strength (Wood *et al.*, 2012). T is a steroid hormone secreted by the Leydig cells of the testes under hypothalamic and pituitary control defining the hypothalamo-pituitary-testicular (HPT) axis and in small amounts from the adrenal cortex (Guignard *et al.*, 1980 ; Hayes *et al.*, 2010). In both sexes, T follows a diurnal rhythm with peak concentrations in the morning followed by progressive decline over the course of the day, rising again at night during sleep (Dabbs, 1990; Guignard *et al.*, 1980; Hayes *et al.*, 2010).

It has been reported that increases in strength performance were always related to the increases of T levels in athletes (Kim *et al.*, 2009; Shakeri *et al.*, 2012). T is biomarker of particular interest in sport, exhibit circadian rhythmicity, and is known to correlate with athlete performance (Le Panse *et al.*, 2012; Cook *et al.*, 2014). T may promote athletic performance, not only through its long-term anabolic actions, but also through rapid effects on behavior. Cardiovascular exercise and resistance training generally transiently increase T levels in athletes although a few studies report null effects (Kraemer & Ratamess, 2005 ; Enea *et al.*, 2009). Sinar *et al.* (2011) reported that the increase of T is higher in people who exercise compared to people who do not exercise.

Acutely, a training stimulus (e.g. strength/hypertrophytraining) can raise post-exercise T levels; however, the impact of training on circadian changes in T is unclear (Kraemer & Ratamess, 2005). The majority of studies exploring the effects of exercise on androgens have focused on acute effects in short-term exercise protocols, and most of these demonstrate that exercise bouts are associated with an acute transient increase in T, with variable effects on other androgens when these are measured (Hawkins *et al.*, 2008; Kumagai *et al.*, 2016). Sedliak *et al.* (2007) reported that training in the morning or evening hours had no significant effect on resting serum T levels. However, few studies to date have examined the effects of time-of-day-strength training on hormonal adaptations. Therefore, the purpose of this study was to determine the effect of strength training duration on T levels in Tunisian male football players and if any differences in level occur during the day.

II. Methods

2.1. Participants

With university ethical approval, forty-four Tunisian male football players (mean ± SD, age: 22.34±0.62 years; height: 1.74±0.41 m; weight: 72.31±1.89 kg) were carefully selected to participate in the present study. All players had at least 4 years of (monitored/recorded) training history. Players volunteered to take part in this study and all were informed of the potential risks associated with the study prior to giving their informed consent. To be eligible to participate in the study, players were required to meet the following criteria: (a) not consume any supplements or drugs; (b) no injury history for the lower and upper-body; (c) no history of use of medications that could alter the hypothalamic-pituitary-gonadal (HPG) axis, such as anabolic steroids; (d) no history of chronic disease, including reproductive disorders; (e) regular eating patterns; (f) no history of depressive illness and (g) no severe cognitive impairment. Throughout the periods of training, the players maintained their normal dietary regime, were not permitted to use nutritional supplementation and did not consume anabolic steroids or any other anabolic agents known to increase performance. The study protocol was reviewed and approved by the Ethical Committee at the High institute of Sport and Education of Sfax, Tunisia.

2.2. Experimental approach to the problem

The study was conducted from January to March, so the pre-training tests were carried out in January 2018 and the post-training tests in March 2018. Players were randomly assigned into three groups: morning strength training group (MSTG, 07:00-08:00 hours, n=14), afternoon strength training group (ASTG, 16-17 hours, n=15) and morning and afternoon strength training group (MASTG, 07:00-08:00 and 16:00-17:00 hours, n=15). For the purpose of this study, the concentration of T for each athlete was evaluated in three stages collected in the morning, noon and afternoon, before and after 8 weeks of strength training.

2.3. Training program

The training program used in this study was reported by Souissi *et al.* (2014). The selected players participated in concentric and strength training for 8 weeks. They were subjected to strength training of both the knee extensor and flexor muscles for three sessions per week. Two successive sessions were separated by at least 48 hours. Three sessions of 'Leg Extension', 'Leg Curl' and 'Squat', were used. The training program was preceded by determining the one-repetition maximum (1-RM) for each exercise. The 1-RM was adjusted after every four weeks of training. During the first four weeks of training, participants were subjected to concentric strength training (60-70% of 1-RM, 8 repetitions per set, 6 sets, 2 minutes of rest between sets) to prepare for the eccentric protocol. In the last four weeks, the athletes were subjected to an eccentric strength training protocol (100, 110 and 120% of 1-RM, 3-5 repetitions per set, 3-5 sets, 6-8 minutes of rest between sets).

2.4. Blood collection and testosterone analysis

All players were admitted to the laboratory of biochemistry at the University Hospital Center of Habib Bourguiba, Sfax, Tunisia, at the same time of day for blood collection. The blood samples (5 ml) were collected in the morning (07:00-08:00 hours), at noon (12:00-13:00 hours) and the afternoon (16:00-17:00 hours) from each athlete before and after the eight week training protocol. Players rested in the supine position for at least 10-min before blood collection. Blood was collected by a qualified phlebotomist into tubes from the antecubital fossa in a restful sitting position using standard venipuncture techniques (Kramer & Ratamess, 2005). Plasma was separated by centrifugation within 15-min of collection and divided into two aliquots and subsequently frozen and stored at -80°C for subsequent analysis. The plasma levels of T were measured by immunochemical methods using Chemiluminescent Microparticle Immunoassay (CMIA), in line with manufacturers instructions. It was determined with a sensitivity of 0.026 ng/ml and an intra-essay coefficient of variance (CV) of 1.9%.

2.5 Statistical analyses

The effects of group, time-of-day and pre- to post-training were verified by a 3-way analysis of variance (ANOVA) with repeated measures (3 [training group] × 2 [training] × 3 [time of day]). To allow a better interpretation of our results, the effect sizes were calculated (η^2). Bonferroni test was used as post-hoc. A significance level of P≤ 0.05 was used for all analyses. All statistical analyses were carried out using the commercial software "Statistical Package for Social Sciences" (SPSS Inc., Chicago, IL, USA, version. 16.0).

III. Results

Mean values ± SD for T in Tunisian male football players evaluated before and after training program in each group during eight weeks at different time-of-day are presented in figure 1. Basal T levels differ significantly between groups (P< 0.01; η^2=0.63), pre- to post-training (P< 0.01; η^2=0.59) and time-of-day (P< 0.01; η^2=0.56). However, an effect for groups × pre- to post-training × time-of-day interaction (P< 0.05; η^2=0.57) was detected. The Post-hoc comparisons indicated higher values for T level in the ASTG (P <0.001), MSTG (P< 0.01) than in the MASTG

IV. Discussion

T is normally present in the circulation of both men and women (Le Panse *et al.,* 2012; Slimani *et al.,* 2018). Due to the dynamic regulation of endogenous testosterone production, including the acute effects of competition and exercise, T levels may vary considerably within and among individuals (Bouazizi *et al.,* 2019; Slimani *et al.,* 2017). T, a steroid hormone produced primarily by the gonads, is believed to play a key role in modulating physiological and behavioral processes critical to survival and reproduction (Ketterson & Nolan, 1992). The purpose of the present study was to examine the effects of time-of-day strength training on plasma T levels in Tunisian male football players. Our study showed that the submission of Tunisian male football players to strength training during 8-weeks either in the morning, in the afternoon or successively in the morning and in the afternoon at the rate of one hour per session influences significantly resting T levels. Indeed, the adaptation to strength training is associated with higher improvements in resting T concentration for the ASTG and MSTG than the MASTG. In fact, with only one training session per day either in the morning or in the afternoon, daily concentrations of this hormone increase in athletes but this increase was significant only for the last group. The rise of T level may be the result of the reduction in plasma volume with hemoconcentration and increased blood viscosity on the one hand, and a rise in testicular perfusion on the other hand as was reported by Souissi *et al.* (2014). However, by imposing two training sessions daily to athletes, successively in the morning and in the afternoon, which probably led to depletion, a significant decrease in daily production of this androgen was observed. Plasma T showed a diurnal circadian rhythm in all trained athlete groups when measured before and after strength training during eight weeks. In fact, plasma T level decreases significantly during the day which reflects normal cyclic biological variations which indicate that the hormone is less anabolic in the afternoon. The circadian profile of T, which peaks in the morning before slowly declining throughout the day and elevating again within the first few hours of sleep has been reported by Guignard *et al.* (1980). These findings agree with Hayes *et al.* (2010), who showed that T levels are higher in the morning, an increased resistance exercise-induced T response has been found in the late afternoon, suggesting greater responsiveness of the hypothalamo-pituitary-testicular axis. Whereas, excess training for two sessions in the day, caused an inverse effect. Indeed, our results agree with those of other studies (Hayes *et al.*, 2010; Kumagai *et al.*, 2016; Callard *et al.*, 2000). Particularly, strength exercise performed in the afternoon (McMurray *et al.,* 1995), but not in the morning (Kraemer *et al.,* 2001), has been reported to temporarily alter overnight T release.

V. Conclusion

This study showed that the time-of-day-strength training influences significantly the T levels in Tunisian male football players. Daily workouts of short exercise durations in the morning or in the afternoon merely promote the T level. From an applied perspective, this study suggests that Tunisian male football players should train at the afternoon to maximize their performance gain.

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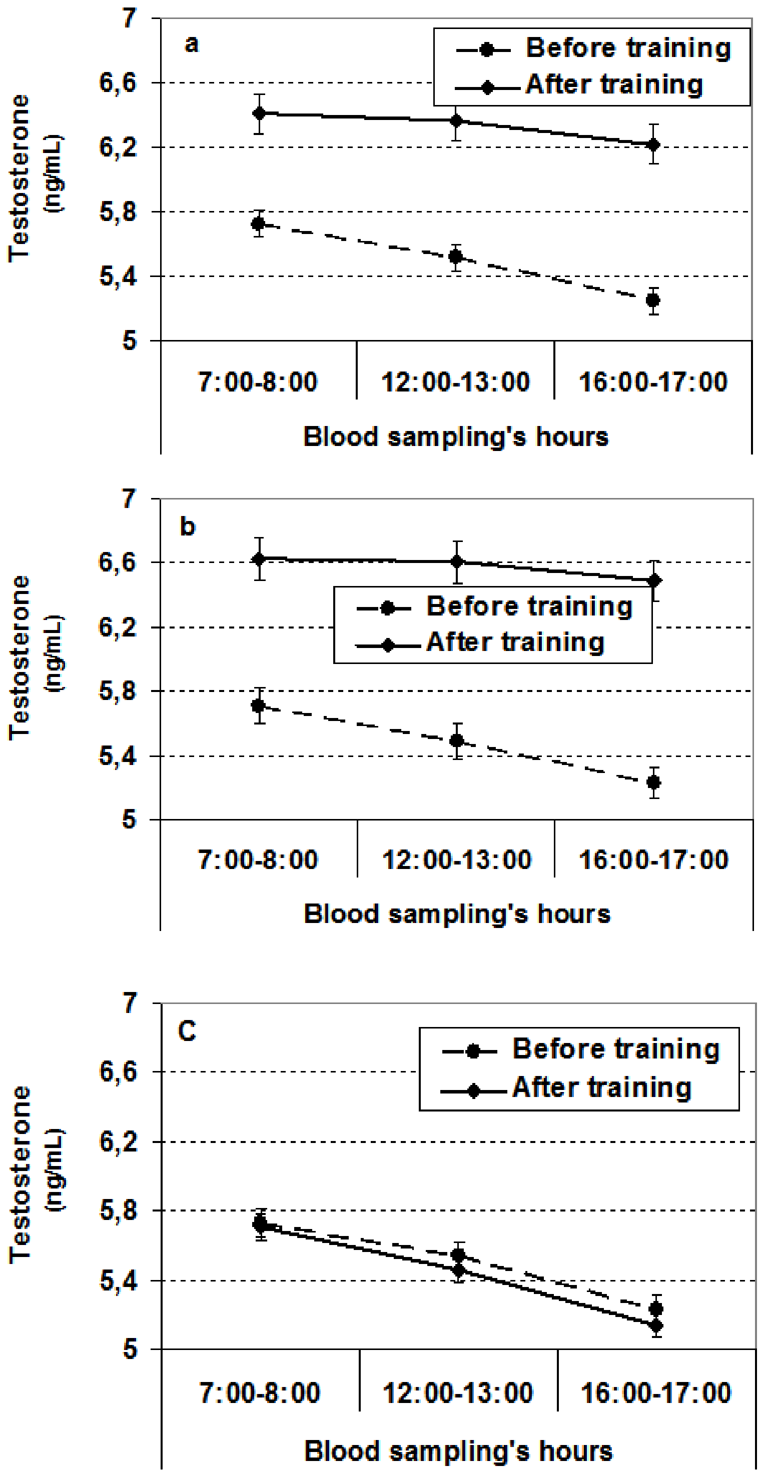

Figure 1. Daily change of testosterone (ng/mL) levels in Tunisian male football players before and after submission to eccentric strength training in the morning (a), afternoon (b) or morning and afternoon (c) during 8-weeks.

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