

The effects of four weeks aerobic training on saliva cortisol and testosterone in young healthy persons

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Abstract. [Purpose] The purpose of this study was to evaluate the effect of 4 weeks moderate aerobic exercise on outcome measures of saliva stress hormones and lactate levels in healthy adult volunteers. [Subjects and Methods] Sixteen healthy students with an age range of 15–25 years participated in this study. The participants performed an exercise test of moderate intensity for 4 weeks, three times per week. The exercise was treadmill walking. Saliva concentrations of cortisol, testosterone and lactate dehydrogenase (LDH) were measured before and after the 4 weeks of moderate aerobic training using immunoassay techniques. [Results] After 4 weeks of exercise, there were significant increases in cortisol, free testosterone levels, and LDH activity along with a significant decrease in the ratios between testosterone and cortisol levels. No significant correlations were found among the studied parameters in the resting stage, a result which supports the positive effect of exercise on stress hormones following 4 weeks of training. [Conclusion] The results suggest that four weeks exercise of moderate intensity significantly affects the salivary stress hormones of young healthy volunteers. The data support the importance of salivary stress hormones as potential biological markers especially for older ages. However, more research is required to validate these biological markers which determine the host response to physical activity.

Key words: Aerobic exercise, Saliva Stress hormones, Physical activity

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INTRODUCTION

Cortisol is principally glucocorticoid secreted from the adrenal cortex into human serum or saliva and plays a major role in metabolism, the immune function, and the regulation of physiological stress responses¹⁾. A strong association has been reported between salivary cortisol and blood sample ratios which provides an accurate assessment of unbound cortisol levels^{2, 3)}.

Stress is explained as physiological changes occurring in the human body in relation to physical or external stress conditions which drastically and negatively affect the homeostasis^{4–6)}, and differences in psychological and physical stressors resulting in hormonal changes^{7, 8)}.

It has been reported that accommodation of psychological and physical stressors mainly depends on the activity of the adrenal glands. Bodies under stress need higher rates of metabolic processing along with glucocorticoid release which may affect physical activity^{9–11)}.

Physically active people of different ages show better

emotional health, and it has been found that regular physical activity increases the fitness of persons and their ability to conquer depressive mood¹²⁾. The change of endocrine hormones among individuals exposed to different types of stress has been investigated. A significant increase in the cortisol level, a marker of endocrine response was reported in subjects stressed by high intensity physical activity⁹⁾. Also, it was reported that participants with low physical activity showed larger cortisol responses to physical exercise than those participated regular exercise¹³⁾, and that regular exercise modulates the response of cortisol elevation over time⁹⁾. The change in cortisol level depends mainly upon the type of exercise. Decreased levels of cortisol were reported in individuals performing both low intensity¹⁴⁾, and acute exercise training^{15, 16)}.

Many exercise interventions are designed to manipulate the mechanistic effects of physical activity such as behavioral and physiological actions on cortisol levels. Recent studies have reported that the adaptation of endocrine system to exercise training occurs physiologically via buffering of anabolic and catabolic processes¹⁷⁾. For example, the change in the cortisol/testosterone ratio, as an indicator of the anabolic-catabolic balance, has been used with limited success to determine the physiological strain of exercise training¹⁸⁾. The free-testosterone/cortisol ratio (fr-T/C) has been proposed as a major indicator of anabolic and catabolic effects due to over training especially among athletes. It was reported that, a reduction in the testosterone/cortisol ratio of

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more than 30% is related to overtraining status¹⁹). Also, in another study, there was a noticeable increase in the levels of testosterone without a big difference in cortisol levels after maximum aerobic training²⁰, and this variation in adrenal hormones may be related to subjects having differences in their response to exercise²¹. While, cortisol has a catabolic effect, testosterone is responsible for the stimulation of the anabolic process of skeletal muscle growth which increases linearly in response to exercise²²).

The increase of cytosolic proteins in the circulation including lactate dehydrogenase (LDH) after exercise reflects muscular injury whereas both exercise intensity and duration independently affect enzymatic activity and muscular pain with intensity being the variable with the greatest effect^{23, 24}. It has also been reported that adjusting lactate levels plays an integral part in overtraining prevention, since it is an accessible and widely used technique of overtraining diagnosis in the sports field²⁵).

Many previous studies have investigated changes in cortisol and testosterone hormones in response to exercise training and the differences in adaptation to exercise among subjects however; their results are contradictory regarding the intensity and type of exercise^{26–28}).

Both cortisol and testosterone show significant linearity with peak concentrations in response to exercise once a specific intensity threshold is reached²⁹, even under low intensity exercise with a long enough duration^{30, 31}. Previous, studies have reported the change in stress hormones at different durations of training programs of varying intensities^{32–35}), their results suggest that salivary stress hormones are a potentially valid outcome measures of responses to exercise training programs. While, there are many studies on the effects of short training periods on stress hormones^{36, 37}), little is known about the effect of short term exercise of moderate intensity on the status of salivary stress hormones even though it may be of use in diseases related to older adults such as cognitive impairment and cardiovascular diseases^{38, 39}). Therefore, the present study assessed the effects of 4 weeks of moderate aerobic exercise on saliva stress hormones and lactate levels in healthy adult volunteers because short term exercise of moderate intensity may be suitable for most sedentary and elderly subjects

SUBJECTS AND METHODS

Sixteen healthy students with ages between 15 and 25 years were recruited for this study (Table 1). None of the participants were involved in any specific training program or normal physical activity during the study schedule. Subjects with a BMI of more than 25 (kg/m²), ischemic heart disease, or with severe orthopedic problems were excluded from this study. Prior to the exercise test, the risks and benefits of the study were explained, and written informed consent was obtained from each participant after a medical check-up to ensure that they were fit, healthy and had no physical limitations. All participants were advised to keep to their normal habits of eating during the entire period of data collection. Dietary information was obtained from food diaries or by extensive dietary interviews. The experiment was conducted in accordance with the ethical guidelines of

Table 1. Anthropometric characteristics of the participants (mean \pm SD)

No.	Anthropometric Parameters	Participants (No.= 16; mean \pm SD)
1.	Age (years)	22.8 \pm 2.14
2.	Height (cm)	172.0 \pm 4.92
3.	Weight (kg)	69.5 \pm 3.7
4.	BMI (kg/m ²)	23.7 \pm 1.41

the 1975 Declaration of Helsinki, and was reviewed and approved by Ethical Committee of the Rehabilitation Research Chair (RRC), King Saud University, Riyadh, KSA.

Each subject participated in an exercise training program three times per week for 4 weeks. The exercise program consisted of treadmill walking. Each individual's training intensity was calculated as the training heart rate (THR) based on the subject's age and predicted maximum heart rate and resting heart rate according to Karvonen's formula⁴⁰), (THR = HRrest + (HRmax – HRrest) \times TF, where HRrest = resting heart rate in bpm. HRmax = maximum heart rate in bpm and TF = training fraction (65 to 75% for the moderate intensity used in this study). This formula has previously been shown to be valid for the effect of exercise on stress hormones^{41, 42}). Each exercise session consisted of three phases; the warm-up, active, and cool-down phases. During the warm-up phase, the subjects performed simple stretching exercise for all large muscle groups and walked for 5 to 10 minutes at TF equal to 30–40%. During the active phase, the subjects were encouraged to reach their pre-calculated training heart rate (THR) in bouts with a total time of 30 to 45 minutes. The last phase was cool-down which continued for 10 to 15 minutes during which the workload gradually decreased until HR and blood pressure had nearly returned to their resting levels. Throughout the entire training session, the subjects were monitored by a portable heart rate monitor to keep the exercise intensity within the precalculated training heart rate for each subject.

Samples of saliva were taken from the participants during a rest day (24 hours after training) in the mid-morning (9:00–11:30 AM), following overnight fasting. Samples were separated using centrifuge, numbered, and then stored at -80°C until used for analysis.

After the saliva had been separated, the lactate levels were measured by the UV method, provided with the Caymans L-lactate assay kit (Cat No 700510, USA). Cortisol and testosterone levels (pg/ml) were measured in the saliva samples of participants using the immunoassay technique according to the instructions of the cortisol ELISA-kit (Diagnosics Biochem Canada, Inc.) and testosterone ELISA-kit (Cat no., 11-TESHU-E01-SLV, ALPCO Diagnostics, Inc.), respectively.

Statistical analysis was performed using SPSS 7.5 for Windows (SPSS Inc., Chicago, IL, 1989 1996). Repeated measures analysis of variance (ANOVA) was used to analyze the effect of the exercise on the levels of the studied stress hormones. The statistical relation among hormone levels pre- and post-exercise were calculated using Pearson

Table 2. Differences between pre- and post-training concentrations of selected biomarkers

Variables	Pre-training value (Mean \pm SD)	Post- training value (Mean \pm SD)	Mean difference
Cortisol [C] (pg/ml)	17.51 \pm 3.18	37.2 \pm 4.17	19.71 \pm 4.66**
Testosterone [T] (pg/ml)	26.42 \pm 3.1	41.03 \pm 3.04	14.62 \pm 3.52**
[T/C] Ratio	1.6 \pm 0.22	1.12 \pm 0.15	-0.45 \pm 0.22**
LDH (IU/L)	4.71 \pm 0.57	18.23 \pm 3.55	13.51 \pm 3.56***

All values represent as mean \pm SD.* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 3. Correlation of cortisol ratios with other stress biomarkers between pre- and post-test

Variables	Pre-training value		Post- training value	
	r	(95% CI)	r	(95% CI)
Cortisol vs. Testosterone	0.01	75 (73–98)	0.25**	94 (87–100)
Cortisol vs. [T/C] Ratio	0.06	80 (75–100)	0.53**	89 (87–100)
Cortisol vs. LDH	0.09	68 (56–100)	0.21**	90 (86–100)

$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. 95% confidence interval

coefficients, and the significance of differences between the mean values before and after each exercise session were tested using the paired samples t-tests. P values < 0.05 were considered significant.

RESULTS

The effect of short term exercise on the levels of stress hormones was assessed after 4 weeks of training. The participants were recommended to keep to their normal routine diet throughout the study to avoid dietary effects on the measured variables. The data of stress hormones in the pre- and post-training recovery stage are shown in Table 2. The levels of stress hormones were normal and within acceptable clinical ranges for the conditions under which they were collected. Cortisol and free testosterone concentrations were significantly increased ($p < 0.01$) in the post-exercise recovery samples compared to their pre-training values after four weeks of moderate intensity of training, and there was a significant decrease in the T/C ratio ($p < 0.01$) (Table 2). Four weeks of moderate exercise training also elicited significant increases in lactate dehydrogenase (LDH) concentration ($p < 0.001$). The correlation analysis revealed that the increase of cortisol was positively ($p < 0.001$) related to the changes in total free testosterone, the T/C ratio, and the lactate dehydrogenase level in the post-training samples. The pre-training samples showed no relationship of cortisol with other stress biomarkers ($p > 0.05$) (Table 3).

DISCUSSION

The endocrine system, by modulation of anabolic and catabolic processes, plays a major role in the physiological adaptation to exercise training⁴³. Hormonal assays particularly anabolic (testosterone) and catabolic (cortisol) hormones have been suggested as being valuable indicators of the exercise intensity and work load. The ratio between anabolic and catabolic hormones has been used to determine the readiness status of individuals¹⁰. Whereas, the free tes-

tosterone to cortisol ratio is used as an adaptation exercise index for males⁴⁴.

This study was performed to investigate the efficacy of 4 weeks of moderate aerobic training on the levels of saliva stress hormones and lactate (LDH) activity as an early detection of muscle injury among young adult men. The results show that 4-weeks of moderate aerobic training by young men led to significant changes in saliva stress hormones and lactate level (LDH).

LDH is the most useful serum markers of muscle injury. It is an enzyme protein that converts pyruvate to lactate, with concomitant conversion of NADH to NAD⁴⁵. In this study, LDH activity significantly increased after 4 weeks of aerobic exercise. Measurements of LDH following exercise have been examined to seek evidence in support of the theory of muscle soreness. Distinguishable elevation in aerobic enzyme capacity was observed following long or short-term aerobic training in mammals^{46, 47}. Previously, it was reported that in active muscles the rate of lactate removal from the muscle cells into the blood increases following active exercise intervention⁴⁸. Therefore, the increase of blood lactate is due to the release of lactate from muscles into the blood following exercise.

Exercise training produces changes in the concentration of several biologically active molecules including cortisol and testosterone^{49–51}, which play pivotal roles as catabolic and anabolic agents in gluconeogenesis via the proteolytic pathway. The storage of glycogen and muscular protein synthesis is stimulated by testosterone⁵².

Four weeks of moderate exercise training produced significant positive increases in the concentrations of cortisol and free testosterone. Salivary testosterone increases found in this study, coupled with the observed change in the resting cortisol level suggest an enhanced anabolic environment. These results were in agreement with previous studies that reported that aerobic training increased the levels of cortisol and serum testosterone^{53, 54}. The increase noted in testosterone was probably induced by adrenaline stimulation, the stimulatory effect of lactate, or the compatibility of testos-

terone secretion⁵⁵).

Changes in the levels of cortisol and testosterone depend on the intensity of exercise training^{56, 57}, as it was reported that exercise intensity of more than 60% 1RM (one repetition maximum) increased the cortisol concentration significantly⁵⁸).

The testosterone-to-cortisol ratio (T/C) is considered to be a sign of the hemostatic balance between both anabolic and catabolic states in athletes; and this ratio plays a significant role as a prognostic biological marker for insufficient recovery and overtraining syndrome⁵⁹).

In this study, significant decrease in the T/C ratio was found in young men following 4-weeks of exercise training, a result that was in agreement with other studies^{60, 61}, and that the increase in cortisol following intensive endurance exercise was accompanied with significant lowering of the T/C ratio as previously reported^{62, 63}. However, the increase in the level of testosterone was significantly related to the increase in the level of gonadotropin hormone after a marathon⁶⁴. In this study, positive relationships were found among cortisol, free testosterone, T/C, and LDH activity in young men following 4-weeks of exercise training, as previously reported⁶⁵. However, in the resting stage (pre-exercise) no significant correlations were found among the studied stress hormones, similar to previous reports^{33, 34}.

The positive relationship between cortisol and free testosterone may be related to stimulation of the adrenal gland in response to physical stress, since both hormones are synthesized in the same cascade of reactions⁶⁶, indicating that stimulation of the adrenal gland via stressors results in concurrent secretion of both hormones which ultimately increase in the blood⁶⁷. Also, the increase in free testosterone may be related to changes in the binding affinity of the carrier protein as a result of change in pH and temperature due to exercise. This in turn would have decreased the level and uptake of the carrier protein to hormones which would be finally liberated as free hormones⁶⁸).

In the same manner, the positive correlation between post-exercise cortisol concentration and LDH activity may be affected by several mechanisms including blood lactate accumulation⁶⁹. The findings of this study also show that the increase of lactate at the end of exercise can elevate levels of cortisol during recovery. Thus, the changes of salivary stress hormones along with LDH detected in the present investigation may be related to the effect of short term aerobic exercise.

In conclusion, four weeks of moderate aerobic training three times per week, significantly elevated the levels of stress hormones, cortisol, free testosterone, and LDH in young men, with significant relationships among levels of cortisol, free testosterone, T/C, and LDH activity. However, more research is needed to validate these hormones as biological markers of physical activity stress.

Several limitations must be considered when interpreting the results of this study. The major limitation of this study is its low sample size, as only participants who were measured at pre- and post intervention and follow-up were included. Also, this study included only males which mean the results are gender specific. Furthermore, the exercise duration was short, and the study should be repeated with longer exercise

periods to measure the effect of long term moderate aerobic exercise on stress hormones. Finally, many studies define exercise intensity by heart rate which requires measurement of oxygen consumption, and this is an avenue of investigation we intend to pursue.

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