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The effect of one session Concurrent training on biochemical and hormonal composition and salivary flow rate in male student-athletes

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ABSTRACT

Saliva is one of the body's biological fluids, composed of proteins, glycoproteins, electrolytes and small organic molecules. The identification and the study of structural and functional changes of it is important for the health of athletes. The present study investigated the effect of one Concurrent training session (concurrent strength and endurance) on flow rate and biochemical composition of saliva (sodium, calcium, magnesium, potassium, phosphate, urea and total protein) and salivary hormone Cortisol and DHEA. In this study, 20 male athletes of Qaemshahr free University (mean aged 2 ± 23 years) participated for 100 minutes in Concurrent exercises. Participants' Diet and activities were controlled, 48 hours before and 24 hours after the race and non-stimulated salivary samples were collected for measurement in three time stages, before, immediately and 24 hours after the race. To analyze the data, Mauchly test, repeated ANOVA test, post hoc tests and SPSS16 software (significant level $P > 0/05$) were used. Findings showed that one session Concurrent exercise caused a significant decrease in concentration of electrolytes such as Sodium, calcium, magnesium, salivary flow rate and a significant increase in such electrolytes as potassium, total protein following the exercise. On the other hand the amount of phosphate and salivary urea has increased following exercises, but not statistically significant. Cortisol and DHEA levels have also a significant increase and decrease simultaneously after the activity ($P > 0/05$). 24 hours after exercise, except the amount of salivary flow rate, cortisol and DHEA, all electrolytes were partially returned to baseline. According to the results of this study, it can be said that physical activity, suppresses hormone production and Hydration may result into the reduction of salivary flow and undesirable changes in salivary electrolytes and provide the base for suppression of the mucosal immune system of athletes.

Keywords: salivary flow rate, biochemical composition of saliva, training Concurrent, DHEA

INTRODUCTION

Saliva, a heterogeneous fluid composed of proteins, glycoproteins, electrolytes, small organic molecules and blood transferred compounds, and plays an Important role in maintaining the immune system of upper respiratory tract, that any changes in its quantity and quality can have a significant effect on body health (1).Salvia is one of the biological fluids which is noninvasive and easily accessible, and can be considered in early detection of a wide range

of diseases. But unfortunately, like other biological samples such as blood plasma has been not commonly used for diseases diagnosis. Studies have shown that exercises may cause changes in the amount and activity of salivary proteins, hormones, and enzymes, so understanding the molecular and biochemical features of saliva contents and the study of structural and functional changes in its composition due to various factors, including physical activity can be very important and be applied in the prevention, diagnosis and treatment of various diseases of the respiratory tract(3).

Salivation in human is done in two ways, one is non-stimulated saliva and the other is stimulated saliva. In a healthy person non-stimulated salivation is between 0/1 to 0/5 ml min with a mean of 0/32, and stimulated salivation will be 1/1 to 3 ml per minute with a mean of 1/7ml. Meanwhile non-stimulated saliva has significant effects on the health of the immune system and generally is more important than the stimulated salivary (4).

In general, the base and principles of body health and best performance of body functions and the operation of various body systems including respiratory, heart, nerves, etc. depends on the transport systems i.e., fluids and electrolytes and endocrine changes. By exercising more than 3% of body water is lost and therefore many types of salivary electrolytes such as sodium, potassium, chloride, magnesium, etc. will be lost(5). Kyungall et al, in a study have reported a slight increase in serum magnesium levels in 7 minutes of vigorous physical activity (6). Rose also found that the short-term and heavy exercise increases the serum calcium concentration (7). In a research by Coester and colleagues, they have also found that after an exhaustive exercise serum sodium levels in participants will increase(8). Rovira et al., in an interesting study on dogs found that performing a 100-second agility training in dogs caused no significant changes in the concentrations of sodium and potassium ions(9).

On the other hand, researches have shown that as athletes do heavy and vigorous trainings such as training Concurrent (endurance and strength simultaneously) or bear high pressure to bear, they will experience hormonal changes, which are prominent in their Anabolic- Catabolic hormones. The balance between catabolic hormones including cortisol and Anabolic like DHEA¹ has an important application in the study of how to suppress the immune system, particularly the mucosal immune system (10,11). DHEA is one of adrenal specific hormones that can have anabolic effects on some issues through converting to sexual steroids such as testosterone and estrogen (10). DHEA and cortisol changes depend on the intensity and duration of exercise and training environmental conditions. Ratio of cortisol to DHEA (DHEA / C) is used as an indicator of training pressure in athletes. This ratio is influenced by the intensity and duration of exercise and any change in this ratio can be accompanied by potential changes in immune function (12, 13). Hosseini et al., in a study to assess the effects of Concurrent, strength exercises in different groups of non-athlete women showed that strength training caused no significant change in the levels of salivary cortisol but increased the levels of DHEA and ratio of DHEA to cortisol was considered as one of the body anabolic status indexes in participants the participants. While in parallel exercises (endurance training performed after resistance training) weaken the positive findings of strength training alone (10).

Abraham Pour et al., also conducted a study to investigate the effects of two sessions volleyball match on salivary DHEA and cortisol indexes in volleyballist women and found that competence in unprofessional volleyball have no effect on salivary DHEA levels while the changes (decrease) in Salivary cortisol was significant and tangible(14). Changes in DHEA concentration and nature of DHEA / C in response to exercise despite limited research that has been done in this area, is still unclear. Due to the variety of sports fields, training features, the specific responses of immune function to the type of trainings and lack of relevant researches, particularly in the areas of safety adaptations following Concurrent trainings which is a new type of training compared with endurance and strength new type of training, and also the cross-sectional nature of studies, different statistical samples and communities and various research methods, the need to do more research is necessary(15, 16, 17).

Totally the amount of each biochemical and hormonal compounds in saliva changes under the influence of physiological conditions including age, sex, diet, day and night cycles, salivary flow rate, hydration, exercise nature and of the subjects' initial fitness level. As no research has been done independently and directly on the impact of Concurrent trainings on saliva biochemical compounds and in regard to this fact that the balance of these electrolytes in the saliva is one of the main causes of mucosal immune homeostasis, therefore the present study investigated the effects of Concurrent training (endurance and strength simultaneously) on the flow rate and biochemical composition of saliva and also the changes in cortisol and DHEA hormones.

MATERIALS AND METHODS

Research participants:

According to the nature and objectives of the research, the present study is a quasi-experimental and applied research. The population consist of entire boy athlete students of Free University of Qaemshahr, among which 20 persons with an age range of 20 to 25 years were selected Voluntary and purposeful as subjects of the study. The subjects selection criteria was having a healthy heart - vascular, without any disease, no hormonal disturbances, no drug consumption, at least one month before the study is initiated.

Data Collection:

Following statistical sampling, the subjects participated in one session training Concurrent (aerobic and resistance) for 100 min. the first saliva sample was taken 15 minutes before the start of the practice in resting time, second one was immediately after exercise and the third one was collected 24 hours after exercise. And due to the need of collecting non-stimulation saliva, saliva samples collected from subjects after oral rinse for 4 minutes. The subject also avoided intense and heavy exercises for 48 hours before and 24 hours after the competition. According to training protocol diet in test day was 2500 kcal before the test.

Training Protocol:

Parallel group protocol 100 minutes includes: 10 minute warm-up (5 minutes running smoothly with moderate, 5 minutes of static stretching) after 20 minutes of running with 85 -80% of maximum heart rate (MHR), then 10 minutes of active recovery between strength and endurance exercise (5 minutes' walk, 5 minutes rest); followed by 50 minutes of strength training including movements, respectively (opening leg, chest press, back thigh, underarm stretch, triceps and biceps) in three sets of 6-8 repetitions at 85-90% of one repetition maximum (1RM), and finally a cooling step to 10 minutes (3 minutes walking, 7 minutes stretching) protocol is finished. Each set takes 30-60 seconds, the rest interval between each set is 90 seconds and rest interval between each period is 2 minutes. Stretching exercises include: bending and reaching hands to tip toes, stretching legs to the chest, pulling legs up the rear, turning waist to the sides, stretching hands, stretch the leg muscles, chest muscles tension, underarm muscle tension, arm front and back muscles tension.

Subjects Diet:

In order to match the nutritional status of subjects before test and its probable impact on some variables, the subjects were advised not to consume coffee, drug or smoke 12 hours before the first saliva sampling. Researcher controls the diet during tests days, which includes a simple breakfast (biscuits and juices with about 350 kcal) at 10:30 am and lunch (about 2500 kcal) 2/5 to 3 hours before the test.

Laboratory measurement methods:

To measure the amounts of sodium and potassium photometer film technique with (Corning 480) is used, calcium and urea were measured by CPC methods, Acetyl-D mono calcium, salivary magnesium and phosphate by Xylidyl blue, ammonium molybdate, with Pars test kits, and biochemistry methods respectively. Salivary cortisol and DHEA were measured by commercial kits Demeditec made in Germany using ELISA Model Start fax2100 made by Awareness Company. Salivary flow rate was also obtained by dividing saliva volume on sampling time.

Statistical Analysis:

To investigate the homogeneity of baseline data and evaluate the differences between the sample and population, Mauchly test is used. Then in order to determine the effect of one session training Concurrent on measured variables, repeated ANOVA test is used, that by observing difference between the three time periods (three sampling stages), the post hoc test will be used. SPSS16 All statistical analysis was done by SPSS16 and Excel (2007) at the significance level $P < 0/05$.

RESULTS AND DISCUSSION

The results of the present study showed that one session training Concurrent exercise results into a significant decrease in the concentration of such electrolytes as Sodium ($P=0/0004$), calcium ($P=0/000$), magnesium ($P=0/000$), salivary flow rate ($P=0/000$) and a significant increase in electrolytes such as potassium ($P=0/000$), total protein ($P=0/000$) following training. On the other hand the amounts of phosphate ($P=0/000$) and salivary urea ($P=0/000$) after the activity has increased but not statistically significant. And also Cortisol levels ($P=0/000$) and DHEA ($P=0/000$) after the activity has increased and decreased respectively. 24 hours after exercise, with the exception of salivary flow ($P=0/000$), cortisol ($P=0/000$) and DHEA ($P=0/000$), the amount of sodium ($P=0/000$), calcium ($P=0/003$), magnesium ($P=0/000$), potassium ($P=0/000$), phosphate ($P=0/000$) and urea ($P=0/000$) partially returned to baseline levels.

Table 1. Personal characteristics of subjects (n=20)

The measured parameters	Mean \pm SD
Age (years)	2 \pm 23
Height(m)	4.2 \pm 179.3
Weight (kg)	7.1 \pm 77.5
Body fat percentage (%)	3.4 \pm 18.6
Body mass index (Kg/m ²)	2.6 \pm 23.1
Maximum oxygen consumption (ml/kg ⁻¹ /min ⁻¹)	2.3 \pm 48.1

Table 2. The mean and standard deviation of parameters (sodium, calcium, magnesium and total salivary urea) in 3 stages of sampling (before, immediately after and 24 hours after exercise)

Group Index	Sodium (mEq/l)			Calcium (Mg dl)			Magnesium (Mg dl)			Urea (Mg dl)		
	before	after	After 24h	before	after	After 24h	before	after	After 24h	before	after	After 24h
	4.3 \pm 0.3	3.4 \pm 0.3	3.6 \pm 0.2	5.6 \pm 0.9	4.3 \pm 0.7	4.8 \pm 0.6	3.6 \pm 0.7	4.4 \pm 0.7	4.4 \pm 0.7	5.5 \pm 0.4	6.1 \pm 0.5	6 \pm 0.5

Table 3. The mean and standard deviation of parameters (potassium, phosphate, and total protein in saliva) in 3 stages of sampling (before, immediately after and 24 hours after exercise)

Group Index	Potassium (mEq/l)			Phosphate (Mg. ml)			Total protein (g.l ⁻¹)		
	before	after	After 24h	before	after	After 24h	before	after	After 24h
	6.3 \pm 0.7	7.6 \pm 0.6	7.5 \pm 0.5	18.3 \pm 0.8	19.6 \pm 0.7	19.1 \pm 0.7	0.43 \pm 0.18	0.73 \pm 0.29	1.01 \pm 0.27

Table 4. The mean and standard deviation of parameters (salivary flow rate, salivary cortisol and DHEA) in 3 stages of sampling (before, immediately after and 24 hours after exercise)

Group Index	Saliva flow rate (μ l . min ⁻¹)			Cortisol (ng.ml ⁻¹)			DHEA (ng.ml ⁻¹)		
	before	after	After 24h	before	after	After 24h	before	after	After 24h
	537 \pm 56	454 \pm 57	433 \pm 62	2.60 \pm 0.45	3.24 \pm 0.6	3.74 \pm 0.6	21.4 \pm 2.3	21.1 \pm 2.4	26.6 \pm 3.2

The results of the statistical analysis of biochemical compounds amounts in saliva have shown that one session training Concurrent (aerobic - resistance) significantly reduce the levels of salivary sodium and calcium and significantly increase the levels of potassium, magnesium, phosphate, urea and total protein in male athletes ($p < 0/05$).

Given that in the field of sport science very little research was done on the biochemical composition of saliva has been, so the research results cannot be compared with each other. Therefore, similar studies that have examined the blood serum are used. Negler et al (2005) in a study on the biochemical composition of non-stimulated saliva in non-athlete men and women, reported salivary volume loss and changes in salivary composition including reduction of all salivary elements(18). Bruins and his colleagues (2008) examined the effects of exercise on salivary secretion and quantity and quality of salivary compounds and reported that due to physical exercises the salivary IgA concentration decreased, protein concentration fixed and salivary cortisol levels increased. Concentration of sodium, potassium, calcium and phosphate also increased slightly or remained unchanged. According to this study, the researcher found that several factors such as diet, duration of physical activity, fluid intake and ... can be effective on changes in salivary composition (19). Imelik, (1983) in a study on the impact of aerobic activity on serum electrolytes had reported the increase of serum electrolytes (sodium, potassium, calcium and magnesium) (5).

Mashiko et al (2004), for the impact of exercise in the heat on potassium electrolyte concentration showed that serum potassium levels after a summer training program significantly increased in rugby players(20). Verde T., Shepherd (1983) also found that 20 minutes of physical activity in three conditions, outdoors, indoors and sauna, can be followed by a significant decrease in serum magnesium level. According to these researchers, the findings inconsistency can be due to the differences in the type of activity, intensity, duration and also study environment. In other words, the reason of no change in serum magnesium can be associated with inadequate intensity and duration of activity, enough changes in subjects and unsuitable laboratory temperature (21).

On the other hand, factors such as time intervals between the exercise completion and blood sampling, temperature and humidity in training environment, the amount of secretion, regular intake of fluids before, during and after physical activity have also been found as the other factors of changes in the biochemical composition of saliva(22).

The results of the present study showed a significant decrease in the levels of salivary flow by one session training Concurrent (aerobic-resistance), which is in consistent with Sari-Sarraf *et al*(2007) [23], with research results (2007) [23], Williams *et al* (2004) [24], Walsh *et al* (2004) [25] based on reduced salivary flow after a long and intense activity, but on the other hand contradicted with the results of the research by Blannin *et al* (1999) [26], who had reported severe and prolonged activity (cycling race) caused significant changes in salivary flow rate. In most cases researchers agree that the salivary flow rate decreased by exercise and the inconsistency of some researches may probably be due to differences in the intensity, duration, type of activity, age, hydration, exercise environment, temperature and stress. Hydration is one factor that can affect the salivary flow rate. If body fluids reduced by 80 percent, salivary flow will be reduced to zero[4]. Physical activity can reduce salivary flow due to increased activity of the sympathetic nervous system, which in this case decreases arterial diameter and so volume of saliva decreases. However, these changes are more noticeable in hot weather [27,28].

In the present study, DHEA levels following one session training Concurrent (aerobic and resistance simultaneously) had significantly decreased, this reduction remained constant until 24 hours after the activity. The results suggest that the sensitivity of these variables in response to aerobic exercise and endurance will be higher, as performing endurance trainings in Cancarnt exercises inhibits DHEA increase and causes its decrease. Hosseini *et al* (2010), in a study conducted on 29 non- athlete female students have found that 8 weeks of strength training compared with aerobic and endurance trainings significantly increased DHEA rates while had no effect on cortisol levels(29). On the other hand Chatard *et,al* (2002) after 37 weeks of swim trainings in 4 male and 5 female elite swimmers reported the increase of salivary DHEA. According to this researcher, one of the mechanisms involved in the changes of DHEA concentration is hemodynamic differences between the exercise environments. Blood volume changes during swimming due to water pressure result into hormonal changes in response to physical exercises (30). Overall, the reason for DHEA responses differences in different studies could be a result of exercise type, different duration and intensity of exercise, activity environment, differences in laboratory methods, and the level of physical fitness in subjects and the amount of cortisol secretion.

Finally the values of salivary cortisol in the present study showed a significant decrease until 24 hours after the activity, which coincide with the results of Dally (27), Sari-Sarraf *et al* (23) and in contradictory with findings by Filaire(1998)[16],Corral(1994)[31]. There are several mechanisms that show the reason for salivary cortisol concentrations increase after exercises with different intensities. One of these mechanisms is increased hormone secretion through hypothalamic-pituitary-adrenal axis stimulation which results into the increase of ACTH secretion from pituitary and ACTH secretion increase is an important factor in stimulating cortisol secretion [3].

Other reasons may be changes in PH of sympathetic nervous system, hypoxia, lactate accumulation and Psychological Stress. Totally, Factors such as the intensity, duration and volume of exercise, level of physical fitness of subjects, nutrition status and recovery period between training sessions or competition have important influence on levels of cortisol concentrations. Finally it can be said that several physiological factors may affect the immune system, particularly the mucosal immune system. The most important factors can be the reduction of saliva secretion level during exercise, hydration, intensity and duration of physical activity, age, fitness level, the interval between the exercise completion and sampling and the environment temperature. Physical activity and hydration by stimulation of sympathetic nervous system activity can reduce the salivary flow, through which, the levels of several different salivary electrolytes such as sodium, potassium, chloride, magnesium, etc., will be changed, that can be very dangerous and important for the development of Mucosal immune system performance repression in athletes.

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