

Original Research

The effect of exercise mode and intensity of sub-maximal physical activities on salivary testosterone to cortisol ratio and α -amylase in young active males

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ABSTRACT

Int J Exerc Sci 4(4) : 283-293, 2011. We examined the effect of exercise intensity and mode on the acute responses of free testosterone to cortisol ratio and salivary α -amylase. We also evaluated the relationship between cortisol and salivary α -amylase. Ten healthy young active males participated voluntarily in this study in six single sessions. They exercised on a cycle ergo meter, treadmill, and elliptical instrument at intensities of 70% and 85% maximum heart rate for 25 minutes. Saliva samples were collected 5 minutes before and 5 minutes after each exercise session. No significant changes were observed for cortisol. Free testosterone to cortisol ratio increased during each exercise session ($F_{5, 45}=3.15$, $P=0.02$). However, these changes are only significant after exercise on the treadmill at 70% maximum heart rate ($t=2.94$, $P=0.02$) and 85% maximum heart rate ($t=0.53$, $P=0.03$). Salivary α -amylase significantly varied among exercise sessions ($F_{5, 45}=3.97$, $P=0.005$), and a significant decline was observed after exercise on the elliptical instrument ($t=2.38$, $P=0.04$) and treadmill ($t=3.55$, $P=0.006$) at 85% maximum heart rate. We found that the free testosterone to cortisol ratio is dependent on the exercise mode, while the salivary α -amylase response is dependent on the intensity of exercise. The increase of free testosterone to cortisol ratio in this study may indicate lower physiological stress in response to performing these exercises. Applying muscular strength with moderate intensity weight-bearing exercises possibly activates the anabolic pathways. Although the cortisol and salivary α -amylase responses were opposite in the majority of the exercise sessions, no significant inverse relationship was observed.

KEY WORDS: Physiological stress, sympathetic, adrenocortical, elliptical, cycle ergometer, treadmill

INTRODUCTION

Contradictory results from studies of hormonal responses to physical activities can be due to discrepancies between the different protocols used, including differences in intensity, duration, or exercise mode. Comparison of such studies employing exercise on a cycle ergometer

(25, 42) or treadmill (33, 38) indicate that exercise mode or the type of muscle involved likely influences the hormonal responses. One way to understand the effects of physical activity is to assay salivary biomarkers.

The use of saliva as a diagnostic tool in exercise physiology studies is increasing rapidly. This is due to its simplicity and the

ability to take frequent samples, given that it is non-invasive and non-stressful. Numerous studies have demonstrated the sensitivity of some salivary biomarkers in response to various exercises that are associated with physiological and psychological stress (3, 6, 7, 23, 24), and a significant relationship was observed between salivary and serum levels (15, 27, 32, 40).

Salivary cortisol is a good physiological biomarker for assessing stress level and hypothalamic-pituitary-adrenocortical axis function (10, 11, 12). The cortisol concentration changes in response to exercise and competition (3, 21, 44). If the salivary testosterone concentration is also assayed along with cortisol concentration, another valuable index of exercise-associated physiological stress can be obtained (24, 45). In fact, the free testosterone to cortisol ratio is used in assessing metabolism in relation to anabolic-catabolic balance (24). Another non-invasive biomarker used in assessing exercise stress and the sympathetic-adrenal-medullary system is salivary α -amylase enzyme activity (2, 3, 8, 9, 23).

The salivary components do not all vary in concert, and various factors such as salivary flow rate and time of day (diurnal course) have different effects on them. The salivary flow rate reaches its peak in the afternoon and declines to its lowest level during the sleep (36). The highest testosterone and cortisol concentrations occur at the beginning of the day and reach their lowest levels in the afternoon and late at night (31, 43). In contrast, the lowest levels of amylase and total protein are found in the morning, and the highest levels are found in the afternoon (31, 36).

It is established that there is a significant relationship between amylase, catecholamine, and the anaerobic threshold (8). This relationship provides an impetus to investigate α amylase activity. Because cortisol has been accepted as an exercise-induced physiological stress biomarker, it could be useful to study the relationship between cortisol response and α -amylase activity.

Although there have been various studies on the responses of cortisol, testosterone, and α -amylase to exercise, there are limited data available regarding their patterns of response to different exercise modes. Additionally, while these biochemical factors have been studied as exercise-related physiological stress biomarkers, each has a unique response to physical exercise due to their different origins.

With these limitations in mind, this study investigated the effects of intensity, duration, diurnal course, and exercise mode. In addition, few studies have investigated the hormonal response to exercise on an elliptical instrument. Therefore, the present study sought to investigate the effects of intensity and exercise mode on the acute responses of the selected biomarkers.

METHODS

Participants

The participants were ten young active male volunteers with three regular exercise sessions per week. They were considered to be in healthy condition based on medical screenings (table 1). We used a standard treadmill test with the Bruce protocol to measure the aerobic capacity of the participants. Prior to the Bruce test

performance, volunteers participated in a 5-min warm up program that included a 4-min run at 6 Km/h on a treadmill with a 3% gradient followed by a 1 min run of 8 Km/h with the same slope.

Table 1: Characteristics of the participants including anthropometric data and VO₂ max measurements.

3.69±24	Age (year)
6.12±178.7	Height (cm)
9.59±72.5	Weight (kg)
1.88±22.62	BMI (kg/m ²)
4.86±47	VO ₂ (ml/kg/min)

Protocol

The participants exercised in six single sessions on an elliptical instrument (Technogym Wellness in motion, Italy), a cycle ergo meter (Technogym, Bike Race HC 600, Italy) and a treadmill (Technogym run race HC 1200, Italy) at 70% and 85% of maximum heart rate for 25 min (table 2). To remove the effect of the previous exercise session, participants rested for a period of 48 hrs during which they avoided any heavy physical activities. All the sessions were held between 2 pm and 4 pm to limit any time of day effects (diurnal variations) on the variables. To determine the maximum heart rate of the participants, the equation from Tanaka et al. (2001) was used [208- (0.7×age)] (41). During the exercise sessions, the participants maintained the volume of exercise at the estimated HR range for each person.

Unstimulated whole saliva was collected to measure the concentration of cortisol, testosterone, and total protein as well as α-amylase activity collected using the passive drooling method 5 min before and 5 min after each exercise session.

Table 2: Heart rate (beat/min) of the participants for each session (mean ± standard deviation).

Sessions	Heart rate (beat/min)
Exercise on elliptical instrument at 70% maximum heart rate	134.5±3.89
Exercise on cycle ergometer at 70% maximum heart rate	138.6±4.07
Exercise on treadmill at 70% maximum heart rate	133.7±2.17
Exercise on elliptical instrument at 85% maximum heart rate	158.9±4.46
Exercise on cycle ergometer at 85% maximum heart rate	163.0±2.27
Exercise on treadmill at 85% maximum heart rate	159.7±3.98

The participants were asked to rinse their mouth 5 min before sampling to remove residue. They gradually drank 500 ml water 2 h before the first sampling (pre-test) to maintain normal hydration. The samples were stored at 4°C and were sent to the laboratory in less than 2 h.

DIAMETRA kits were used to assay cortisol, testosterone, and amylase. The competitive immune enzymatic colorimetric method was used for assaying salivary cortisol and testosterone concentrations. The kinetic colorimetric method was used to measure amylase activity. The inter-assay coefficients of variability for cortisol, testosterone, and amylase measurement were calculated to be 7%, 5.5% and less than 1.5%, respectively. The intra-assay coefficients of variability for cortisol, testosterone, and amylase were calculated to be 9.5%, 9.3% and less than 1.5%, respectively. Total protein was measured by Bradford method using Coomassie blue G 250 dye. An ELISA reader (Stat Fax model 2100 Awareness Technology, U.S.A.) was used for all tests. In 4 out of 120 samples, the testosterone and total protein concentration could not be detected.

Statistical Analysis

All data were found to be normally distributed using a Kolmogorov-Smirnov test for normality; therefore, the analysis was carried out using parametric statistical tests. Differences between the mean values before and after each exercise session were tested by paired samples t-tests. To examine the effect of intensity and exercise mode, the pre-test and post-test levels were measured for each variable and were analysed by repeated measures analysis of variance (ANOVA). Sphericity of the data was confirmed by performing variance analysis (Mauchly's Test of Sphericity). In cases where significant changes were observed, we determined pairwise differences using a Bonferoni correction. In addition to the significance level of ANOVAs, we present the observed power and effect size. To determine the relationships between variables, Pearson's correlation test was used. A level of P less than 0.05 was considered significant and the SPSS 18 (PASW Statistics 18) computer program was used for statistical analysis.

RESULTS

The cortisol concentration decreased in treadmill sessions and increased in other sessions (elliptical and cycle ergo meter). However, no significant changes were observed for cortisol (table 3). Testosterone increased at all sessions except for exercise on the cycle ergo meter at an intensity of 70% maximum heart rate (table 3). Testosterone levels were significantly increased on the elliptical instrument ($t=0.07$, $P=0.02$) and the treadmill ($t=0.55$, $P=0.03$) at the intensity of 85% maximum heart rate.

The free testosterone to cortisol ratio increased in almost all cases. These changes

were significant after exercise on a treadmill at an intensity of 70% maximum heart rate ($t=2.94$, $P=0.02$) and 85% maximum heart rate ($t=0.53$, $P=0.03$). There was a significant difference in pre-test level of free testosterone to cortisol ratio among exercise treatments ($F_{5, 45}=3.14$, $P=0.04$, effect size=0.22, observed power=0.75). Please see table 4. The post-test free testosterone to cortisol ratio also varied significantly among exercise sessions ($F_{5, 45}=3.14$, $P=0.02$, effect size=0.31, observed power=0.82). Please see table 4.

Table 3: The change in pre- and post-test concentration of selected biomarkers. *Indicates a significant difference.

		Elliptical instrument 70% maximum heart rate	Cycle ergo meter 70% maximum heart rate	Treadmill 70% maximum heart rate	Elliptical instrument 85% maximum heart rate	Cycle ergo meter 85% maximum heart rate	Treadmill 85% maximum heart rate
Cortisol (nmol/l)	Pre	13.71±4.24	11.86±6.47	11.86±4.83	9.66±4.52	12.80±5.26	11.81±6.6
	Post	15.75±7.68	13.49±8.12	9.93±4.26	11.04±7.73	13.27±6.95	10.07±6.53
Testosterone (nmol/l)	Pre	0.60±0.3	0.89±0.65	0.73±0.25	0.87±0.56	0.89±0.54	0.5±0.37
	Post	0.99±0.38	0.76±0.23	0.90±0.51	1.07±0.33*	1.19±0.51	0.87±0.68*
Free testosterone to cortisol ratio (nmol/l)	Pre	0.049±0.02	0.07±0.03	0.06±0.02	0.1±0.054	0.09±0.03	0.04±0.02
	Post	0.068±0.02	0.074±0.02	0.09±0.04*	0.17±0.13	0.1±0.05	0.09±0.06*
α-amylase (U/ml)	Pre	36.31±23.82	30.77±23.01	39.4±23.32	34.2±20.53	18.2±21.37	29.74±22.07
	Post	29.65±24.75	24.31±22.03	31.55±21.09	19.53±15.91	26.45±19.56	4.1±3.41*
Total protein (mg/dl)	Pre	68.63±20.81	68.81±17.21	69.14±16.95	58.58±19.08	58.74±14.46	53.97±24.06
	Post	90.8±22.75*	79.54±16.03	74.89±26.58	82.51±44.81	73.33±18.87*	79.28±13.33*

Total protein showed more sensitivity to exercise in that an increase in total protein concentration was noticed in all exercise sessions. Total protein levels noticeably increased in response to exercise on the elliptical instrument ($t=2.84$, $p=0.02$) at an intensity of 70% maximum heart rate and on the cycle ergo meter ($t=3.55$, $p=0.006$)

and on the treadmill ($t=3.92, p=0.006$) at an intensity of 85% maximum heart rate.

Table 4. The results of repeated measures analysis of variance (ANOVA) with the effect size and observed power. *indicates significant differences.

		F	P	Effect size	Observed power
Cortisol (nmol/l)	pre	1.15	0.34	0.11	0.37
	post	2.15	0.07	0.19	0.65
Testosterone (nmol/l)	pre	2.02	0.93	0.18	0.62
	post	1.40	0.24	0.16	0.43
Free testosterone to cortisol ratio (nmol/l)	pre	3.14	0.03*	0.22	0.75
	post	3.14	0.01*	0.31	0.82
α-amylase (U/ml)	pre	1.48	0.21	0.14	0.47
	post	3.97	0.005*	0.30	0.92
Total protein (mg/dl)	pre	1.63	0.17	0.15	0.51
	post	0.99	0.43	0.14	0.30

Amylase activity after exercise on the elliptical instrument ($t=2.38, P=0.04$) and on the treadmill ($t=3.55, P=0.006$) at an intensity of 85% maximum heart rate showed significant declines (table 3). Amylase activity increased (non-significantly) only in the case of cycle ergo meter exercise at an intensity of 85% maximum heart rate (table 3). Amylase varied significantly in response to the exercise mode and intensity ($F_{5, 45} = 3.97, P=0.005, effect\ size=0.306, observed\ power=0.92$) please see table 4.. In response to exercise on the treadmill at an intensity of 85% maximum heart rate, amylase was significantly lower than that of the cycle ergo meter at an intensity of 85% maximum heart rate ($t=3.66, P=0.005$), on the treadmill at an intensity of 70% maximum heart rate ($t=3.96, P=0.003$) and on the elliptical instrument at an intensity of 85% maximum heart rate ($t=3.15, P=0.01$).

No significant relationship was found between cortisol and amylase. However, in the majority of the cases they were inversely related. Amylase declined in 5 sessions, and cortisol increased in 4 sessions. Two significant inverse relationships were observed between salivary total protein and amylase. Exercise on the cycle ergo meter at an intensity of 70% maximum heart rate and on the elliptical instrument at an intensity of 85% maximum heart rate increased total protein and decreased amylase.

DISCUSSION

Cortisol

It has been reported that the response of cortisol to exercise is affected by the intensity (7) and duration (6) of physical activity, exercise state (such as competition; 21), psychological stimuli (10), and time of day (31). The findings of the present study are in accordance with studies indicating a lack of significant changes (38) or declines (4, 22, 26, 33) in cortisol in response to physical activity. However, in many studies, the cortisol concentration does increase with sub-maximal exercise (17).

Because intensity is a major factor governing the response of cortisol to exercise (7, 18), the recruitment of active young males that regularly engage in exercise may have been important in causing the lack of significant changes. It has been reported that cortisol response in elite athletes is less sensitive (21). In contrast, some believe that the hypertrophy of the adrenal glands in elite athletes may lead to an increase of cortisol response (44).

On the other hand, because time is also a determining factor in cortisol secretion (6),

cortisol might increase during exercise and then later decrease. Considering the 25-min duration of exercise in this study, the lack of significant changes to cortisol may be explained by the short duration of exercise.

Some reports also indicate that states like competitive stress (21, 29) and anticipatory stress (19) lead to an increase in cortisol secretion. These findings suggest that cortisol is a main factor during exercise and is related to physiological stress. Exercise in a laboratory setting, as in this study, is essentially independent from factors such as competitive or anticipatory stress.

The responses of cortisol to physiological stress might be different from that of psychological stress. The peak salivary cortisol concentration was observed 2 hrs after exercise (18), whereas the cortisol concentration continues increasing until 24 hrs after remembering a sad event (10). Therefore, the findings of the present study are limited because of infrequent measurement of cortisol.

The time of exercise in the present study (2 pm to 4 pm) is when cortisol concentration starts declining (31). Therefore, the somewhat non-significant increase of cortisol level in this study should not be neglected. In contrast, it has been reported that the exercise method influences the cortisol response but that exercise in daytime has no effect on the cortisol response (4). These contradictory results show that further purposive studies are needed to determine the effects of exercise mode and intensity on cortisol response.

Testosterone and free testosterone to cortisol ratio

The findings of the present study indicate that the free testosterone to cortisol ratio is affected by the exercise mode. Therefore, exercise at intensities of 70% and 85% maximum heart rate may not lead to anabolic-catabolic disorder. The main factor contributing to the increase in free testosterone to cortisol ratio in all sessions was the increase of testosterone. A similar increase was reported in other studies (45). One report indicated that intense exercise leads to an acute increase in testosterone that declines one to six hrs after exercise (5). A boost in salivary T was also observed in the present study. The results of the study confirm the hypothesis that the free testosterone to cortisol ratio is affected by exercise intensity (7, 42, 45).

The pre-test findings showed that although the free testosterone to cortisol ratio before exercise on the elliptical instrument at 70% maximum heart rate was similar to the ratio before exercise on a treadmill at 85% maximum heart rate, the increase of free testosterone to cortisol ratio during the treadmill exercise session was significant. These data indicate that free testosterone to cortisol ratio is affected by exercise intensity and exercise mode.

This is likely due to the selection of sub-maximal exercise intensities and short duration of exercise, which leads to the increase of anabolic processes or prevents activation of catabolic processes. These findings suggest that applying muscular strength with moderate intensity weight-bearing exercises such as treadmill and elliptical instrument in comparison to cycle ergometer not having a weight bearing character activates anabolic pathways. Evidence for this phenomenon lies in the increase of testosterone during exercise on

the treadmill and elliptical instrument at 85% maximum heart rate.

Total protein and amylase

Amylase has different responses to exercise. The main factor driving these differences was the comparatively low level of amylase response to exercise on the treadmill at 85% maximum heart rate.

It has been reported that amylase is the main protein contributing to the measurement of total protein (14). This study showed that an increase in total protein was associated with decreased amylase activity, which indicates an inverse relationship, perhaps due to sub-maximal exercise intensities (8). Regarding the contradiction between the results of the present study with previous results, it is worth mentioning that an amylase response is not always obvious in saliva because the measurement is affected by both the secreted amylase (sympathetic response) and the amylase stored in the salivary gland granules (2).

The likely reason for the inverse relationship is that the total protein secretion pattern is not affected by intensity or stimulation period, and this pattern is different in each salivary gland (35). An immediate reduction of total protein was observed after stimulation in the parotid gland, whereas an immediate increase was observed after stimulation in the sub-maxillary gland (12, 13).

The effects of duration and intensity of stimulation on the response to salivary protein were previously studied (35). One reason for the difference in findings between the present study and other works is the use of an intensity profile and sub-

maximal duration (4), which is increased by the sympathetic-adrenal-medullary response when exercise is performed near the anaerobic threshold (8). In contrast, it is reported that amylase activity was induced after a short period of exercise of 20 min at 50% VO_2 max (1).

The majority of studies emphasise intensity more than duration of exercise to explain the amylase response. One study stated that amylase initially declined in response to incremental exercise and later increased (8). This acute decline was also noticed in our previous study, in which we found an acute decline of amylase 5 min after a football competition ended and an increase 30 min later (3).

It has been reported that the effects of exercise likely lead to an increase in catecholamine (4). During exercise, sympathetic-adrenal-medullary stimulation is sufficient to reduce saliva secretion. It has been suggested that physical activities result in a reduction of saliva flow rate (39). Amylase activity is under the influence of the β -receptors, and consequently, a continuous increase in epinephrine can have an inhibitory effect on salivary secretion and leads to a cottonmouth appearance. In contrast, some reports indicate that amylase activity is not affected by the saliva flow rate (37). Thus, this study may be limited by not having measured the saliva flow rate and catecholamine levels.

Relationship between cortisol and amylase

A non-significant inverse relationship between amylase and cortisol was observed. In most of the exercise sessions, the cortisol and amylase responses were inversely proportional. This result confirms those of previous studies (20, 23). Previous

reports indicate a relationship between cortisol and amylase (9). The variation among studies in the responses of amylase and cortisol is probably due to the fact that the amylase response to psychological stress is acute (20, 36).

Cortisol is secreted from hormonal glands, and via the circulation, it diffuses to the saliva. However, amylase is secreted from the epithelial acinar cells of the salivary gland itself. It has also been found that the amylase secreted during sympathetic activity also includes the amylase stored in the parotid gland granules (2). Also in contrast to cortisol, the amylase level is at its lowest level in the morning and highest in the afternoon (31, 34). This effect may also have contributed to the non-significant relationship between the two biomarkers under investigation.

The findings of the present study suggest that the response of exercise biomarkers to the different exercise modes at two sub-maximal intensities was not the same; the free testosterone to cortisol ratio was dependent on the exercise mode, whereas the amylase response was dependent on the intensity of exercise. The increase in the free testosterone to cortisol ratio could indicate lower physiological stress in response to performing the exercise protocol in this study. Therefore, exercise at intensities of 70% and 85% maximum heart rate is safe with regard to health maintenance.

Applying muscular strength with moderate intensity in weight-bearing exercises probably activated the anabolic pathways. Therefore, exercise at 70% and 85% maximum heart rate may not lead to anabolic-catabolic disorder. While in the majority of the exercise sessions, cortisol

and amylase changed in opposite directions, no significant inverse relationship was observed between them. These findings also confirm that physical exercises can lead to salivary compositional variation, particularly of salivary steroids.

The results of this study indicate that people, especially healthy young active males, could perform these exercises to maintain health. In addition, athletes can use these exercises in their post-training recovery periods.

Considering the development of various popular exercises and exercise machines, it is recommended that other exercise modes such as the use of a rowing ergometer, swimming, and military training should be considered. In addition to controlling exercise intensity, duration, and mode, future studies should also monitor the instruments' resistance, calorie consumption, and distance covered. In future studies, it is also recommended to use frequent sampling or to evaluate salivary flow rate, blood lactate, and catecholamine levels.

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