The Responses of Muscle Damage Markers and Growth Mediator to Different Concurrent protocol of Endurance and Resistance Training

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Abstract
Introduction: The aim of this study was to investigate the muscle damage markers and growth mediator responses to concurrent training with different endurance and resistance training order in healthy males.

Methods: Thirty-nine healthy male were randomly assigned into three equal homogeneous groups; endurance-resistance training (ER), resistance-endurance concurrent training (RE) and control (C). The training group’s subjects performed eight weeks 3sessions per week concurrent training sessions in the same intensity and duration but different by endurance and resistance exercise orders. Tow incremental exhaustive treadmill tests was performed before training and 72 h after the last training session. Blood samples for the measurement of creatine kinase (CK), lactate dehydrogenase (LDH), insulin-like growth factor (IGF)-1 and insulin-like growth factor binding protein (IGFBP)-3 were collected at baseline and immediately after the tow exhaustive treadmill tests.

Results: The response of CK, LDH, IGF-1 and IGFBP-3 to the first and second incremental exhaustive treadmill test showed significant increases in both exercise orders when compared to baseline values (p<0.05). Also, comparison of pre and post-training responses showed a significant decrease in CK and LDH in both exercise order and a significant increase in IGFBP-3 and IGF-1 only in the RE exercise order (P<0.05). However, the present study results didn’t show any significant difference between the ER and RE groups.

Conclusion: According to the results, there were significant decreases in muscle damage markers after both types of concurrent training. However, higher growth mediator’s responses were seen when resistance exercise precedes endurance exercise.

Keywords: Concurrent Training, Muscle Damage, Growth Mediator

Introduction

Endurance and resistance training modalities independently cause different physiological adaptations. These adaptations are occasionally contradictory in different body systems (1- 4). In this context, combining both types of training, in the same training session, known as concurrent training (5) may result in gain in both health benefits of endurance training and resistance training (6- 12). However, the effects of resistance and endurance exercise protocol of concurrent training programs on human body is not well investigated. Previous studies have shown doing endurance training first is in favour of improving VO_{2max} (13, 14) and increasing excess post-exercise oxygen consumption (EPOC) (15), but doing resistance training first could improve muscle strength, power and its size (16).

On the other hand, it is well known that physical activities are often associated with
muscle damage (17, 18). The muscle damage can be tracked by muscle enzymes such as creatine kinase (CK) and lactate dehydrogenase (LDH) in the bloodstream (19). To the best of our knowledge, no study in the literature on muscle damage markers response has compared the effects of exercise protocol in concurrent endurance and resistance training on muscle damage markers. It is well indicated that skeletal muscle damage increases the circulating of proteins necessary for long-term hypertrophy (17). Anabolic hormones such as IGF-1 have a mediating role in muscle protein synthesis signalling and gene expression (20). However, some studies investigated the effect of intra-session exercise order in concurrent endurance and resistance training on anabolic (11, 20-22) and catabolic (21, 22) hormones. In a study, Rosa et al. (2015) showed that doing resistance training in a concurrent training program resulted in a significant increase in IGF binding protein-3 (IGFBP-3) (11). Another study showed that no significant difference was observed in plasma levels of IGF-1, endurance-resistance and resistance-endurance concurrent training in men and women (20). There has probably been no research on the effects of concurrent and resistance training on muscle damage markers. Furthermore, there is not enough information about the effects of concurrent endurance and resistance training protocol on anabolic hormones. Therefore, this study aimed to determine the responses of muscle damage markers and growth mediator to different concurrent protocol of endurance and resistance training.

**Methods**

Thirty-nine healthy males who had no regular physical activity during the past year participated in this study. They completed and signed a consent form which was approved by Ethic Committee of Kurdistan University of Medical Sciences and received details of the possible risks of participation in the exercise training protocol. The subjects completed the Par-Q form for their health condition assessment. Those with a history of diseases such as liver disease, muscle disorders and myocardial infarction were excluded. The subjects were randomly assigned into three groups (n=13 in each group) of resistance-endurance (RE), endurance-resistance (ER) concurrent training, and control. The characteristics of the participants are presented in Table 1.

Experimental groups carried out both endurance and resistance training in each session, concurrently. Training programs were performed for eight weeks, three times a week in the evening on Sundays, Tuesdays and Thursdays. The endurance training program included aerobic training on treadmill with 55 to 85% of maximum heart rate (HRmax) for 25 to 45 minutes. The training was increased by 10% intensity and by 5 minutes every two weeks. The resistance training program included exercises with weights including bench press, biceps and triceps flexion-extension with weights, underhand cable pull-down, leg press, scot and sit-ups, which were performed with 50 to 80% of 1-RM. The intensity of resistance training was increased by 10% every two weeks. In order to imply likely improvements, the 1RM measurement was repeated in the next of four week and the new 1RM was calculated. ER and RE group’s subjects were asked to warm up for 10 min by voluntary running. The endurance training program was performed first in the ER group and the resistance training program was performed first in the RE group. The endurance training was carried out on standard treadmills (RUN700, TechnoGym, Italy) and the resistance training was performed with standard weights and machines (Ningjin Xinrui, Shandong, China). Control group only participated in daily activities. Anthropometric measurements were performed one day before random assignment of individuals of all groups. The height and weight was measured by using standard scale with integrated measuring rod (Secca 704s, Germany) and the
body fat percent was measured by skinfold technique (Harpenden skinfold caliper, Baty, UK). Subjects were asked to perform the maximal Bruce protocol on treadmill (23) before the training program and 72h after the last training session. For blood sampling and analysing pre-training blood sample collected one day prior to the Bruce protocol test after 12 hours of fasting in the morning. Tow incremental exhaustive treadmill tests performed before and 72h after the last training session. Other blood samples were collected immediately after the tow exhaustive treadmill tests. Quantitative measurement of serum CK, was done by commercial test kits (Randox ®-UK), Human LDH was measured with Lactate Dehydrogenase Activity Assay Kit (Randox ®-UK) and serum IGF-1 and IGFBP-3 were measured by using ELISA kit (Quantikine High-Sensitivity Kit; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Descriptive Data were presented as Mean ± SD. The Shapiro-Wilk test result showed all variables were normally distributed. Therefore, two-way (condition × time) repeated measures analysis of variance (RM-ANOVA) using the Bonferroni correction was used to analyse between and within group differences. Paired sample t test was used to compare before and after training values in each group. When RM-ANOVA indicated a significant difference, Bonferroni's post-hoc test was used for pairwise comparisons. To identify the differences between before training values of variables in ER, RE and control groups, one-way ANOVA was utilized. All analyses were tested at an alpha level of 0.05 (α = 0.05). SPSS 21 (Statistics IBM SPSS, Inc., Chicago, IL, USA) was used for statistical analysis and MS- Excel 2013 software was employed to draw figures.

**Results**

One-way ANOVA results didn’t show any significant differences in pre-training values of age, height, weight and BMI between the groups (p>0.05). Physical characteristics of ER, RE and control groups is shown in Table 1.

Data analysis showed significant differences in maximum oxygen consumption between the groups (p=0.003). Also, significant differences were seen in time (training) and time × group interaction for both VO$_{2max}$ and body fat percent (p<0.05). Bonferroni post hoc test showed significant difference between ER and CON for VO$_{2max}$ and body fat percent. Furthermore, a comparison within group showed significant differences in ER and also RE groups in case of both variables (Table 2). RM-ANOVA results showed that the response of CK, LDH, IGF-1 and IGFBP-3 to the first and second incremental exhaustive treadmill test were significant increases in both exercise orders when compared to baseline values (p<0.05). Also, comparison of the first (pre training response) and second (post training response) incremental exhaustive treadmill test showed a significant decrease CK and LDH in both exercise order and a significant increase in IGFBP-3 and IGF-1 in the RE exercise order (p<0.05).

**Table 1.** Physical characteristics of subjects at the start of the study

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>RE</th>
<th>Con</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>22.00±3.00</td>
<td>21.66 ±2.08</td>
<td>22.61±3.05</td>
<td>0.551</td>
<td>0.583</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>177.17±4.85</td>
<td>174.63±3.48</td>
<td>176.89±4.00</td>
<td>1.124</td>
<td>0.340</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.72±4.38</td>
<td>68.70±3.35</td>
<td>71.85±4.14</td>
<td>2.069</td>
<td>0.146</td>
</tr>
<tr>
<td>BMI(kg/m$^2$)</td>
<td>21.88±1.01</td>
<td>22.54±1.28</td>
<td>22.95±0.80</td>
<td>2.593</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Data are presented as M±SD. BMI; body mass index.
Table 2. RM-ANOVA statistical results in ER, RE and Con groups for VO₂max and Body Fat percent before and after the training program

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Between Groups</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>ER</td>
<td>37.79±11.09</td>
<td>46.03±13.68#</td>
<td>0.003*</td>
<td>0.001* 0.002*</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>38.74±16.30</td>
<td>44.80±17.69#</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>37.95±10.22</td>
<td>38.28±11.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>ER</td>
<td>15.70±5.21</td>
<td>12.75±5.00#</td>
<td>0.184</td>
<td>0.001 0.024*</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>17.57±6.58</td>
<td>13.73±4.09#</td>
<td>*†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>16.92±5.74</td>
<td>16.71±6.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as M±SD. T×G= interaction effect of Time × Group. *= Significantly different at 0.05 level (α=0.05); †= significantly different at 0.01 level (α=0.01). #=significant difference between the pre and post-training.

Table 3. RM-ANOVA statistical results in ER, RE and Con groups for muscle damage and growth factors before and after the training program

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Baseline</th>
<th>Pre-training response</th>
<th>Post-training response</th>
<th>Between Groups</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/l)</td>
<td>ER</td>
<td>198.43±94.23</td>
<td>492.80±236.99</td>
<td>311.90±141.78#</td>
<td>0.001*</td>
<td>0.038† 0.001*† &amp;</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>213.60±126.04</td>
<td>466.30±278.04</td>
<td>343.80±150.03#</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>229.48±122.21</td>
<td>511.70±263.21</td>
<td>518.10±211.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>ER</td>
<td>158.70±78.21</td>
<td>424.70±132.21</td>
<td>295.75±111.00#</td>
<td>0.01*</td>
<td>0.001* † 0.003*</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>181.57±73.58</td>
<td>398.57±165.58</td>
<td>262.73±129.09#</td>
<td>*†</td>
<td></td>
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<tr>
<td></td>
<td>CON</td>
<td>167.92±81.78</td>
<td>439.92±218.78</td>
<td>427.91±157.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>ER</td>
<td>287.34±121.64</td>
<td>342.50±189.42</td>
<td>440.50±213.92</td>
<td>0.08</td>
<td>0.001* 0.085*†</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>242.70±98.32</td>
<td>351.90±193.21</td>
<td>493.30±168.96#</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>271.55±142.48</td>
<td>366.20±202.84</td>
<td>351.80±173.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>ER</td>
<td>34.48±15.86</td>
<td>61.32±17.34</td>
<td>68.09±23.22</td>
<td>0.39</td>
<td>0.001* 0.003*†</td>
</tr>
<tr>
<td></td>
<td>RE</td>
<td>29.75±13.13</td>
<td>59.57±19.83</td>
<td>83.71±25.15#</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CON</td>
<td>27.83±18.23</td>
<td>67.12±21.03</td>
<td>64.89±19.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as M±SD. T×G= interaction effect of Training × Group. *= Significantly different at 0.05 level (α=0.05); †= significantly different at 0.01 level (α=0.01). #=significant difference between the pre and post-training response.
However, there was significant difference between the group for CK and LDH concentration \( (p<0.05) \). Bonferroni post hoc test showed significant difference between ER and CON for CK and between ER and CON and also between RE and CON for LDH and IGF-1 \( (p<0.05) \). Time (training) effect was significant in all variables and time × group interaction was significantly different for CK and LDH \( (p<0.05) \). However, the present study results didn’t show any significant difference between the ER and RE groups. Statistical analyses for CK, LDH, IGF-1 and IGFBP-3 were presented in table 3.

**Discussion**

The aim of present study was to investigate the responses of muscle damage markers and growth mediator to different concurrent protocol of endurance and resistance training in sedentary men. In this study CK and LDH measured as a marker of muscle damage (19). We illustrated a non-significant but remarkable decrease in CK for ER and RE by 38% and 26%, respectively. However, no significant difference was found in CK and LDH between ER and RE groups of this study. Although the exact mechanisms to explain muscle damage have not been delineated, Armstrong et al. stated that eccentric part of contraction in resistance training is thought to be involved. Because smaller numbers of motor units are recruited in these types of contractions, then the proportion of the imposed load on the muscle fibre is increased (24). Muscle damage usually occurs in untrained individuals, but it’s likely that if the exercise intensity is beyond the level they are adapted to, it may occur in trained individuals, too. Recently one study showed that ultra-endurance marathon race can cause highly level of muscle damage in athletes (25). To the best of our knowledge, no research has been done on the effects of intra-session exercise sequence in concurrent endurance and resistance training on muscle damage markers. Insulin-like growth factor-1 and insulin-like growth factor binding protein-3 were measured as growth mediators in the present study. Although IGF-1 and IGFBP-3 responses were significantly different after training in RE group, there weren’t any significant difference between the ER and RE groups. This results support the finding of previous studies which showed the order of concurrent endurance and resistance training which caused no significant difference in the plasma level of IGF-1 in men and women (20). However, one study found a significant in IGFBP-3 in ER but not RE concurrent training methods (11). Despite the present study result, one investigation have reported that IGF-1 mRNA content was decreased (-42%) when cycling activities precedes the resistance exercise (26). Generally, the mechanisms of training effect on IGF-1 are not clear. It is expressed that muscle tissue damage in the z-planes, structural proteins, and connective tissues are needed for muscle growth. It is possible that exercises with sufficient intensity results in muscle cell damage, which is related to the increase in growth factors such as IGF-1 (27).

Body fat percent decreased significantly and \( \text{VO}_{2\text{max}} \) increased significantly in both ER and RE concurrent training groups after 8 weeks of training. All of above mentioned results shows that 8 week concurrent training, regardless to exercise protocol, is sufficient for physiological adaptation in human body. Nowadays many non-athletes and competitive athletes are doing endurance and resistance training in their daily training programs routinely. According to our study results we can recommend to inactive people doing concurrent endurance and resistance training regardless to exercise protocol. However, our research has examined the effects of two types of concurrent training by different exercise protocol, certain intensity, and duration. It is likely the examining of the effects of concurrent training protocol with different
intensity and duration would have different results.

**Conclusion**
According to the results of this study, there are no significant differences in muscle damage markers and growth mediators between RE and ER concurrent training. However, we observed the beneficial effects of both concurrent methods on enzymatic cell damage markers and growth mediators.

**Ethical issues**
No applicable.

**Authors’ contributions**
All authors equally contributed to the writing and revision of this paper.

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**References**


