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HORMONE RESPONSES TO INTENSIVE INTERVAL TRAINING IN MIDDLE-DISTANCE RUNNERS

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Abstract. This study examined the effects of intensive interval training session on the post-exercise level of cortisol and testosterone in a relatively homogeneous group of college-level middle-distance runners. The subjects performed a typical set of four 400 m intensive interval runs with individually chosen high speed. Recovery intervals between runs were 5 min. During recovery, runners were asked to walk slowly on the track. Blood samples were taken before, immediately after the training session and 30 min post-exercise. Cortisol, testosterone and sex hormone binding globulin were measured, and free testosterone and the free testosterone : cortisol ratio calculated. The determined hormone concentrations were not changed as a result of interval training session. However, to further analyse the hormone responses, the subjects were divided into two subgroups according to the cortisol responses to training. Group 1 (n=5) and Group 2 (n=5) consisted of subjects whose cortisol concentration was increased and decreased immediately after the training session, respectively. Resting cortisol and testosterone concentrations were significantly lower in Group 1 compared to Group 2. In Group 1, the cortisol concentration was higher after training by 102±86 nmol.l^{-1} ($P<0.05$). Group 2 demonstrated a decrease in cortisol concentration after the training by 296±77 nmol.l^{-1} ($P<0.05$). No further changes were noted during the first 30 min of recovery in both groups ($P>0.05$). Testosterone was significantly decreased immediately after training and remained suppressed for the first 30 min of recovery in Group 2, while no significant changes in testosterone concentration were observed in Group 1. No significant changes were also noted in free testosterone and free testosterone: cortisol ratio as a result of training in both groups. However, both groups demonstrated similar body composition and 1500 m running performance values as well as the results of 4 x 400 m runs were similar in both groups of runners. In conclusion, the findings of this study suggest different regulation of pituitary-adrenocortical activity as a result of intesive 4 x 400 m interval running training session in middle-distance runners, expressed either by intensified or suppressed endocrine functions. Intensified and suppressed endocrine functions

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were observed in subjects with low and high resting hormone concentrations, respectively.

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Key words: Cortisol – Testosterone - Pituitary-adrenocortical activity - Anaerobic interval exercise

Introduction

The acute responses in the endocrine system during physical exercise are related to the intensity and duration of the specific exercise [12,17,21] as well as to the level of specific physical condition of the subjects and/or athletes [18,22,30]. The blood concentrations of the catabolic hormone cortisol and anabolic hormone testosterone demonstrate a response threshold, identified as the percentage of aerobic power [3,30]. The concentration of cortisol increases when the intensity of exercise exceeds 60% of aerobic power [3,30]. However, further increase of exercise intensity may not be accompanied by a parallel increase in the cortisol concentration [26]. Some results have indicated the possibility that high-intensity anaerobic exercises may even suppress the cortisol response [4,26]. Furthermore, the cortisol concentration may even decrease during exercise when the pre-exercise level of cortisol is relatively high [8]. These results together suggest that cortisol response to acute exercise is highly specific.

Inconsistent results have also been reported regarding the training-related changes in resting cortisol levels [5,7,31]. For example, a study involving 361 athletes demonstrated great variability in resting cortisol levels [5], while another study failed to demonstrate a significant difference between endurance athletes and untrained males [16]. Furthermore, it has been reported that sprint-trained athletes present significantly higher resting cortisol levels in comparison with endurance-trained athletes [7]. While endurance-trained men show lowered [7,18] or similar [17] resting testosterone levels in comparison with untrained men. These results could suggest some sport-related specificity, while a great inter-individual variability in the resting concentrations of these hormones still exists.

Testosterone and cortisol have been used to evaluate the anabolic and catabolic hormone balance in athletes [3,17,18,21,22]. However, free testosterone, the ratio between testosterone and sex hormone binding globulin levels, may be more specific indicator of anabolism as it represents the biologically available testosterone [1,19]. Therefore, the ratio between free testosterone and cortisol can be used to indicate the

balance between anabolic and catabolic metabolism [21,22,28]. A decrease in the free testosterone: cortisol ratio indicates a metabolic strain in the organism [1,19].

This investigation employs an intensive interval exercise protocol modelled on those which have frequently been used by athletes involved in middle- and long-distance running [27,29]. The effects of such a protocol to anabolic and catabolic hormone concentrations are largely unknown. Unfortunately, many previous investigations, which have investigated the effects of interval exercise on the endocrine system have employed protocols of little relevance to the training of middle- and long-distance runners. Therefore, the aim of the present study was to investigate the effects of four 400 m intensive interval runs on the post-exercise level of cortisol and testosterone in a relatively homogeneous group of college-level middle-distance runners.

Material and Methods

Subjects: Ten male college-level middle-distance runners volunteered to participate in this study. They had trained regularly for the last 3.3±1.1 years. All participants trained regularly 5-6 times a week. All subjects were healthy and displayed no symptoms of acute or chronic diseases. The study was conducted during winter pre-competition period in January, when the basic aim of training was special preparation for the indoor competitions. The runners were familiarized with the procedures and possible risks before providing their consent to participate in the experiment as approved by the Medical Ethics Committee of the University of Tartu. Training session was carried out from 10.00 to 12.00 hours at their usual training time and subjects were asked not to participate in any physical activity in the 24 hours before testing. In addition, the runners 1500 m indoor running competition time was obtained one week later as their performance parameter.

Testing procedures: Interval training session was performed on a 150 m indoor track. After voiding, baseline blood samples were obtained followed by a warm-up that included stretching and jogging for 15 min. The participants then performed four 400 m intensive interval runs with individually chosen high speed [27,29]. Recovery intervals between runs were 5 min. During recovery, runners were asked to walk slowly on the track. Heart rate was measured continuously and stored at 5 s intervals by Sporttester Polar Vantage NV (Kempele, Finland). Other blood samples were taken immediately after the training session and 30 min post-exercise. Ratings of perceived exertion were also determined after the training session using the Borg (6-20) Scale [6].

Blood analysis: A 10 ml blood samples were obtained from an anticubital vein with the participant in the upright position. The plasma was separated and frozen at –20 ^{0}C for later analysis. Testosterone, cortisol and sex hormone binding globulin were measured in duplicate using an automated immuno-chemoluminescence system Immunolite [2]. Samples from one individual were run on the same assay. The inter- and intra-assay coefficients of variation were less than 5%. Free testosterone was determined from the ratio of testosterone to sex hormone binding globulin; the ratio of free testosterone to cortisol was also calculated [21,22,28]. All hormone concentrations reported are not corrected for the plasma volume shifts induced by the exercise bout [17,18,21] as we deemed it important to know the concentration of hormone that the target tissues were exposed to regardless of the means by which the change in concentration occurred [13,23]. In addition, blood lactate concentration was analysed from fingertip capillary blood samples enzymatically in duplicate with Lange microanalyzer (Lange, Germany) [15].

Body composition assessment: The height (Martin metal anthropometer) and body mass (A&D Instruments Ltd, UK) of the participants were measured to the nearest 0.1 cm and 0.05 kg, respectively. Body composition was measured using dual-energy X-ray absorptiometry. Scans of the whole body were performed on each of the subjects using a Lunar DPX-IQ scanner (Lunar Corporation, Madison, USA) [10] and analyzed for fat, lean and bone mineral tissue mass.

Statistical methods: Means and standard deviations (SD) were determined. Friedman analyses of variance were used to examine changes, as the raw data and their logarithmic transformations were not normally distributed. The Wilcoxon matched-pairs signed-ranks test was used where post-hoc analysis was relevant and also between group comparisons. The Kendall rank correlation coefficients were computed to determine the relationships between dependent variables. In all analyses, the accepted probability level was $P<0.05$.

Results

The hormone concentrations were not significantly altered during training or in the first 30 min of recovery (Fig. 1). Sex hormone binding globulin before the training session was 32.5±11.6 nmol.l^{-1} and was not significantly altered immediately after (28.1±10.9 nmol.l^{-1}) and after 30 min of recovery period (29.7±7.0 nmol.l^{-1}) in middle-distance runners. Blood lactate concentration was significantly increased immediately after interval training session and remained elevated after first 30 min recovery period (Table 1).

Fig. 1
Mean (±SD) cortisol (C), testosterone (T), free testosterone (T_{free}) and T_{free}:C ratio before (PRE), immediately after (POST) and 30 min (POST-30') after interval training

Fig. 2
Mean (±SD) cortisol (C), testosterone (T), free testosterone (T_{free}) and T_{free}:C ratio before (PRE), immediately after (POST) and 30 min (POST-30') after interval training in Group 1 and Group 2; *Significantly different from Group 1; $P<0.05$, #Significantly different from PRE; $P<0.05$

Table 1
Mean (±SD) 4 x 400 m interval running times, blood lactate concentration and ratings of perceived exertion

	Group 1 (n=5)	Group 2 (n=5)	Pooled (n=10)
1st 400 m (s)	63.0±2.3	64.3±3.3	63.7±2.8
2nd 400 m (s)	64.7±3.1	64.2±1.5	64.5±2.3
3rd 400 m (s)	65.5±4.0	66.0±1.0	65.8±2.8
4th 400 m (s)	67.6±4.8	70.8±4.6	69.2±4.7
Lactate before (mmol.l^{-1})	1.7±0.7	2.1±0.6	1.9±0.7
Lactate after (mmol.l^{-1})	19.0±3.3	22.3±3.5*	20.7±3.6
Lactate 30'recovery (mmol.l^{-1})	7.2±2.0	11.6±2.5	9.4±3.2
Rating of perceived exertion	17.2±1.5	17.4±1.7	17.3±1.5

*Significantly different from Group 1; P<0.05

Correlation analysis revealed significant relationships between cortisol and testosterone before training (r=0.84) and in 30 min recovery period (r=0.75) but not immediately after training. Free testosterone was also related to cortisol concentration only after 30 min of recovery period (r=0.76). All other relationships between hormone concentrations measured at different time points were not significant (r<0.48; P>0.05). Free testosterone: cortisol ratio was related to the blood lactate concentration before (r=-0.68), immediately after (r=-0.64) and after 30 min of recovery period (r=-0.66). In addition, resting free testosterone: cortisol ratio was also related to the 1500 m competition time (r=-0.67) and lean body mass (r=0.76).

In attempt to analyze the hormone responses further, the subjects were divided into two groups according to the cortisol responses to the training session [32]. Individual cortisol changes were considered significant when they exceeded intra-individual variations of ±25 nmol.l^{-1} [31]. Group 1 (n=5) and Group 2 (n=5) consisted of subjects whose cortisol concentration was increased and decreased immediately after the training session, respectively (Fig. 2). Resting cortisol and testosterone concentrations were significantly lower in Group 1 compared to Group 2. In Group 1, the cortisol concentration was higher after training by 102±86 nmol.l^{-1} (P<0.05). Group 2 demonstrated a decrease in cortisol concentration after the training by 296±77 nmol.l^{-1} (P<0.05). No further changes were noted during the first 30 min of recovery in both groups (P>0.05). Testosterone was significantly decreased immediately after training and remained lowered for the first 30 min of

recovery in Group 2, while no significant changes in testosterone concentration were observed in Group 1. No significant changes were also noted in free testosterone and free testosterone: cortisol ratio as a result of training in both groups.

The results of 4 x 400 m runs were not different between groups (Table 1). Running time was significantly increased after fourth run in comparison with the first three runs in both subgroups as well as in the whole group of runners. However, blood lactate concentration immediately after the training was higher in Group 2, where cortisol concentration was significantly decreased after the training. Blood lactate concentration immediately after training was significantly related to post-exercise cortisol (r=-0.88) and testosterone (r=-0.90) levels only in Group 2 runners. In addition, rating of perceived exertion responses were similar in both groups. No differences between groups were also observed for body composition parameters and 1500 m competition results (Table 2).

Table 2
Mean (±SD) body composition and 1500 m performance parameters

	Group 1 (n=5)	Group 2 (n=5)	Pooled (n=10)
Height (cm)	185.2±8.5	179.6±4.5	182.4±7.1
Body mass (kg)	70.1±6.6	65.0±7.8	67.5±7.3
Body fat% (%)	7.5±1.3	8.3±2.0	7.9±1.6
Fat mass (kg)	4.9±1.0	5.2±1.8	5.1±1.4
Lean mass (kg)	61.2±6.0	56.4±5.3	58.8±5.9
Bone mass (kg)	3.7±0.6	3.1±0.4	3.4±0.6
1500 m time (s)	271.4±13.8	273.4±7.3	272.4±10.5

Discussion

The present investigation showed that intensive 4 x 400 m interval training, involving considerable anaerobic energy production [27], is associated with a no significant cortisol, testosterone, free testosterone and free testosterone: cortisol ratio responses in the whole group (n=10) of middle-distance runners with similar performance and body composition parameters (Fig. 1). Furthermore, free testosterone: cortisol ratio was related to the blood lactate concentration at rest, immediately after and after 30 min of recovery period ($r=-0.64$; $P<0.05$). This indicates that overall anaerobic energy production of interval running influenced the concentration of specific anabolic and catabolic hormones in blood. It is well known

that the decrease in the ratio of these hormones indicates a metabolic strain of the organism [1,19]. In addition, resting free testosterone: cortisol ratio was significantly related to the 1500 m performance time ($r=-0.67$; $P<0.05$) and lean body mass ($r=0.76$; $P<0.05$) in our middle-distance runners. These results demonstrate that the determination of specific anabolic and catabolic hormones can provide useful information on the adaptation of certain levels of exercise intensity and duration as well as monitoring whole training process in college-level middle-distance runners.

The primary finding of the present study was that the lack of the whole group hormone responses to 4 x 400 m interval training session did not mean that there was a lack of changes in individuals. In some runners, the cortisol concentrations increased and in others they decreased immediately after the training session (Fig. 2). Viru *et al.* [32] have suggested to further explore individual trends of hormones within the data as a result of exercise. The criteria of Viru *et al.* [31] were used in our study to classify runners into two subgroups and cortisol was chosen as the classification hormone due to its links to various "fatigue mechanism" hypotheses [24,32]. Resting levels of cortisol were relatively low and different (124 ± 58 vs 485 ± 112 nmol.l^{-1}; $P<0.05$) in Group 1 ($n=5$) compared to Group 2 ($n=5$) when using the criteria of Viru *et al.* [31]. Therefore, resting cortisol concentrations in all our runners were within the normal range for adult males [7,20]. However, it is very interesting to note that the classification of the subjects into these two subgroups revealed no differences in either 4 x 400 m running times, 1500 m performance times nor different body composition parameters between subgroups.

For Group 1, the observed increased cortisol concentration immediately after the interval training session is in accordance with previous investigations [17,30]. However, the cortisol response in Group 2 was opposite. It appeared that the relatively high resting cortisol concentration in Group 2 provoked a feedback suppression of the cortisol responses as demonstrated by Brandenberger *et al.* [9]. Testosterone and free testosterone responses were quite similar in this respect (Fig. 2). Together these findings may demonstrate a different regulation of pituitary-adrenocortical activity as a result of intensive interval training in two subgroups of middle-distance runners who present similar performance and body composition parameters. Group 1 with relatively low resting cortisol concentration involved an intensified mobilization of pituitary-adrenocortical function, while Group 2 with relatively high resting cortisol concentration reflected the inhibition of activity within the system.

A typical acute anabolic hormonal response to maximal anaerobic interval training session can be demonstrated as an increased level of post-exercise testosterone [14,17]. In contrast, testosterone responses to intensive 4 x 400 m

interval runs demonstrated non-significant and significant decrease immediately after training in Groups 1 and 2, respectively. These differences could be explained by the fact that the total amount of work performed during acute training session (i.e. the level of exercise volume and threshold) by our subjects was not enough to elicit a significant increase in post-exercise testosterone level and/or the performance capacity of our middle-distance runners was different. Furthermore, it has to be considered that the subjects in Group 1 could also be regarded as trained runners with lowered resting testosterone levels in comparison with untrained subjects according to the results of other studies [14,18,25]. In contrast, some studies have not demonstrated lowered resting testosterone concentrations in well-trained runners [17]. This was also the case for our subjects in Group 2. However, the resting testosterone levels in Group 1 (11.9±2.8 nmol.l^{-1}) were even lower compared to the resting values in endurance-trained subjects (16.6±2.4 nmol.l^{-1}) found in the Hackney *et al.* [18] study. These differences in resting hormone concentrations and also different responses to anaerobic interval training session between subgroups in our study and with the results of other studies [17] could explain different reactions of hypothalamo-pituitary-testicular axis to anaerobic interval training session. Interestingly, DeSouza *et al.* [11] suggested that the development of hypothalamo-pituitary-testicular axis alterations seems to depend upon a "volume-threshold" effect with respect to the exercise training that male runners perform. This could suggest that the hormonal data of Group 1 may reflect the physiological responses of runners who have already reached such a threshold. In contrast, the performance parameters between subgroups were not different. Alternatively, there could be inter-individual differences for the development of such a threshold [14,18] as the actual magnitude of the individual response also depends on the functional capacity of the endocrine system [30]. Taken together, additional research is necessary to further address this point and consequently determine the magnitude of training needed to reach the proposed threshold in different athletes.

It is interesting to note that the training stimulus for both subgroups of subjects was similar and relatively high as demonstrated by very high post-exercise values of blood lactate (19.0±3.3 vs 22.3±3.5 mmol.l^{-1}; $P<0.05$) and ratings of perceived exertion (17.2±1.5 vs 17.4±1.7; $P>0.05$). Similar post-exercise ratings of perceived exertion demonstrated that overall body fatigue was relatively high in all subjects. Despite this, post-exercise blood lactate concentration was related to post-exercise cortisol and testosterone values ($r>-0.88$; $P<0.05$) only in runners of Group 2. No such relationships were observed for Group 1 runners. This demonstrated that the substrate of anaerobic energy metabolism was related to the suppressed activity of

pituitary-adrenocortical function in middle-distance runners with high resting cortisol and testosterone levels. In contrast, an intensified mobilization of pituitary-adrenocortical function did not influence the anaerobic metabolic process during intensive interval running training session in middle-distance runners. However, further studies are needed before any conclusions can be drawn.

Viru *et al.* [32] have suggested that the comparison of the overall mean responses within their data may mask individual differences that could have occurred (i.e. subjects who are highly responsive negate those who are not). Similarly, the overall (n=10) mean anabolic and catabolic hormone response to the interval training session demonstrated no significant post-exercise changes in these hormone concentrations (Fig. 1). However, individual re-examination of the effect of 4 x 400 m runs demonstrated that the level of cortisol in blood was increased in subjects with low resting cortisol level (n=5; Group 1) and decreased in subjects with high resting cortisol level (n=5; Group 2) (Fig. 2). Analogous findings have also been reported for the hormone responses to 2 h of endurance exercise [31]. Specifically, the prolonged aerobic exercise caused an immediate post-exercise cortisol increase in some subjects, while cortisol levels fell below initial resting values by the end of exercise in other subjects [31]. The results of both these studies together would suggest different types of resetting within pituitary function regardless of the specificity of acute exercise (i.e. "volume-threshold") and previous training history of subjects.

In conclusion, the findings of this study suggest different regulation of pituitary-adrenocortical activity as a result of intensive 4 x 400 m interval running training session in college-level middle-distance runners with similar body composition and performance parameters. In some cases, an intensified mobilization of certain endocrine functions occurred, while endocrine function was suppressed in other cases.

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