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Effects of time-of-day strength training on plasma testosterone and cortisol concentrations in male amateur athletes

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Conflicts of interest

None declared.

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Abstract

BACKGROUND: The purpose of the present study was to examine the effects of time-of-day-strength training on plasma testosterone (T) and cortisol (C) concentrations in male amateur athletes. ***METHODS:*** Forty five male athletes (age, 21.2±0.3 years; height, 1.75±0.14 m; weight, 72±1.52 kg) participated in the present study. They were subjected to strength training of the knee extensor and flexor muscles. Following this, they were randomly assigned into three groups: morning strength training group (MSTG, 07:00–08:00 hours, n=15), 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). The concentration of T and C for each athlete 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/C ratio in both groups of athletes who have always trained for only one session per day mainly in the afternoon compared with the MASTG ($P < 0.05$). However, a reduction of the plasma T/C ratio was observed in the athletes who have always trained successively in the morning and in the afternoon ($P < 0.05$). Plasma T/C ratio exhibit circadian rhythmicity in all groups showing a low level in the morning, whereas in the afternoon an increased value was more noted ($P < 0.05$). ***CONCLUSIONS:*** From an applied perspective, this study suggests that amateur athletes should train at the afternoon to maximize their performance gain.

Key words: strength training, testosterone, cortisol, diurnal variation, time-of-day, amateur athletes.

Introduction

Physical exercise has a relevant impact on endocrine functions.[1, 2, 3] A single bout of exercise activates certain endocrine systems required to maintain body homeostasis. [4, 5] Moreover, the effects of exercise persist after the end of exercise, continuing during the recovery period. Training, i.e. regularly repeated exercise, can affect endocrine functions by modifying hormonal responses to exercise and/or by modifying endocrine functions in resting conditions.[3, 5] Indeed, it was found that increases in strength performance were always related to the increases of testosterone (T) levels in athletes. [5, 6] Particularly, T is a steroid hormone secreted from the Leydig cells of the testes under hypothalamic and pituitary control defining the hypothalamo-pituitary-testicular axis. T has many physiological roles within the body, all of which can be placed into two categories: androgenic and anabolic.[1, 2, 7] Thus, cortisol (C) is a steroid hormone released by the adrenocortical glands under hypothalamic and pituitary control defining the hypothalamo-pituitary-adrenal (HPA) axis. C increases as a response to any and all stresses which include exercise.[7, 8] The functions of C include stimulation of gluconeogenesis by the liver, stimulation of the glucose-alanine cycle, decreased glucose use by cells, protein breakdown, increased free amino acid pool, stimulation of erythropoiesis, and anti-inflammatory effects.[9-12] However, the ratio between testosterone and cortisol levels (T/C ratio) is frequently used as an index of stress level in exercise training.[13,14]

Few investigations have studied the acute hormonal changes after resistance and strength protocols. Ahtiainen et al. [15] showed that changes in maximal isometric force after a 21-week resistance training protocol were correlated with the mean serum basal total T concentration and T/C ratio. Hakkinen and Pakarinen [16] reported increases of 22.4% and 23.8% in free serum T and total T, respectively, in response to a hypertrophy training protocol. In addition, Beaven et al. [17] demonstrated a decrease in C concentration with strength, hypertrophy, power, and strength endurance protocols, decreasing C by 38.2±20.6%, 33.6±20.6%, 44.3±20.6%, and 22.2±20.6%, respectively. These findings conflict with a number of studies that have reported acute increases in C in response to resistance protocols.[16, 18, 19] This contradiction can be due to the specific loading parameters of resistance/strength training, such as intensity, duration, total work, repetitions, rest periods, and time-of-day.[20]

It is well known that diurnal variation of sports performance usually peaks in the late afternoon, coinciding with increased body temperature and T levels.[21] Hayes et al. [21] showed that evening resistance training may result in greater muscular adaptations, since gains in hypertrophy and strength have been partly attributed to the responsiveness of anabolic hormones and the metabolic pathways they signal. Sedliak et al. [22] investigated the effects of time-of-day–specific resistance training on diurnal rhythms of T over a 20-week period. They reported that only C levels decreased significantly in subjects who regularly trained in the morning hours, while training in the morning or evening hours had no significant effect on resting serum T concentrations. The authors suggested that this reduction in serum C concentrations may be due to a decreased anticipatory psychological stress before the morning sessions rather than to adaptations induced by a regular training at this time-of-day. Furthermore, 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 and C concentrations in amateur athletes. A secondary aim was to determine if any differences in concentration occur during the day.

Methods

Experimental approach to the problem

For the purpose of this study, the concentration of T and C for each athlete was evaluated in three stages collected in the morning, noon and afternoon, before and after 8 weeks of strength training. Subjects were randomly assigned into three groups: morning strength training group (MSTG, 07:00–08:00 hours, n=15), 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). The test sessions were conducted from December to February, so the pre-training tests were carried out in December 2014 and the post-training tests in February 2015.

Participants

Forty five male amateur athletes (age, 21.23±0.3 years; height, 1.75±0.14 m; body mass, 72.00±1.52 kg) were carefully selected to participate in the present study. They were specialised in various activities (e.g., soccer, hand-ball, boxing). To be eligible to participate in the study, participants 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. Participants were informed of the experimental risks and signed an informed consent document prior to the investigation. Throughout the periods of training, the subjects 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, Sfax.

Training program

The Training program used in this study was reported by Souissi et al. [2] The selected athletes 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 at the gymnasium of the Higher Institute of Sport and Physical Education of Sfax, Tunisia, namely '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).

Blood collection and hormonal analysis

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. Blood was collected into tubes from the antecubital fossa in a restful sitting position using standard venipuncture techniques [20]. Subjects were made comfortable for at least 10 minutes before blood collection. All subjects 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. Plasma was separated by centrifugation within 15 minutes of collection and divided into two aliquots, Frozen and Stored at -80°C for subsequent analysis. Plasma T and C levels were measured by immunochemical methods using Chemiluminescent Microparticle Immunoassay (CMIA). Plasma T was determined with a sensitivity of 0.1 ng/ml and an intra-essay coefficient of variance (CV) of 2.6%. Plasma C was determined with a sensitivity of 6.8 ng/ml and an intra-essay CV 4.6%. The results of plasma T and C levels were expressed in ng/ml. Plasma T/C ratio was also calculated.

Statistical analyses

The data were analyzed using multivariate analysis of variance (MANOVA). Kolmogorov–Smirnov test (K-S) was used to determine the normal distribution of data. The effects of group, time-of-day and pre- to post-training were verified by a 3-way analysis of variance with repeated measures (3 [training group] × 2 [training] × 3 [time of day]). Bonferroni test was used to conduct *post-hoc* comparisons. The data weare presented as mean±standard deviation (SD). Effect size was determined by using eta squared calculations (η^2). A significance level of $P \leq 0.05$ 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).

Results

Table 1 shows the mean values of T and C levels in male amateur athletes evaluated before and after each group during eight weeks at different time-of-day.

*** Table 1 here***

Testosterone

There was a significant main effect for groups ($F_{(2,41)}$=201.62; P=0.001; η^2=0.72), pre- to post-training ($F_{(2,41)}$=110.55; P=0.002; η^2=0.61) and time of the day ($F_{(2,41)}$=62.12; P <0.01; η^2=0.57). Thus, there was a main effect for groups × pre- to post-training × time-of-day interaction ($F_{(2,41)}$=92.45; P=0.04; η^2=0.54). Post-hoc comparisons revealed that T level was higher in the ASTG (P <0.001), MSTG (P=0.03) than in the MASTG.

Cortisol

There was a significant main effect for groups ($F_{(2,41)}$=3.62; P=0.04; η^2=0.15), pre- to post-training ($F_{(2,41)}$=2.55; P=0.03; η^2=0.11) and time-of-day ($F_{(2,41)}$=4.12; P <0.01; η^2=0.37). In contrast, there was no main effect for groups × pre- to post-training × time-of-day interaction ($F_{(2,41)}$=2.11; P >0.05; η^2=0.10). *Post-hoc* comparisons revealed that C level was higher in the MASTG than in the ASTG (P=0.02) and MSTG (P=0.008).

Testosterone/cortisol (T/C) ratio

Strength training for the male amateur athletes in the morning or in the afternoon for eight weeks induced an increase in plasma T/C ratio compared with pre-training (Figure 1). This increase was only significant for the group of subjects who strength-trained in the afternoon ($P < 0.01$). Nevertheless, inverse effects were observed in athletes who participated in the same strength training period successively in the morning and in the afternoon. (Figure 1). Plasma T/C ratio increased during the day regardless the time of training in athlete groups (Figure 1). Nevertheless, the magnitude of T/C ratio increase was more pronounced in the athletes who were training during the afternoon ($P < 0.01$).

*** Figure 1 here***

Discussion

The aim of the present study was to examine the effects of time-of-day strength training on plasma T and C concentrations in male amateur athletes. The major result of this study was that adaptation to strength training is associated with higher improvements in resting T concentration and T/C ratio for the ASTG and MSTG than the MASTG. In contrast, the higher improvement of resting C concentration was observed in the MASTG when compared to ASTG and MSTG.

Testosterone

T is an androgenic and anabolic hormone secreted mostly from the Leydig cells of the testis and in small amounts from the adrenal cortex. T release is stimulated by luteinizing hormone (LH) from the anterior pituitary.[20] Our study showed that the submission of male amateur athletes to strength training during eight 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. 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. [2].

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, during the day plasma T level decreases but not significantly. Thus, decrease in plasma T level reflect normal cyclic biological variations which indicate that the hormone is less anabolic in the afternoon. These findings agree with Hayes et al. [21], 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.[21, 23] Particularly, strength exercise performed in the afternoon [24], but not in the morning [25], has been reported to temporarily alter overnight T release.

Cortisol

C is a glucocorticoid hormone secreted from the zona fasciculata of the adrenal cortex. C release is stimulated by adrenocorticotropin hormone (ACTH) from the anterior pituitary [1]. It has been reported that the response of C to exercise is affected by the intensity and duration of physical activity, exercise state such as competition, psychological stimuli, and time of day.[26, 27] In fact, athletes who have been submitted successively to two sessions of strength training in the morning and the afternoon during eight weeks have always showed greater C levels than those who realized only a single session especially in the afternoon. Submission of athletes to physical activities successively in the morning and the afternoon could cause stress increases and therefore could provide a mechanism for the increase in C concentrations observed. Our results are in disagreement with those of Viru and Viru [9] and Hackney et al. [28] who reported a lack of significant changes or declines in C in response to physical activity.[29] In fact, it has been reported that C response in elite athletes is less sensitive.[26] However, others studies showed that C level increases with sub-maximal exercise.[30] Smaller changes in C levels in elite athletes may be due to their resistance to physical stress, and that they are accustomed to physical activity. This may be the opposite too: amateur athletes who are possibly still vulnerable to physical stress. It also has been reported that the exercise method, and this may also influence the C response. However it may be possible that exercise in daytime has no effect on the C response.[9] Conversely, time is also a determining factor in C secretion.[31] These contradictory results show that further purposive studies are needed to determine the effects of training mode and intensity on C concentration. Our investigation showed a significant diurnal decrease in C level measured before and after strength training during eight weeks in amateur athletes. This decrease in plasma C level reflect normal cyclic biological variations for maintenance of body homeostasis. This suggests that the hormone is less catabolic in the afternoon. Since C primarily affects protein degradation, a decrease in C is expected to enhance skeletal muscle hypertrophy through reduction in protein degradation.[24, 32] In addition, few scientific studies appear to have dealt with possible phase shifting properties of strength training on hormonal rhythms. However, 10 weeks of morning time-of-day-specific strength training resulted in reduced morning resting C concentrations, presumably as a result of decreased masking effects of anticipatory psychological stress prior to the morning testing.[22]

Testosterone/cortisol (T/C) ratio

The T/C ratio is frequently used as an index of the stress level in exercise training. Changes in this ratio are responsible for several training responses such as hypertrophy and strength gain.[33] The findings of the present study indicate that the plasma T/C ratio increased in the athletes who were active in strength training either in the morning or in the afternoon. In contrary, submission of athletes to two training sittings leads to significant decreases. The main factor contributing to the increase in plasma T/C ratio in all sessions was the increase of plasma T level. Our study also shows that the submission of athletes successively in the morning and the afternoon to the exercise leads to the decline of plasma T/C ratio compared with pre-training. The results of the study confirm the hypothesis that the plasma T/C ratio is affected by exercise intensity and time of day.[33, 354, 35] In fact, the more prolonged period of intense exercise leads to a physiological stress on the subjects causing the dramatic reduction in the plasma T/C ratio.[1] The selection of sub-maximal exercise intensities leads to the increase of catabolic state in the athletes.[36] These findings suggest that applying muscular strength with moderate intensity activates anabolic pathways.

Our investigation showed also a significant increase in plasma T/C ratio during the day for all athlete groups before and after training. The increase in plasma T/C ratio is due especially in to diurnal decrease of C levels. In fact, T/C ratio still higher among athletes who were submitted to the training in the afternoon whatever the sampling time. However, it is less important when the athletes were submitted to two training sessions. Rhythms in the release of hormones constitute a common feature of almost all endocrine systems, with periodicities varying from minutes to a few hours.[37, 38] Both T and C exhibit circadian rhythmicity with peak concentrations in the morning and reduced levels in the evening and overnight.[39] The effects of C and T on the metabolic processes should not to be considered separately. In fact, it is the balance between these two hormones that ultimately determines their impact on anabolic and catabolic processes. Changes in T/C ratio are always responsible for several training responses such as hypertrophy and strength gain [14]. As C exhibited significantly higher concentrations in the morning than in the evening, and T demonstrated no significant variance during the day, we suggested that variation in the T/C ratio is due to variation in C. Thus, these hormones are less catabolic in the afternoon, experiencing an increase in relative anabolism via reduced C rather than an increase in T being responsible for the change in anabolic/catabolic balance. Skeletal muscle can be regulated through changes in either protein synthesis or degradation. Ultimately, muscle mass is the net result of these protein turnover processes. Since C primarily affects protein degradation, a decrease in C is expected to enhance skeletal muscle hypertrophy through reduction in protein degradation rather than increase in protein synthesis as the primary mechanism.[24, 32] From our results therefore we suggest that athletes experience more protein degradation in the morning than in the afternoon which probably may be more favourable for adaptations to resistance exercise. In fact, higher C levels and lower T/C ratio are mildly suggestive that morning hours are less suitable for training activities that are highly catabolic. The physical exercise practice may be more appropriate in the afternoon when T/C ratio is at its highest ratio of the day.

Conclusion

In conclusion, this study showed that the time-of-day-strength training influences significantly the T/C ratio in male amateur athletes. Daily workouts of short exercise durations in the morning or in the afternoon merely promote the T/C ratio. The findings of the present study are interesting as they also confirm previous works in the area in that endocrine markers of catabolism are highest in the morning and lowest in the late afternoon hours. From an applied perspective, this study suggests that amateur athletes should train at the afternoon to maximize their performance gain.

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Table 1. Testosterone (ng/mL) and cortisol (ng/mL) levels in male amateur athletes before and after submission to eccentric strength training in the morning, afternoon or morning and afternoon during 8 weeks.

			Blood collection time		
Training time	**Hormone**	**Training**	**Morning**	**Noon**	**Afternoon**
Morning	**Testosterone**	Before	5.47±1.4	5.47±1.5	5.44±1.4
		After	6.38±1.6	6.26±1.3	6.23±1.6
	Cortisol	Before	105±16	101±13	98±15
		After	112±14	107±11	103±14
Afternoon	**Testosterone**	Before	5.47±1.3	5.45±1.4	5.43±1.1
		After	6.82±0.9*	6.81±1.3*	6.74±1.3*
	Cortisol	Before	104±15	100±11	97±12
		After	108±17	104±16	99±13
Morning and Afternoon	**Testosterone**	Before	5.46±1.1	5.44±1.3	5.41±1.3
		After	5.21±1.3	5.19±1.2	5.42±1.2
	Cortisol	Before	104±16	101±13	98±16
		After	124±14**#	119±13**#	115±15**#

*: Significant difference at post-training compared with pre-training at P<0.05

**: Significant difference at post-training compared with pre-training at P<0.01

#: Significant difference from morning and afternoon groups at P<0.05.