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Effects of morning and afternoon high-intensity interval training (HIIT) on testosterone, cortisol and testosterone/cortisol ratio response in active men

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Abstract
Introduction. The time of exercise (morning and afternoon) can lead to changes in hormonal responses and exercise performance. Aim of Study. The current study aimed to investigate the effects of morning vs afternoon high-intensity interval training (HIIT) on testosterone (T), cortisol (C) and the testosterone/cortisol (T/C) ratio in active men. Material and Methods. Eleven active male students (aged: 19.0 ± 1.0 yrs., height: 177.5 ± 9.0 cm, weight: 70.6 ± 8.3 kg and BMI: 22.19 ± 1.88 kg/m^2) completed two trials of the 40-m maximal shuttle run test (which incorporates 5 × 40 m shuttle sprints with 30 s between the start of each sprint), with seven days between the trials. All the trials were conducted indoors. Blood samples were taken before and immediately after each exercise session from the antecubital vein by repetitive venous puncture in a sitting position. Results. Data evidenced that the T concentration increased after HIIT in the afternoon (pre: 9.86 ± 0.42 vs post: 10.3. ± 0.61, P = 0.02). The significant difference was observed between pre-tests (T: 10.4 ± ± 0.67 vs 9.86 ± 0.42, P = 0.009; C: 898.38 ± 199.51 vs 355.53 ± 92.95, P = 0.001; T/C ratio: 0.012 ± 0.002 vs 0.03 ± 0.012, P = 0.001) and also post-test (T: 10.68 ± 0.53 vs 10.3. ± 0.61, P = 0.01; C: 990.64 ± ± 293.07 vs 452.73 ± 307.34, P = 0.001; T/C ratio: 0.011 ± 0.003 vs 0.056 ± 0.065, P = 0.04). Conclusions. It seems that performing high-intensity interval training (HIIT) in the afternoon may be more suitable in terms of the level of anabolic processes.

KEYWORDS: high-intensity interval training (HIIT), testosterone, cortisol, testosterone/cortisol ratio.

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Introduction

Scientific evidence shows that physical exercise plays a key role in improving hormonal function [27], that its long-term practice has beneficial effects on the body physiological functioning [19] and that acute physiological responses to exercise differ depending on the activity type [9]. However, exercising at different times of the day has different consequences depending on the exercise type and duration, and on hormone adaptation [14]. Also, there are differences in physical performance for exercise performed in the morning and evening, which is the reason why these factors should be considered by athletes, coaches and scientists [25].
Although physical exercise has many beneficial effects, it can also act as a stressor, with several indicators being proposed for its diagnosis (e.g. the testosterone/cortisol ratio) [5]. Testosterone is a sex hormone that is produced and secreted in men's testicular cells, with an important anabolic effect on muscle tissue development and growth [33]. Cortisol is a glucocorticoid secreted

from the adrenal cortex in the adrenal glands, playing a key role in protein degradation and preventing muscle building [6]. Since testosterone and cortisol are anabolic and catabolic hormones, respectively [5], their ratio is used to indicate anabolic/catabolic balance and it is influenced by exercise intensity and duration.

The testosterone/cortisol ratio decreases as a result of high-intensity training and regular participation in competitive events, indicating physiological stress [29]. In healthy men it was found that testosterone concentration increases after submaximal and maximal endurance training concurrently with a cortisol concentration increase after maximal exertion and a decrease after submaximal training, with the testosterone/cortisol ratio being higher for submaximal comparing to maximal exercise [26]. A decrease in cortisol concentration was observed only in the first analysis immediately after exercise compared to the pre-exercise measurement, while in subsequent measurements an increase in cortisol level was noted. The authors conclude that the results of this study showed that repeated short periods of high intensity exercise in both a thermoneutral and moderately hot environment resulted in no significant differences in testosterone or cortisol response. Moreover, no significant differences in recovery after exercise were observed between the two environments [14].

High-intensity interval training (HIIT) is a form of interval training, a cardiovascular exercise strategy alternating short periods of intense anaerobic exercise with less intense recovery periods, until the athlete is too exhausted to continue. Though there is no universal HIIT session duration, these intense workouts typically last under 30 minutes, with times varying based on the participant's current fitness level. The general prescription for interval training is to employ 3- to 5-minute work bouts with an interval to rest. This type of training may not be safe, tolerable or practical for many individuals [8, 24]. In fact, in the last decades some studies have shown that high-intensity interval exercise can accelerate skeletal muscle metabolism adaptations, with sport physiologists implementing it in training programmes [17]. However, since the circadian rhythm effect on training outputs is still scarcely known, we have aimed to evaluate the effect of time of performance of high-intensity interval exercise (morning vs afternoon) on testosterone and cortisol variations and their respective ratio.

Material and Methods

Subjects

Eleven healthy men with a sedentary lifestyle (19.81 ± ± 0.98 years old, 177.9 ± 5.0 cm of height, 70.6 ± 8.3 kg of body mass, 22.2 ± 1.9 kg/m^2 of BMI and 14.4 ± 4.0% of body fat) volunteered to participate. The inclusion criteria were that the subjects did not have infectious, respiratory or cardiovascular diseases or diabetes, and did not use drugs, supplements, consume alcohol or smoke. The study was performed in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the host University (ethics code: IR.MIAU.REC.1396.102).

Study design

Two weeks before starting the experiments the participants were asked to complete medical history and exercise readiness questionnaires (PAR-Q) and those who had abnormal conditions were excluded from the study [22]. Then, demographic measurements were taken, with the subject's weight and height measured using a scale and a Seca 700 height measuring tape (700, Seca, Hamburg, Germany), while body composition and body fat percentage were measured using a bioelectrical impedance device (Boca X1, Medigate, Korea).

The participants performed a familiarization session and then the high-intensity interval exercise was performed on two distinct days separated by a week washout period. In that period (and 24 h before) the participants were asked to refrain from any extra high intensity exercise and not to consume caffeinated beverages (they were allowed to drink water ad libitum during the run). Each participant's heart rate was monitored during all the exercise sessions using a heart rate monitor (FT4, Polar Electro, Finland).

HIIT occurred in the morning (8:30 to 9:30) and in the afternoon (15:00 to 16:00), consisting of a short burst of high-intensity activity followed by a brief low-intensity activity [12]. All the participants were allowed to warm up for 5 min before starting the exercise. The subjects were asked to complete five repetitions of a 40-m shuttle run with a 30-s interval between the start of each sprint, i.e. recovery time between sprints was 30 s minus the time taken to complete the previous sprint. The subjects ran between two lines placed 20 m apart with the start/finish line placed at the midpoint of the course. On instruction, each participant sprinted 10 m from the start/finish line to the end of the course, turned 180°, sprinted 20 m to the other end of the course, turned 180°, and sprinted 10 m back through the start/finish line. For all the tests, sprints were initiated from a line 50 cm behind the start line to prevent false triggering of the first timing gate. The subjects were instructed to place at least one foot over the line at the end of each shuttle, the adherence to which was monitored to ensure full compliance (Figure 1). During each trial the subjects were verbally encouraged to give a maximal effort [23]. Average exercise intensity and maximum heart rate at all the exercise sessions were measured using the Polar FT4 device.

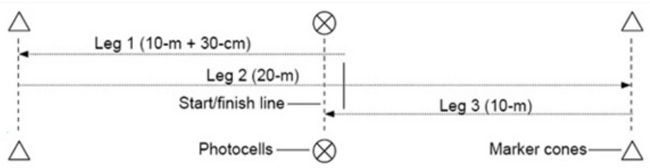

Figure 1. Schematic illustration of the 40-m maximal shuttle run test

Blood samples were taken before (PRE) and immediately after (POST) each exercise session from the antecubital vein by repetitive venous puncture in the sitting position. To separate serum, the blood was allowed to clot at room temperature and then it was centrifuged at 3500 g for 20 min. The serum was drawn off and stored at −70°C for subsequent analysis of testosterone and cortisol levels. The testosterone concentration was measured using ELISA kits made by Monobind, USA (Kit Lot No. EIA-.37K4I6). The kit sensitivity was 576.0 picograms, equivalent to a testosterone concentration of 0.0576 ng/ml. The ELISA kits made by DBC Corporation, Canada (Lot No. 161380) were used to measure serum cortisol concentration. The kit sensitivity was 0.4 micrograms per decilitre (calibrator range: 0.5-60 μg/dl). The ELISA kits were analysed based on the protocol given by the manufacturer. The kits were read using ELISA readers (Awareness Technology Inc., USA). The intra-assay and inter-assay coefficients of variation were as follows: testosterone 10.22%, 5.60%; cortisol 4.50%, 9.49%.

Statistical analysis
In this study the minimum, maximum, and the 25th and 75th quartiles were used as descriptive statistics. The normal distribution of data was examined using the Shapiro–Wilk test. The Wilcoxon signed-rank test was used to compare pre- and post-test measurements, as well as the differences between morning and afternoon. The significance level was considered to be $P < 0.05$ and all the analyses were performed using the SPSS version 17 software.

Results

Testosterone
The results showed that testosterone concentration increased significantly only after HIIT in the afternoon

Table 1. Findings for changes in testosterone, cortisol and T/C ratio in the morning and afternoon

Variable	Time of training	Test	Mean ± SD	quartiles		(a)	(b)	(c)
				25th	75th	P	P	P
Testosterone (nmol/L)	morning	pre-test	10.4 ± 0.67	9.708	10.748	0.09	0.009*	0.01*
		post-test	10.68 ± 0.53	10.68 ± 0.53	10.748			
	afternoon	pre-test	9.86 ± 0.42	9.361	10.401	0.02*		
		post-test	10.3. ± 0.61	9.708	10.748			
Cortisol (nmol/L)	morning	pre-test	898.38 ± 199.51	721.103	1120.827	0.23	0.001*	0.001*
		post-test	990.64 ± 293.07	697.655	1263.724			
	afternoon	pre-test	355.53 ± 92.95	327.172	411.586	0.27		
		post-test	452.73 ± 307.34	136.827	806.620			
T/C ratio	morning	pre-test	0.012 ± 0.002	0.010	0.013	0.79	0.001*	0.04*
		post-test	0.011 ± 0.003	0.008	0.015			
	afternoon	pre-test	0.03 ± 0.012	0.024	0.030	0.16		
		post-test	0.056 ± 0.075	0.013	0.075			

Note: T/C ratio – testosterone/cortisol ratio
(a) comparison of pre-tests and post-tests of each activity; (b) comparison of pre-tests in the morning and afternoon; (c) comparison of post--tests in the morning and afternoon
* significance difference of $P < 0.05$

(P = 0.02), while there was a tendency for an increase in the morning (P = 0.09) (Table 1; Part a). Significant differences in testosterone concentrations were observed when comparing pre-tests in the morning and afternoon (P = 0.009) (Table 1; Part b). Significant differences in testosterone concentrations were observed when comparing post-test results in the morning and afternoon (P = 0.01) (Table 1; Part c).

Cortisol

Cortisol concentration tended to increase in the morning (P = 0.23) and afternoon (P = 0.27) after HIIT (Table 1; Part a). Significant differences in cortisol concentrations were observed when comparing pre-test results in the morning and afternoon (P = 0.001) (Table 1; Part b). Significant differences in cortisol concentrations were also observed when comparing post-test results in the morning and afternoon (P = 0.001) (Table 1; Part c).

Testosterone/cortisol ratio

The testosterone/cortisol ratio tended to decrease in the morning (P = 0.79) and to increase in the afternoon (P = 0.16) after HIIT (Table 1; Part a). Significant differences in the testosterone/cortisol ratio were observed when comparing pre-test results in the morning and afternoon (P = 0.001) (Table 1; Part b). The differences in the testosterone/cortisol ratio were also significant when comparing post-test results in the morning and afternoon (P = 0.04) (Table 1; Part c).

Maximum heart rate and peak heart rate

The results also showed a significant difference in activity intensity between morning (85.1 ± 4.0% of maximum heart rate) and afternoon (88.9 ± 4.5% of maximum heart rate) (P = 0.008). However, no significant difference was observed in peak heart rate (195.1 ± 7.2 bpm in the morning vs 195.0 ± 8.3 bpm in the afternoon; P = 0.98).

Discussion

This study found that testosterone concentration increased after HIIT in the afternoon. This is consistent with the results of a number of studies reporting increased testosterone levels after HIIT [13, 18, 20]. Results of a study in obese and overweight children showed a significant increase in testosterone concentration after HIIT [20]. Another study demonstrated that testosterone level significantly increased in response to a single session of HIIT and high-volume training in young athletes [16]. Additionally, it was also indicated that HIIT induced an increase in testosterone level in triathletes/cyclists [23]. In sedentary older men, total testosterone and free testosterone levels were significantly increased as a result of HIIT [11]. In endurance-trained males HIIT caused a significant increase in free testosterone concentration [9]. In turn, in recreationally trained males and females a significant increase was observed in testosterone levels after HIIT compared to pre-exercise concentrations [18].

Conversely, some studies have reported decreases or no change in testosterone concentration after HIIT [1, 13, 28]. These results are not consistent with the findings of this study. The difference in the results can be attributed to the time and duration of training [28], sport level of participants [1], as well as differences in the study protocols [13]. For example, in well-trained young cyclists a decrease was observed in total testosterone and free testosterone levels following four weeks of HIIT [28]. Another study in women showed non-significant changes in testosterone after HIIT [1]. In male master's athletes, a non-significant change was observed in total testosterone and a small increase in free testosterone level following HIIT [13].

Another finding of this study was that testosterone, cortisol and the testosterone/cortisol ratio changes in the morning were not statistically significant. Other studies have shown non-significant changes in testosterone, cortisol and the testosterone/cortisol ratio, in line with the current study. For example, in male master's athletes a non-significant change was observed in total testosterone and only a small increase in free testosterone following HIIT [13]. Another study found non-significant changes in testosterone level after HIIT in women [1]. Another study in young athletes showed a non-significant increase in cortisol and the testosterone/cortisol ratio after HIIT [16].

Conversely, some studies have reported significant changes in testosterone, cortisol and the testosterone/cortisol ratio after HIIT. These results are not consistent with the findings of this study [11, 16, 20, 31]. The difference in the reported results can be attributed to the time and duration of training [16, 20], sport level of participants [11, 20] and differences in the study protocols [16, 20, 31]. For example, total testosterone and free testosterone levels were significantly increased as a result of HIIT in sedentary older men [11]. A significant increase in testosterone and significant decreases in cortisol concentration and the testosterone/cortisol ratio was observed after HIIT in obese and overweight children [20]. In young athlete's testosterone level significantly increased as a result of HIIT training [16]. In triathletes/cyclists, HIIT induced increases in testosterone and the testosterone/cortisol ratio, and a decrease in cortisol level [31]. In well-trained male cyclists cortisol concentration increased significantly as a result of HIIT [21]. In turn, in endurance-trained males HIIT caused a significant increase in free testosterone [9]. In recreationally trained males and females significant increases in testosterone and cortisol were observed post-HIIT compared to pre-HIIT concentrations. Additionally, the testosterone/cortisol ratio was significantly reduced compared to pre-HIIT values [18]. In overweight adults, significant decreases in cortisol levels and significant increases in the testosterone/cortisol ratio were recorded in the HIIT group [30]. Various studies have shown that exercise intensity is one of the factors that can increase testosterone levels.

Although no difference was found in peak heart rate between morning and afternoon training sessions in this study, a significant difference was found in exercise intensity (based on average heart rate) between morning and afternoon. Average heart rate was higher in the afternoon than in the morning, which may significantly increase the testosterone concentration in the afternoon. This difference in average heart rate between morning and afternoon training was also reported in another study [2]. Catecholamine stimulates the Leydig cells in the testes and the secretion of testosterone following intense exercise activity [9]. Nevertheless, the findings showed that HIIT can dramatically increase concentrations of adrenaline and noradrenaline [21]. Hormone changes (e.g. testosterone) are variable and may depend on the exercise paradigm (resistance vs aerobic), the exercise intensity, the study population (young vs older men) and/or the catecholamine concentration [3, 10, 15].

On the other hand, as reported, testosterone increases may acutely influence the central nervous system (e.g. mood, behaviour, aggression, cognition), substrate metabolisms (e.g. glycogen and amino acid metabolism) and neuromuscular electrophysiological-contractile properties [4].

The training intensity therefore reverses the cortisol response to training. An increase in the concentration of this hormone in the current study can be attributed to the high-intensity exercise protocol, but the short intervals of the exercise may explain the non-significance of this increase. The reason for the difference in the results can be attributed to exercise duration [11], the subjects [13, 20, 28] and gender [1]. In the current study, although HIIT increased the testosterone/cortisol ratio, the increase in the afternoon was not statistically significant. The testosterone/cortisol ratio was used as an indicator of the physiological pressure response to exercise and as an indicator of anabolic/catabolic balance [29]. The higher this ratio in the afternoon, the more superior the anabolic metabolism after HIIT in the afternoon. Wilk et al. [32] indicated that the highest testosterone values may occur during exercise or at any other time when no measurement was taken.

In summary, in the current study HIIT significantly increased testosterone concentrations only in the afternoon. Significantly higher testosterone and cortisol concentrations in the morning compared to the levels in the afternoon may be attributed to the normal secretion rhythms of these hormones [7]. However, the testosterone/cortisol ratio was higher in the afternoon than in the morning, which is probably due to lower cortisol concentrations in the afternoon. A lower cortisol concentration and a higher testosterone/cortisol ratio in the afternoon therefore show a favourable anabolic-catabolic balance in the afternoon. Consequently, HIIT should ideally be performed in the afternoon due to the favourable anabolic-catabolic balance. Since HIIT simulates the successive sprints that are part of most exercise activities, the time of these activities should be considered by athletes, coaches and developers of academic and non-academic sports programmes when implementing such programmes. Lack of control diet/exercise/bedtime patterns during the study are some of the limitations of the present study. It seems that performing HIIT in the afternoon may be more suitable in terms of the level of anabolic processes. However, further studies are needed to measure these hormones at consecutive intervals during recovery to generalise these findings to the entire population and to better explain them.

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Conflict of Interests
The authors declare no conflict of interest.

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