IJBPAS, August, 2014, 3(8): 1863-1876

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE EFFECTS OF DIFFERENT INTENSITIES OF AEROBIC ACTIVITIES ON THE LEVELS OF SALIVARY CORTISOL, TESTOSTERONE AND TESTOSTERONE TO CORTISOL RATIO AMONG ACTIVE YOUNG MEN

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ABSTRACT

The purpose of the present study was comparison of different intensities of sub-maximal aerobic activities on responses of salivary cortisol and testosterone hormones, among active young men. Eight volunteer active young men with average age of 23.33±1.56 years old, height of 176±1.76 cm and weight of 67.16±3.14 kg participated in 3 aerobic activity sessions, contained of running on treadmill in duration of 25 min and with three intensities of 70, 80 and 90 percents of maximal heartbeat. Salivary samples collecting was carrying out before and immediately then the activity sessions, and cortisol and testosterone hormones and testosterone to cortisol ratio were measured and calculated, for each sample. In order to compare and investigate changes of variables, factor analysis of variance test with repeated measurements was used. Salivary testosterone to cortisol ratio increased with increment of activity intensity, but this enhancement wasn't statistically significant (P>0.005). It seems, there isn't any significant difference in responses of anabolic to catabolic equilibrium of bodies of active young men, between the three mentioned sub-maximal aerobic exercises. However, short span of the exercises and being active situations of the subjects could be some probable reasons of observing insignificant differences, between those three intensities. So, in order to clarify the facts, it's necessary to accomplish further researches.

Keywords: Cortisol, Testosterone, Aerobic Activity, Intensity of Activity, Testosterone to Cortisol Ratio, Saliva

INTRODUCTION

The worthwhile hand of sport about fitness maintenance and spending leisure isn't hidden to anyone. The sub-maximal aerobic activities are the ones that used by people of the society to improve the physical readiness and especially the cardiovascular system. Sedentary groups of the humankind society should follow and perform this type of exercise, to improve quality of life and make a healthy and airily living. In the other hand, axis of hypothalamus-hypophyse-adrenal and also hypothalamus-hypophyse-testicle are affected by physical activity. Survey of these influences, in companion of introducing effective parameters, could be useful for the athletes and coaches **[1]**.

During sport, the body confronts many needs, which would cause plenty of physiologic changes. Homeostase must be kept constant, for survival. Whatever intensity of activity rises, homeostase preserving will be more difficult. The hormones always control internal environment of the body. They record entire changes and response to them, rapidly, to confide the homeostase wouldn't expose a severe disruption. The metabolic hormones [anabolic and catabolic] have been rather paid attention, among the hormones. If catabolic hormones increase following the activity, it's the sign of exceeded pressure of sport, and whether there's a balance between anabolic and catabolic hormones, it means the athlete has a desirable readiness or sport has either a moderate or downward intensity **[2]**.

In this relation, testosterone has been propounded as an anabolic hormone, and cortisol is propounded as the most important catabolic one. The ratio of these two mentioned hormones to each other is a very handful index for determining person's readiness situation **[2]**. Testosterone is a steroid hormone and has anabolic influences on muscle tissue **[3]**. Also, cortisol is the most important humankind glucocorticoid and the best-known hormone with catabolic influences **[4]**.

For the first time, Adlercreutz et al (1986) proposed testosterone to cortisol ratio as a diagnostic tool and an index of activity and exercise pressure **[2]**. Accurate pattern of the response of this ratio to physical activity isn't clear-cut, and decrease **[2]**, increase **[5]** and nonbeing variation **[6]** of this index following activity and exercise have been reported. One sport session might cause temporary changes in equilibrium of anabolic and catabolic processes. The variations in this balance are dependent to intensity and span of exercise. Many changes in testosterone and cortisol

levels have been observed, under physical pressure conditions **[7, 8, 9]**.

The physical activity is a strong stimulant of sympathetic nervous system, and generally automatic nervous system. Studies show that cortisol secretion increases with increment of exercise intensity. Rudolph et al (1998) reported increment of cortisol concentration following an activity with 60 percent of maximal consuming oxygen. The relation of cortisol with sub-maximal intensities of exercises hasn't been cleared, well. Though, few researches have reported a significant correlation between cortisol and sub-maximal intensities **[11]**.

Doan et al (2007) stated cortisol had significant increases, even in response to sub-maximal activities **[12]**. Rudolph et al (1998) reported increases in cortisol concentrations of 13 runners, who ran on treadmill in span of 30 min and with intensity of 60 percent of maximal consuming oxygen **[10]**. Tremblay et al (2003) evaluated testosterone and cortisol, in rest and the recovery after 40 min running with 50 to 55 percent of maximal consuming oxygen **[13]**. They observed slight variations in hormones concentrations, which weren't statistically significant **[13]**. Moya-Albiol et al (2003) reported similar results, too **[14]**.

Though, Budde et al (2010) declared; a short–term (but longer than 15 min) adequate working load of 70 to 85 maximal heartbeat would increase post-exercise cortisol levels of adult and youngster subjects **[15]**. They showed cortisol linearly increases with increment of exercise intensity **[15]**. Indeed, Budde et al (2010) propose threshold intensity of cortisol increment as 50 percent of maximal consuming oxygen **[15]**. Galbo et al (1977) reported remarkable increment of testosterone concentration after last step of a progressive sport activity **[16]**. Reduction of testosterone concentration has been observed among 45 healthy men, who performed activities such as running, rowing and bicycling, in duration of 60 to 90 min and with intensities of 65 to 75 percent of maximal consuming oxygen **[17]**.

Jezova et al (1985) stated testosterone concentration hadn't any increase in moderate intermittent activities **[18]**. Bosco (1996) reported that intense sport activity would lead to immediate increment of testosterone, and then its reduction from 30 min to 6 hr after the exercise **[19]**. Both decrease **[20, 21]** and increase **[22]** in testosterone have been reported, following continuous and enduring activities. Also, there aren't many researches about salivary levels of these mentioned hormones. However, because of inoffensive

attribute of salivary sample gathering, this method could be rather applicable.

The aim of the present research is comparison of the effects of different intensities of sub-maximal aerobic intensities on salivary levels of testosterone, cortisol and testosterone to cortisol ratio, among active young men.

SUBJECTS AND METHODS

Subjects

After invitation announcements in universities of Tehran city and explanation of the targets, ten young male students are purposefully and in access chosen, among 23 volunteers of participating in this study. During the research, three people of the chosen students withdrew their participation, and 7 persons, with average age of 23.33±1.56, height of 176±1.76 cm, weight of 67.16±3.14 kg, maximal consuming oxygen of 48.6±3.96 [ml per kg body weight per min], BMI of 21.6±0.91 kg/m^3 and relaxation heartbeat of 68.55±3.74 beat/min, remained to the end of the research. They have had 2 weekly regular physical exercise sessions, at least, and hadn't any decease precedent. Also, they didn't take any medicine for medical purposes, during the research. It should be mentioned the selected subjects received necessary information about the methods of the research execution, and they signed the consent forms.

Exercise Program

Five days before the research protocol, aerobic power of the subjects was measured using Bruce test on treadmill **[23]**. Then, they held the first exercise session. Three sessions of aerobic exercise consisted of 25 min running on treadmill with three different intensities. These three exercise sessions were interfered with 48-hour rest periods. In order to avoid misleading results, caused by the effects of exercise sessions on each other, the order of exercise sessions was chosen on a random basis. Each participant attended 30 minutes of running on treadmill at 70, 80, and 90 percents of maximal heartbeat. Maximal heartbeat has been calculated using the equation of *208-(0.7×ages)* **[24]**. During the study, the subjects were kept in a house prepared for them, and they were asked to rest and avoid any miscellaneous physical activity.

It should be noticed that the subjects were requested to avoid consumption of caffeine and alcohol at the nights before samples collecting, and generally during the stages of the research. In order to neutralize the circadian effect, entire steps of samples collecting have been carried out in the same conditions, for the whole subjects. Indeed, each person started and finished his entire activity sessions at particular moments, which were the same for his whole activity sessions.

Salivary Samples Collecting and Hormonal Analysis

Salivary samples were taken into specific containers, in three steps of before, immediately then and 1 hr after the activity. The gathered samples were preserved in frozen forms and at -20℃, until arriving to the lab. And there, laboratory examination began, immediately. Testosterone and cortisol hormones and testosterone to cortisol ratio were measured and calculated, for each sample. In order to measure testosterone and cortisol levels by ELIZA method, de medi tec kits were utilized, with sensitivity amounts of 2.2 (pg/ml) and 0.014 (ng/ml), respectively. After unit conversion of hormones amounts to nanomole per liter [nmol/l], testosterone to cortisol ratio was calculated, too. To convert testosterone and cortisol units, two formulas of *testosterone×3.48* and *cortisol×2759* were implemented, respectively **[24]**.

Statistical Approach

In order to describe the data, mean and standard deviation were applied. Kolmogorov-Smirnov test was implemented, to determine the type of statistical method (either parametric or non-parametric), and it has been cleared that the data had natural distributions. So, factor analysis of variance with repeated measurements was used, to compare changes of under study variables at two times of samples collecting, between the three activity sessions with 70, 80 and 90 percents of maximal heartbeat. Before using the variance analysis, data sphericity was investigated, to perform Greenhouse-Giggs modification approach on degree of freedom, in necessary cases. Significance level was considered as $P \leq 0.05$, for entire statistical test. Also, the statistical software SPSS v.16 was utilized for performing the statistical tests.

RESULTS

Statistical descriptions of salivary cortisol, testosterone and testosterone to cortisol ratio have been presented in table 1. The values have been reported as mean and standard deviation. Also, to compare changes of the variables between the three activity sessions with three different intensities, the results of factor variance analysis test with repeated measurements have been represented in tables 2, 3 and 4. Time operation, session operation, and time and session cooperation were insignificant (P was 0.463, 0.752, and 0.115, respectively), about cortisol. Generally, salivary cortisol had a little increase in activities with 70 and 80 percents of maximal heartbeat intensities, and a little decrease in activity with 90 percent of maximal heartbeat intensity, though these variations weren't significant ($P>0.05$). Time operation, session

operation, and time and session cooperation were insignificant (P was 0.308, 0.951, and 0.172, respectively), about testosterone.

Overall, salivary testosterone decreased in activity with 70 percent of maximal heartbeat and increased in activities with 80 and 90 percents of maximal heartbeat, but these changes weren't significant (P>0.05). About testosterone to cortisol ratio, time operation, session operation, and time and session operation were insignificant (P was 0.168, 0.348, and 0.110, respectively). Generally, salivary testosterone to cortisol ratio decreased in the activity with 70 percent of maximal heartbeat and increased in the activities with 80 and 90 percents of maximal heartbeat, though these changes weren't significant (P>0.05). In a rather overall statement, testosterone to cortisol ratio increased versus increment of exercise intensity, but this increase wasn't statistically significant (P>0.05).

Table 1: Statistical Descriptions of Salivary Cortisol, Testosterone and Testosterone to Cortisol Ratio

Variables	Session	Pre	Post
Cortisol]ng/ml[	70% MHR	5.191±10.057	5.345±0.950
	80% MHR	4.988±1.196	5.316±1.468
	90% MHR	5.438±1.427	4.277±1.755
Testosterone [pg/ml[	70% MHR	92.625±3.248	87.750±13.719
	80% MHR	86±13.617	92.625±13.968
	90% MHR	87.375±6.435	94.50±17.113
Testosterone to Cortisol Ratio	70% MHR	0.0229±0.0049	0.0208±0.0048
	80% MHR	0.0228±0.0075	0.0241±0.0105
	90% MHR	0.0209±0.0059	0.0360±0.0258

Table 2: Statistical Results of Factor Variance Analysis Test with Repeated Measurement to Compare Changes of Salivary Cortisol in the Three Sessions

Factor	Sum of Square	df	Mean Square	F	P	Effect Size	Observed Power
Time	0.617	1	0.617	0.559	0.463	0.026	0.110
Session	1.430	2	0.715	0.289	0.752	0.027	0.090
Time* Session	5.301	2	2.651	2.405	0.115	0.186	0.431

Table 3: Statistical Results of Factor Variance Analysis Test with Repeated Measurement to Compare Changes of Salivary Testosterone in The Three Sessions

Factor	Sum of Square	df	Mean Square	F	P	Effect Size	Observed Power
Time	105.021	1	105.021	1.091	0.308	0.049	0.169
Session	21.167	2	10.583	0.051	0.951	0.005	0.057
Time* Session	368.67	2	184.333	1.916	0.172	0.154	0.352

Table 4: Statistical Results of Factor Variance Analysis Test with Repeated Measurement to Compare Changes of Salivary Testosterone to Cortisol Ratio in the Three Sessions

Factor	Sum of Square	df	Mean Square	F	P	Effect Size	Observed Power
Time	0.000	1	0.000	2.04	0.168	0.089	0.276
Session	0.000	2	0.000	1.109	0.348	0.096	0.218
Time* Session	0.001	2	0.000	2.462	0.110	0.190	0.440

DISCUSSION

According to the results of the present study, none of the activity sessions produced any significant change in salivary cortisol. In addition, there wasn't observed any significant difference in variations of cortisol, between the three sessions. Most researches, which have presented cortisol increment following activity, had borne rather high intensities. Usually, whenever intensity of activity exceeds a threshold, cortisol is going to increase, too. It's believed that cortisol would decrease, in lower intensities. And, it's thought the required intensity for cortisol stimulation is 60 percent of maximal consuming oxygen **[26]**.

If exercise intensity exceeds the threshold, the body has to summon free fat acids by decrease in glycogen of the muscles, which means the cortisol concentration would increase for decomposition of triacilglicerols and production of fat acids. It's natural that the recent process wouldn't occur, in exercises with low intensities and short spans. Perhaps, one of the reasons of nonbeing difference and the insignificant change of salivary cortisol in some activities is their short spans. Snegovskaya et al (1992) surveyed the cortisol response following long interval exercises, among men. In their research, the subjects performed four steps of 30 min exercises with 50 percent of maximal consuming oxygen. During the first two steps, cortisol concentration tended toward lower amounts, but it rose up during 3^{rd} and 4^{th} steps **[27]**.

In their study, the below threshold cortisol stimulation might be the probable reason about reduction and nonbeing change of cortisol concentration, at the two first steps. Maybe, because the aggregate activity span increased during the 3^{rd} and 4^{th} steps, this matter led to increase in the cortisol secretion stimulation. So, activity span is a determent factor about cortisol secretion, too **[28]**. Intense physical activity would cause stimulation of hypothalamus-hypophyse-adrenal axis and increment of the central temperature, and thereafter lead to increase in cortisol secretion stimulation and its releasing from the carrier proteins **[28, 29]**.

In this relation, it has been shown the exercise intensity is substantial about determination of the cortisol response **[30]**. Whenever the exercise intensity is at the amount of 40 percent of maximal consuming oxygen, cortisol plasma would decrease, even more than one time. In the other hand, cortisol response is going to reverse versus increment of exercise intensity to 80 percent of maximal consuming oxygen and its plasma concentration will increase **[31]**. It has been

stated, whenever exercise intensity is going beyond 60 percent of maximal consuming oxygen, cortisol plasma will rise up, whereas cortisol concentration would decrease at below 60 percent intensities of maximal consuming oxygen **[30]**. However, some other founds have showed cortisol rising **[31, 32]**. A research indicated cortisol had significant increments, even in response to sub-maximal activities **[12]**.

Maybe, the recent issue is related to exercise situations of the subjects. Whether the subjects of the present study weren't active men, the results might probably be different. The exercise span is also a determent parameter about cortisol excretion **[33]**. Therefore, any increase in cortisol level, which appeared in research of Dan et al (2007) might be as a consequent of long-term duration of the activity. Cortisol is affected by intensity **[34]** and activity conditions. Even, an activity with low intensity may cause an increase in cortisol concentration, if is carried out in a long-term duration **[35]**. This recent note implies the influence of activity duration on cortisol secretion and could be a vindication of some different results in the literature. However, the cortisol response is generally dependent to intensity of activity **[30]**.

Based on the results of the present study, levels of salivary testosterone decreased at 70 percent of maximal heartbeat intensity, though it increased at 80 and 90 percents of maximal heartbeat intensities. However, these variations weren't significant. The salivary testosterone levels insignificantly increased versus increment of exercise intensity. Jensen et al (1991) reported testosterone significantly increased following enduring activity and came back to the basic levels after 2 hr, among men **[22]**. Both decrease **[20, 21]** and increase **[22]** in testosterone levels have been reported, following continuous activities. The differences in these results could be mainly because of the differences in activities masses and/or exercise situations of the subjects **[13]**. The intensity of activity is likely the most important influencing parameter. However, readiness levels of the subjects **[36]**, energy cost, work output, mass, intensity **[26]** and duration of exercise **[37]** probably associate with the observed differences of hormonal responses. Wilkerson et al (1980) didn't report any change in testosterone concentration, following 20 min running with various exercise intensities **[38]**. The exercise intensities might be a probable reason of the disagreement between the results. Sutton et al (1973) observed some responses to maximal exercises (but not to sub-maximal ones), in

androgen serums **[39]**. Kindermann et al (1982) reported 14 percent increase in testosterone plasma concentration, following sub-maximal anaerobic running tests, until reaching to exhaustion **[40]**.

Exercise-induced variations mechanisms of circulation testosterone haven't been completely cleared, yet. But, it has been proposed that the pressure makers would mainly lead to decrease in testosterone levels **[2]**. Catecholamine's levels and other influencing factors of the circulatory affect the amounts of testosterone secretion. The amounts of catecholamines would increase by performing physical activity and enduring exercise **[41]**. It has been stated that the amount of testosterone would increase, by the effect of increment of catecholamine's in animals' blood (without any observation of LH rising) **[42]**. Catecholamine's measurement, beside salivary testosterone gauging that is the indicator of free testosterone levels, could aid the superior understanding of exercise-induced testosterone variations, in future researches. Since, the catecholamine's weren't measured; the recent issue couldn't be discussed with confidence. It seems, the sympathetic system could independently affect [separated from hypothalamus-hypophyse-testicle axis] the production and secretion of testosterone, during the activity **[41]**. Also, it seems the circadian measurements are necessary to realize post-exercise hormonal response, in a superior way.

According to the results of the present study, the salivary testosterone to cortisol ratio would decrease versus 70 percent of maximal heartbeat, whereas this ratio would increase versus 80 and 90 percents of maximal heartbeat. However, these changes weren't significant. Generally, the testosterone to cortisol ratio increased insignificantly, with increment of exercise intensity. Perhaps, the reason of insignificance of these changes was because of short span of the activity and/or being active situations of the subjects. The balance between anabolic and catabolic processes is explained by the testosterone to cortisol ratio, and this ratio has been recommended as an important index of activity and exercise pressure **[2]**.

It has been reported, testosterone and cortisol concentrations are influenced by intensity of activity **[43]**. Some other studies have shown impressionability of the mentioned ratio by intensity of activity **[44, 45]**. In a review article, which was written by Viru (1992), threshold intensity and exercise span have been called as the influencing factors on hormones reactions, consist of testosterone and cortisol reactions to the exercise. This

researcher stated, hormonal reaction depends to various parameters. However, threshold intensity has a substantial importance to stimulate under study hormones, and after that, the exercise duration would take priority. So, being significance or insignificance of the differences of hormonal reactions to the activities, which induced from various exercise intensities and spans, could be propose as the dependent variables **[11]**.

Increment of testosterone to cortisol ratio, during post-exercise recovery period, indicates adequate rest following that the very exercise **[46]**. Inadequate recovery period would lead to the lack of full reconstruction of body energy reserves, failure to establish cellular homeostase, early and acute fatigue, and reduction of the activity threshold **[5, 46]**. Therefore, it's suggested to consider rather specific solutions for the recovery period, when testosterone to cortisol ratio decreases following the activity. Nevertheless, the recent matter has been noticed in the study literature by researchers, though it seems further investigations are required, about effects of different intensities of sub-maximal aerobic activities on responses of anabolic and catabolic hormones and their ratio, to achieve determined results.

CONCLUSION

Regarding to the fact that the activity span of exercise of the present research was 25 min, it appears; although the body response would become further anabolic by raising the activity intensity and increasing it from 80 to 90 percent of maximal heartbeat, but this response wasn't significant. However, short span of the activity and being active situations of the subjects could be some of probable reasons of observing insignificant differences between those three intensities. Hence, accomplishment of further researches is required, to clarify the truth.

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