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The Effect of Concurrent Exercises on Cell Damage Serum Indices and Testosterone to Cortisol Ratio in Non-Athlete Males

Hamzeh Moradi, Ramin Amir Sasan and Vahid Sari Sarraf

Department of Physical Education, Tabriz University, Tabriz, Iran

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ABSTRACT

Concurrent exercises (endurance & resistance) have been utilized in many sports. This study aimed at investigating the effect of concurrent exercise of endurance- resistance versus resistance-endurance on cell damage serum indices (CK, CKMB, CKMM, and LDH) and Testosterone /Cortisol ratio in non-athlete males. Sixteen healthy males in two groups of endurance-resistance group (E-R) and resistance - endurance group (R-E) participated in this study. Blood samples were taken 5 minutes before and one hour after the exercise protocols. The plasma was examined for CK, CK_{MB}, CK_{MM}, LDH, cortisol and testosterone. At the beginning, normality of the collected data was approved through Kolmogorov-Smirnov test and then the data was analyzed by paired and independent samples t test at $p \leq 0.05$. The findings indicated significant changes of all indices before and after the exercise protocols in both groups (except for the testosterone change before and after the exercise in group one, together with cortisol change and the ratio of testosterone to cortisol in both groups). However, there was no significant difference between the groups as far as the mentioned indices were concerned. The results revealed that both exercise protocols caused increase and decrease in some cell damage serum indices, though the percentage of rise and fall of these indices (CK, CKMB, CKMM, and LDH vs. high cortisol; testosterone and testosterone ratio to low cortisol) before and after the exercise protocols was higher in group two (endurance- resistance) compared with group one (resistance-endurance).

Keywords: concurrent exercise**,** Creatine Kinase, Testosterone to Cortisol ratio.

INTRODUCTION

Since endurance and resistance exercises have positive effects on individuals' heath, many studies have reported the beneficial effects of endurance (e. g. increasing power, muscle mass, etc.) and resistance exercises (e. g. cardio-respiratory capacity, muscle resistance, etc.) in athlete and non-athlete individuals [3,5,18]. Therefore, based on the positive impacts of these exercises, several studies have highlighted the higher effectiveness of doing concurrent endurance-resistance exercises, in contrast to one type only, to increase athlete and non-athlete individuals' fitness and health [2,13,21,23,32]. However, research on the effect of the order of performing concurrent exercises on improving endurance and resistance is still under-questioned. It is not clear whether the muscle contraction speed falls in case of doing resistance exercises first. On the other hand, if endurance exercises precede resistance ones, how the excess post-exercise oxygen consumption changes. The researchers believe that the answer to this type of questions depends on acute and chronic compatibility [2,21]. Research has reported that, compared to single type of exercises (endurance or resistance), concurrent exercises have led to interference in endurance and resistance improvement and have shown different results [8,31]. Due to the rise of metabolism as a result of increase in athletic activity, enzymes (one of the vital components of metabolism inside cells) activity goes up in accordance with the intensity of athletic activity.

Normally, many enzymes (e. g. LDH, CK, CK_{MB} ,and CK_{MM}) are surrounded inside cells, but their release in the blood might increase in response to different athletic activities [8,9,30,31]. LDH is one of these enzymes which exists in cellular cytoplasm of all body fibers in different densities. The majority of studies investigating the effects of different athletic activities, particularly endurance and resistance exercises, on LDH enzyme activity amount have revealed significant change of this enzyme [8,30]. For instance, Franca et al, in their study on 20 healthy athlete males, have reported the significant rise of LDH enzyme after the marathon race and even during recovery stage [17].

Creatine Kinase (CK) as one of the key enzymes of energy metabolism in the body that maintains high levels of intracellular ATP through the phosphorylation of creatine is also investigated in many studies examining the impact of athletic activities of endurance and resistance exercises [9,30]. In a relevant study, Kratz et al. examining 37 runners in marathon race (32 men, 5 women, with average age of 49) observed a significant increase in cell damage serum indices of CK and CK_{MB} [25]. CK has three main iso-enzymes, each of which is composed of two polypeptide chain. These chains are called B and M that create dimmer compounds of BB, MB, and MM. This enzyme consists of three iso-enzymes called CK_{BB}, CK_{MB}, and CK_{MM}. The MM iso-enzyme has the highest amount in the skeleton muscles, BB exists in brain, nervous fibers, thyroid, and kidney, and MB type is available in heart muscle and diaphragm, respectively [9,30]. Therefore, we might be able to explain the interference in endurance and resistance improvement of concurrent exercises through investigating the degree of cell damage serum enzymes.

The hormonal system as an effective factor involved in physiological processes of the body plays an important role in growth, maturity and short-term and long-term responses to resistance and endurance exercises [15,31]. The two important hormones in response to skeleton muscle adaptation to exercise are testosterone and cortisol that act as the indices of anabolism and catabolism, respectively [15,31]. Testosterone is a 19-carbon steroid hormone that its metabolism takes place in the liver, testis, and prostate. Testosterone has a crucial role in maintaining muscle and bone mass, and brain function. In addition, testosterone makes more and larger muscle fibers through the synthesis of protein[13,22,26,31]. Cortisol is also one of the main steroid hormones playing a remarkable role in the adjustment and regulation of cardiovascular, immunological, homeostasis, and metabolic functions. This hormone, furthermore, accelerates gluconeogenesis, lipogenesis, and proteolysis in the body. Researchers have pointed out that some factors such as the intensity of athletic activity (more than 75 percent of consumed oxygen), the volume change of plasma and blood, the temperature variation of the environment, and the mental stresses of athletic activities influences the density of serum cortisol in athletes [13,31]. Nevertheless, there is a large variety of hormone responses to endurance and resistance exercises in the literature. Generally, endurance exercises lead to testosterone increase in both phases of exercises and immediately after the exercise which starts to fall during recovery phase [3,6,11,18]. Unlike endurance exercises, resistance ones usually cause a sharp rise in cortisol density after the exercise that, similar to testosterone in endurance exercises, begins to decrease in recovery stage. As a result, cellular and hormone damages happening due to the particular order of performing these exercises may influence the obtained changes (3, 20). Deakin described that muscle damages of concurrent exercises might be because of the fatigue caused by the exercises. In another study, Leveritt et al. indicated that the muscle damage of endurance exercises was more than resistance exercises [13,27]. Contrastingly, not only many studies has shown that resistance exercises also cause cell and muscle damage, but also they have taken resistance exercises as the main cause of muscle damage in concurrent exercises [10,13]. However, because of the importance and novelty of this issue, there is not any single theory in this regard. Therefore, the present study is intended to investigate the effect of concurrent exercise of endurance- resistance vs. resistance -endurance on cell damage serum indices (CK, CK_{MB}, CK_{MM}, and LDH) and testosterone/cortisol ratio in non-athlete males.

MATERIALS AND METHODS

Subjects

The initial sample of this study was male students of 19 to 23 year-old age of Tabriz University. A week prior to the study, among 60 non-athlete volunteer students, 16 students were chosen through participating in a call for research respondents and getting familiar with research details as the final subjects. Furthermore, after elaboration of the study details to the participants and assuring the confidentiality of their records, all subjects were asked to read the agreement and confirm the research terms and conditions before selection stage. The selection requirements of the subjects included absence of drug or supplement use and smoking, individual health, no record of blood disease or illnesses affecting immunity system, lack of infection and allergic conditions, and relative familiarity with weight training. Then, for three repeated days from 10 to 12 in the morning, the following tests were taken from the subjects in order to establish homogeneity: shuttle run test to estimate VO_{2max}, prediction of one repetition maximum (1RM) (in bench press, knee flexion and extension, lat pull-down, and arm exercises), estimation of body fat percentage (using 3Sites Jackson Formula), and body mass index. After assuring homogeneity among subjects, they were randomly assigned into two groups of endurance-resistance (age=23±2, height=173±7.8$_{cm}$, weight=70.9±6.3$_{Kg}$, BMI=21.58±1.23, VO_{2max}=41.01± 2.27, $_{ml/kg/min}$, fat percentage=14.32±1.80) and resistance-endurance (age=23±2, height=175.1±5$_{cm}$, weight=71.2±10$_{Kg}$, BMI=23.26±3.17, VO_{2max}=41.66± 2.68, $_{ml/kg/min}$, fat percentage=16.95±3.81).

Data collection

At the beginning, the demographic information of the subjects was recorded and the participation agreement together with the health questionnaire was distributed and collected. Then, all the subjects were invited to the weight saloon of Tabriz University Stadium to conduct initial measurements and start the tests. Based on a planned time-table, the subjects gone through required instructions on the tests performance and took the tests in certain sessions. Additionally, blood tests were also taken and sent to laboratory in a specific period of time.

Measuring the Maximum Volume of Oxygen (VO_{2max})

In order to measure VO_{2max}, shuttle run test protocol of 20 meters was applied. The subjects had to run a 20-meter flat route in a two-way continuous form. The initial speed of the test was equal to 8 kilometers per hour that every 2 minutes 0.5 kilometer (0.14$_{meter/sec}$) was added to it. The runners speed was adjusted through the broadcasting of the signals on the tape at final lines. When the signals were broadcasting, the subjects ought to stay at the beginning or the end of the lines and try to continue the test and stand on the defined lines at the appropriate time. The first time that the runners lagged behind the signals voice, the first warning was given and written as the first error in the certain form. In case of the third error, the test was stopped and the maximum speed of the runner together with the number of levels and two-way running cycles were utilized to estimate the maximum oxygen volume (VO_{2max}). It should be noted that the heart beat of the subjects was controlled and recorded by Polar heart rate monitors along and at the end of the test. The formula is as follows:

$$VO_{2max}(ml/kg/min) = (5.857 \times \text{maximum speed in Km/h}) - 19.485$$

Measuring One Repetition Maximum (1RM)

For measuring 1RM in six exercises of knee extension, bench press, knee flexion, armpit muscle pulling, and back and front exercises of arm, the subjects moved an under the maximum weight up to the exhaustion (the weight was selected in a way that more than 10 time repetition was impossible). Then, the one repetition maximum (1RM) was calculated according to the following formula:

$$\text{One Repetition Maximum} = \frac{\text{the moved weight (kg)}}{1.0278 - (\text{number of repetition till exhastion} \times 0.0278}$$

The exercise plan

The concurrent exercise of endurance-resistance protocol consisted of 10 minutes warm-up, 20 minutes running of 60 to 70 percent heart beat, and 10 minutes active recovery for the endurance phase. The second phase involving resistance exercises included 50 minutes of power exercises of knee extension, bench press, knee flexion, and finally back and front exercises of arm in three sets of 10 to 12 repetition and 70 percent of one repetition maximum (1RM) and 10 minutes active recovery (stretching exercises). The protocol of concurrent resistance-endurance exercises was performed unlike the previous protocol.

Nutrition and physical activity of the subjects

In order to homogenize the subjects' nutrition conditions and prevent from any effect on the variables before the tests, all subjects were advised to take their meal 2.5 to 3 hours before the test. Furthermore, they were asked to drink adequate water during and after the test if needed. Regarding their physical activities other than the testing days, all subjects were recommended to do their normal daily activities. However, to avoid any possible effect of physical exercises on the research results, they were asked to stop any intensive physical activity 48 hours before the test.

Blood taking and laboratory analysis
Every subject was given a certain time and was asked to be at the testing place an hour before the test for blood sample taking (two groups in two following days). The purpose of this initial blood sample taking was to get the basic levels of enzymes (CK, CK_{MB}, CK_{MM}, and LDH) activity together with testosterone and cortisol hormones activity before the test. Later, in the post test phase, another blood sample was taken from the subjects one hour after the exercises protocol. The blood sample taking is explained in the following. After the arrival of each subject to the testing place, he was asked to sit for 5 minutes. Then, a laboratory technician took 3 ml of blood (without anti-coagulant to separate the serum) from the antecubital vein using 5cc syringes. Next, the blood sample was placed for 30 minutes in laboratory temperature of 22-25c to coagulate. After that, the serum was separated by centrifuge device and the enzymes activity rate and the corresponding hormones changes were estimated through Pars company's kits and Hitachi v.4 auto-analyzer system in liter unit.

The statistical methods
At the beginning, the demographic information of the subjects and research data were collected and elaborated through table. Then, research hypotheses were examined at significance level of 0.05 according to previous studies. The normality of the collected data was approved by Kolmogorov-Smirnov test and then the data was analyzed by paired and independent samples of t test at $p \leq 0.05$ using SPSS 15 software.

RESULTS

The findings revealed a significant increase in the rate of enzymes (CK, CK_{MB}, CK_{MM}, and LDH) change before and after in both endurance-resistance and resistance-endurance groups ($p < 0.05$). However, there was not any significant difference between the groups as far as the corresponding enzymes change domain is concerned (table 1). Regarding the testosterone change, although the results indicated a significant change of testosterone in resistance-endurance group before and after the test ($p < 0.007$), no significant difference was observed in endurance-resistance group between pretest and posttest ($p > 0.15$). On the other hand, the findings showed that there was no significant difference between the groups regarding the testosterone change domain ($p > 0.67$). Furthermore, both groups did not have any significant change in cortisol and cortisol domain before and after the test. Considering the testosterone /cortisol ratio and their change domain before and after the test, no significant difference was seen between the groups. Finally, indices change percentage outcomes revealed that although the second group (resistance-endurance) had a higher percentage in CK, CK_{MB}, CK_{MM}, LDH and cortisol indices, it showed a lower percentage in testosterone and testosterone/cortisol ratio indices [table 1].

Table 1-The Mean of before and after change, rang and change percentage in serum and hormonal indices

Variables														Groups	statistical methods
Testosterone /Cortisol Ratio		Cortisol		Testosterone		LDH		CK_{MM}		CK_{MB}		CK			
p	t	p	t	p	t	p	t	p	t	p	t	p	t		
0/10	1/84	0/44	-0/80	0/15	1/57	0/003	-4/5	0/002	-4/7	0/004	-6/2	0/003	-4/3	E-R	Paired sample t test
0/12	1/77	0/31	-1/09	0/007	3/75	0/002	-4/6	0/004	-4/2	0/005	-8/4	0/007	-3/7	R-E	
0/76	0/30	0/50	-0/68	0/67	0/43	0/43	0/8	0/46	0/7	0/4	-0/8	0/15	1/5	between the groups	independent sample t test
-11/95		6/86		-8/26		70/5		75/38		49/41		83/24		E-R	change percentage
-18/83		11/83		-15/50		71/38		147/19		77/71		110/31		R-E	

DISCUSSION

The findings of the present study demonstrate a significant difference (increase) after the test in CK, CK_{MB}, and CK_{MM} enzymes levels in both groups (endurance-resistance vs. resistance-endurance). In a similar vein, Catherine has reported a significant increase in CK enzyme after the concurrent exercises of resistance-endurance [12]. In addition, this study represented a significant difference between the two groups as far as the enzymes (CK, CK_{MB}, CK_{MM}) change domain is concerned. This study also approved Deakin's findings mentioning a significant rise ($p < 0.026$) in CK enzyme level after resistance-endurance exercises, and an insignificant difference in CK change domain between both groups [15]. Deakin added that his findings might be due to the endurance exercises in the second phase because the change domain of CK after the endurance exercises was more than the change domain after endurance-resistance exercises [15]. Similarly, in another study, Deakin reported that performing concurrent endurance-resistance exercises show a lower CK level compared to doing endurance exercises. This study represented a significant volume of CK in both parts of endurance exercises (upper-body, lower-body); however there was no significant change of CK volume in the endurance-resistance group [14]. The conflicting results between the mentioned studies and the present study could be due to the differences in study groups, evaluation and measurement methods, the intensity and duration of exercises, types of exercises protocols, blood taking time, and rest intervals between two exercises [7,14]. Taking into account LDH enzyme change, many studies on the impact of athletic exercises, particularly endurance-resistance exercises, on LDH enzyme activity rate also have confirmed the significant change of this enzyme. This is in line with the results of the present study indicating a significant increase in LDH enzyme change and an insignificant change in LDH domain after the test. Franca et al. have demonstrated a significant increase of LDH after the marathon and recovery stage [17]. The results of cell damage serum indices illustrated that in spite of the significant increase of all corresponding indices in both groups ($p < 0.05$) in posttest, no significant difference was observed between the groups ($p > 0.05$). A lot of studies have investigated the change of CK, CK_{MB}, CK_{MM}, and LDH enzymes before and after athletic exercises and reported a remarkable growth in these enzymes after endurance-resistance exercises [8,9,13,30]. Therefore, the high percentage of these enzymes in the resistance-endurance group might be because of the endurance part. In other words, the endurance-resistance group had more time for enzymes wash from the blood serum (about 2 hours) in contrast to the resistance-endurance group (around 1 hour time) [9,13].

Considering testosterone change, the findings represented that despite the significant change of this hormone in the resistance-endurance group, it did not have any significant change in the endurance-resistance group. Additionally, no significant difference was seen between the two groups in terms of testosterone change domain. Some quantitative studies have reported testosterone rise and fall after concurrent endurance-resistance and resistance-endurance exercises [13,14]. In a similar study by Hakkinen et al, they reported an insignificant increase of testosterone after performing concurrent endurance-resistance exercises [21]. In his study, Deakin also demonstrated that both groups of endurance-resistance and resistance-endurance had a similar significant fall ($p < 0.022$, and $p < 0.024$, respectively) in testosterone amount [15]. Furthermore, in another study, Deakin found no significant difference was seen in testosterone amount between the two concurrent groups. However, compared to the resistance-endurance group, the endurance-resistance group showed a higher response to testosterone amount [16].

Unlike the findings of the present study, Kraemer et al indicated no change in Testosterone amount after doing endurance-resistance exercises. Similarly, Bell et al and Horne et al also represented the same results as Kraemer et al in their studies [4,22]. The contrast between the findings of this study and the preceding studies could be probably originated from type of the subjects, exercises type and protocol, and intensity and amount of the exercises [13,19].

Regarding cortisol change, the results revealed no significant difference of cortisol amount and cortisol change domain before and after the test in both groups ($p > 0.05$, $p > 0. 5$, respectively). Hakkinen et al demonstrating no significant change in the amount of cortisol after resistance-endurance exercises approve the findings of this study[21]. In this vein, Arazi et al also revealed no significant change of cortisol amount when doing concurrent exercises [1]. Deakin's findings also supported Azeri et al, though the resistance-endurance group represented more response to cortisol amount, compared to its counterpart [16].

Nevertheless, some studies have reported cortisol change (increase and decrease) after concurrent exercises (endurance-resistance vs. resistance-endurance). For instance, Deakin reported a fall in cortisol amount in both concurrent groups[15]. Similarly, Guilherme et al also supported Deakin's results after doing resistance-endurance exercises [20]. On the other hand, Bell et al, Kraemer et al, and Horne et al reported a growth in cortisol amount in endurance-resistance group [4,22,24]. Since the rise of cortisol depends on the intensity of exercises, it responds to a certain amount of the exercise and do not increase in short-term exercises (less than 30 minutes) of average intensity (VO_{2max}= 60 to 70٪). On the other hand, heavy resistance exercises can increase cortisol amount to a considerable degree. Nevertheless, gradual cortisol fall has been observed in cortisol degrees of individuals after doing the exercises of resistance and after under maximum endurance exercises, in contrast to inactive individuals [13,26,29]. Therefore, the rise in the corresponding indices in the endurance-resistance group might be because of the resistance part.

About the changes in the ratio of testosterone to cortisol, the findings did not represent any significant difference between testosterone and cortisol ratio in both endurance-resistance ($p > 0.10$) and resistance-endurance group ($p > 0.12$). Similarly, no significant difference existed between the two groups in terms of change domain of the testosterone to cortisol ratio ($p > 0.76$). In this regard, Deakin reported, in two of his studies, that there was no significant difference between mean difference of the ratio between testosterone and cortisol in both groups [15,16]. In another study by Mark et al, they demonstrated that the ratio of testosterone to cortisol in the resistance group was more than the endurance group, though no significant difference was observed between the two groups [28].

In general, the results of this study indicated that both concurrent exercises protocols have changed the hormone indices, but the degree of percentage increase of these indices in the second (resistance-endurance) group was more than the (endurance-resistance) group in testosterone (G1= -8.26٪, G2= -15.50٪), cortisol (G1= 6.86٪, G2= 11.83٪), and the ratio of testosterone to cortisol (G1= -11.95٪, G2= -18.83٪) respectively. In this vein, Deakin reported in his study that the amount of cortisol, testosterone, and the ratio of testosterone to cortisol increased more in the resistance-endurance group than the endurance-resistance group, though no significant difference existed between the two groups ($p > 0.57$). It means that, in comparison to resistance-endurance exercises, the endurance-resistance exercises have a higher anabolic status[16]. However, in another study, Deakin demonstrated that the endurance-resistance group had a lower ratio of testosterone to cortisol and more catabolic status compared to its counterpart [15].

CONCLUSION

Generally, this study represented that both protocols of exercises (endurance-resistance vs. resistance-endurance) had some changes in hormonal indices. However, the rate of percentage increase of these indices in the second (resistance-endurance) group was more than the (endurance-resistance) group in testosterone (G1= -8.26٪, G2= -15.50٪), cortisol (G1= 6.86٪, G2= 11.83٪), and the ratio of testosterone to cortisol (G1= -11.95٪, G2= -18.83٪) respectively. As a result, although there was not any significant difference in corresponding indices, doing one session of endurance-resistance concurrent exercises had a better anabolic status and less cell damage compared to the resistance-endurance exercises. However, more research is needed to support the results.

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