

# Effect of Exercise Order of Resistance Training on Strength Performance and Indices of Muscle Damage in Young Active Girls

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## Abstract

**Background:** Exercise order is one of the key variables in designing resistance training (RT) that may affect physiological and functional muscle characteristics.

**Objectives:** The purpose of this research was to examine the effect of exercise order of RT on muscle strength, lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes as indices of muscle damage in active young girls.

**Patients and Methods:** For this purpose, 24 active girls aged 20 - 30 years voluntarily participated in six weeks RT. The subjects were randomly assigned into three groups of large to small muscle group (SLM, n = 8), large to small group (LSM, n = 8) and control group (Co, n = 8). The RT performed in bench press, lat pull down, triceps with machine and biceps with barbell. Before and after RT, blood sample was collected to measure the level of LDH and CK enzymes activity.

**Results:** The result of analysis indicated that RT significantly increased the level of LDH enzymes in all three groups ( $P < 0.05$ ). In addition, the result showed that six weeks of RT did not cause any significant change in CK ( $P > 0.05$ ). However, there is no significant difference between SLM, LSM and control in CK and LDH after six weeks RT.

**Conclusions:** Both RT methods cause the same improvement on muscle strength and performance. Also muscle damage indices did not show sensitivity to the exercise order. Therefore, resistance training may be designed regardless of effect of exercise order on muscle damage.

**Keywords:** Weight Training, Creatine Kinase, Strength, Lactate Dehydrogenase

## 1. Background

Resistance training program can improve measures of strength in adults. But the choice of exercises and the order of exercises performed during a resistance training session is an important consideration in program design (1), and when they are combined properly with other RT variables, it can lead to more efficiency, safety, and finally lead to more effective resistance training effects (2) increase the level of power (3-5) and hypertrophy (6). Teague et al. (7) examined the influence of exercise order on strength in untrained young men after 8 weeks of non-periodized resistance training. Authors reported that the small muscle exercises revealed significantly higher strength gains when placed first, demonstrating that small muscle exercises may be particularly important during the initial stages of resistance training in untrained young men. On the other hand, Bellezza et al. (2) examined the influence of exercise order on strength and muscle thickness in untrained men after 12 weeks of linear periodized resistance

training and reported that if an exercise is important for individual training goals, it should be performed at the beginning of the training session, whether or not it is a large or a small muscle group exercise. It appears that there is a need for further investigation of exercise order.

On the other hand, resistance exercise can result in localized damage to muscle tissue. Damaged muscle cells release some of the markers of micro trauma such as lactate dehydrogenase (LDH) and creatine kinase (CK) inside the plasma (7-9). The increase in the accumulation of these enzymes following the activity is due to the cell membrane rupture and is a significant factor for the muscle tissue functioning status (10). It has been reported that both intensity and duration of exercise can affect aspects of muscle damage and muscle performance (11). However, this issue becomes more complicated when the RT with more variables such as the speed of movement, rest interval between sets, and the exercise order influence the outcomes. In regard to the effect of arrangement of RT order on the

cell damage indices, Chaves and associates (2013) examined the issue on the CK and concluded that exercise order is not an effective factor on this enzyme (12). Despite the fact that this research was designed to examine the effect of exercise order of resistance exercise on muscles' CK and LDH, however, sufficient evidence to determine the effect of RT on changes of muscles' CK and LDH during the rest is lacking. It has been reported that creatine kinase enzyme during rest and following exercise training is important for trainers and sport clinics to quantify the type of exercise more suited to athletes (13). In a rare number of researches that have examined the effect of RT on CK and LDH enzyme activities, da Silva and associates (2010) demonstrated that four weeks of RT in the form of delorme and with oxford resistance training with 10 repetition maximum intensity significantly increase the level of CK and LDH enzymes activities with no significant differences observed between these two methods (14). However, the study of CK and LDH in sport medicine allows to obtain information on the state of the muscle. For this reason, it seems like there is a need to examine the effect of exercise order of resistance training programs on muscle cell damage.

## 2. Objectives

This research was designed to determine the effect of exercise order of resistance training programs in the form of large to small and vice versa on the muscles' strength and creatine kinase and lactate dehydrogenase levels of active young women.

## 3. Patients and Methods

### 3.1. Subjects

In semi experimental design, 24 active young women aged between 20 - 30 years with no experience of resistance training volunteered to participate in the study. The primary health status was evaluated by a researcher designed questionnaire in which the cardiovascular disease, history of severe hospitalization, neuromuscular disease in addition to surgery and chronic disease history were identified and excluded from the sample. No subject reported taking exogenous anabolic-androgenic steroids, drugs, medication, or dietary supplements with the potential to affect redox and muscle capacity during study. The selected sample was advised not to consume any nonsteroidal anti-inflammatory drug belonging to aspirin family. All the participants signed a human consent form prior to randomly assigning into three equal groups of small-large muscle (SLM, n = 8), large-small muscle (LSM, n = 8) and control groups (con, n = 8).

### 3.2. Functional and Physiological Measurements

Prior to the start of the RT, subjects visited the laboratory four days at baseline. On the first day, 5 mL of blood from the left hand antecubital vein was gathered after 5 minutes of rest in a in a seat position following 10 hours of fasting in order to measure the activity of CK and LDH enzymes. In their second day, a series of anthropometrics measurements including high and weight (Seca, Mod 220, Germany) and body fat percent which was estimate by skin fold thickness (Lafayette, Mod 01127, USA) were done (15). On the 3<sup>rd</sup> and 4<sup>th</sup> day, the participants took part in familiarization sessions for performing resistance training of the upper extremities. In these two days, participants were familiarized to the resistance training protocol. Free weights and exercise machines were used for performing bench press (BP), lat pull down (LP), triceps (TC) with machine and biceps (BI) with barbell.

The participants received instructions about how to perform the acts correctly and how to inhale and exhale during the workouts. Then, the 1RM was assessed in all these exercises. In this way, prior to the start of the test and performing 1RM, warm-up exercises including 15 - 20 repetitions per exercise were performed. After 2 - 3 minutes, subsequent trial was performed for 1RM with progressively heavier weights until the 1RM was determined within three attempts, with 3 - 5 minutes of rest between trails. The 1RM was determined in fewer than five attempts with a rest interval of 3 - 5 minutes between 1RM attempts while 10 - 15 minutes was allowed before the start of the test of the next exercise. To minimize errors during 1RM testing, the following strategies were adopted: a) standardized instructions concerning the testing procedure were given to participants before the test; b) participants received standardized instructions on exercise technique; c) verbal encouragement was provided during the testing procedure (16). For the purpose of maximizing validity, the one-repetition maximum was performed on two different days in test-retest form. Accordingly, 48 - 72 hours later, 1RM was assessed. Table 1 presents the general characteristics of the participants.

### 3.3. Resistance Training Program

The resistance training program was performed for 6 weeks, three times per week on nonconsecutive days. LSM performed BP, LP, TC with machine and BI with barbell while SLM performed the same sequence of action in an inverse order. In order to eliminate the order effect, another group, serving as the control group, performed this sequence of actions alternatively; switching from SLM to LSM and vice versa. All the experimental groups performed the first and second week of RT programs with 4 sets at 12 -

**Table 1.** Physiological and Performance Characteristics of the Participants Before the Training Program

	LSM	SLM	Control	F	P Value
Age, y	25.25 ± 3.49	25.50 ± 3.74	25.62 ± 3.73	0.022	0.978
High, cm	167.12 ± 6.64	166.75 ± 5.47	167.75 ± 5.36	0.06	0.942
Weight, kg	67.37 ± 5.78	67.37 ± 4.6	67.25 ± 7.02	0.001	0.999
BMI, kg × m <sup>-2</sup>	24.23 ± 0.66	24.27 ± 0.59	23.91 ± 1.21	0.404	0.673
Bench press, kg	23.91 ± 5.31	24.13 ± 5.51	24.03 ± 5.36	0.003	0.995

15 RM load with one-minute rest interval between each set. On the third and fourth weeks, the program included 3 sets at 8 - 12 RM load with 120 seconds of rest interval between sets. The fifth and sixth weeks of the program, the load was increased with 3 - 5 repetition-maximum interspaced with 180 seconds of rest.

Warm-up program included 15 - 20 repetitions for each exercise. Verbal encouragements were given, especially during the performing 3<sup>rd</sup> and 4<sup>th</sup> sets to perform all sets to concentric failure and to completion of successful repetition. The participants were asked to perform every exercise thoroughly and to its highest range of motion. When the subject was unable to complete the move, the number of repetition and sets were recorded in order to calculate the total volume of the RT. The total work was calculated by weight × set number × repetitions in all participants.

#### 3.4. Blood Sampling and Biochemical Analyzing

Four days prior to the start of the RT, after 10 hours of fasting between 8 - 10, 5 cc of venous blood was obtained following 5 minutes of rest for measuring the level of CK and LDH at the pretest condition. In order to avoid the acute effect of last session of training, 48 hours of rest interval was given before collecting posttest venous blood sample in a similar condition to the pretest state.

The blood sample was left to coagulate at room temperature for 30 minutes and was centrifuged at 1500 g for 10 minutes to separate the serum, which was analyzed immediately or was stored at 20°C. CK and LDH were assayed spectrophotometrically through the use of commercially available kits (Pars Azmmon, Iran) that employed optimized conditions in accordance with the recommendations of the international federation of clinical chemistry (1920). Reference range of CK concentration for men using this method is 45 - 130 U/L. The catalytic concentration of CK was expressed as U/L at 37°C.

#### 3.5. Statistical Analysis

Descriptive statistics including mean ± standard deviation were calculated. One-way analysis of variance

(ANOVA) was employed before the start of the training protocol on every act to assure the homogeneity of the groups on repetition maximum, percent of body fat and body mass index of the three groups. In addition, two-way ANOVA repeated measures (2 × 3) was employed to compare the means. The result of analysis indicated that there was no homogeneity in creatine kinase level; therefore, analysis of covariance was used to control the pretest effect. All the analysis was performed by SPSS 17.0 and the level of significance was set to 0.05.

## 4. Results

The result of analysis indicated that there was no significant difference between the groups in performance and LDH before the start of RT program ( $P > 0.05$ ).

In addition the results showed that there was no significant difference between the work volumes of the experimental groups (SLM = 398.7 kg, (LSM = 408.5 kg), (Control = 387.4 kg) ( $P > 0.05$ ).

Also, results showed that strength increased significantly in all exercises in post test than the pretest ( $P < 0.05$ ), however, there was no significant difference between these exercise of the three groups in the post test ( $P > 0.05$ ).

The result of analysis of variance repeated measure showed that there was a significant main effect for the time variable in LDH ( $P = 0.000$ ). However, there was neither significant group main effect nor interaction of time and training conditions ( $P = 0.250$ ,  $P = 0.579$ ). These results are an indication for the significant increase of LDH enzyme during the six weeks but no difference in the order of training program for the enzyme change in three groups (Table 4).

The result of analysis showed that there was no significant main effect for the six weeks of resistance training program (time) ( $P = 0.425$ ) nor was there any significant interaction effect for the time and order on CK ( $P = 0.446$ ). These results are presented in Table 5.

**Table 2.** Presents the Resistance Training Protocol<sup>a</sup>

	1 - 2 wk	3 - 4 wk	5 - 6 wk
<b>Sets</b>	4	4	3
<b>Intensity, RM</b>	12 - 15	8 - 12	3 - 5
<b>Rest interval between Sets, s</b>	60	120	180

<sup>a</sup>Exercise was bench press, lat pull down, triceps and biceps curl.

**Table 3.** 1RM (kg) Tests, Kilogram of Body Mass at Baseline and After 6 Weeks of Resistance Training<sup>a</sup>

Group	Bench Press	Lat Pull Down	Triceps	Biceps
<b>LSM</b>				
Baseline		12.15 ± 2.7	14.6 ± 3.7	7.33 ± 2.796
After training	23.91 ± 5.3	23.03 ± 3.56	25.54 ± 4.1	20.8 ± 3.8
<b>SLM</b>				
Baseline	37.7 ± 3.8	12.37 ± 2.95	14.82 ± 4.0	7.5 ± 2.7
After training	24.1 ± 5.5	23.6 ± 3.82	25.7 ± 4.2	21.1 ± 4.0
<b>Con</b>				
Baseline	38.1 ± 4.5	12.29 ± 3.03	14.78 ± 3.8	7.4 ± 2.7
After training	24.0338 ± 5.3	23.37 ± 3.5	25.68 ± 3.8	20.95 ± 3.7

<sup>a</sup>Data are expressed as means ± SD.

**Table 4.** Lactate Dehydrogenase Enzyme Changes Before and After the Resistance Training Programs<sup>a</sup>

LDH (U/L)	LSM	SLM	Control
<b>Pretest</b>	320.6 ± 80.02	288.7 ± 43.8	308.6 ± 54.04
<b>Posttest</b>	315 ± 54.5	311.8 ± 51.1	311.1 ± 63.8

<sup>a</sup>Data are expressed as means ± SD.

**Table 5.** Creatine Kinase Enzyme Changes Before and After the Resistance Training Programs<sup>a</sup>

CK (U/L)	LSM	SLM	Control
<b>Pretest</b>	60.12 ± 11.59	159.25 ± 43.53	89.25 ± 6.92
<b>Posttest</b>	96.62 ± 23.50	130.12 ± 27.28	125.37 ± 37.15

<sup>a</sup>Data are expressed as means ± SD.

## 5. Discussion

The aim of the present research was to examine the effect of order of RT programs on the muscle strength and changes of LDH and CK enzymes as the markers of cell damage. For this purpose, untrained participants were exposed to different exercise orders of RT programs for six weeks. In order to determine the net effect of exercise order as the independent variable on muscle strength and enzyme changes activity, it was necessary to match the rest inter-

val, work volume, speed of performing the acts, types of muscle contractions, types of exercises, intensity and repetitions in both groups. Thus, the researcher attempted to employ identical exercise condition which differed in the order of performance.

The result of this research indicated that there was no significant differences in the amount of work performed (volume = workload × repetition × sets) by LSM and SLM and control groups, therefore, the groups in all conditions

performed identical resistance training programs.

The result also showed that there were no significant differences in the body fat percent and body mass index of the groups. In addition, the groups were similar in 1RM (1 RM in bench press). It is important to homogenous body fat percentage, because it has been reported that body fat percent and body mass index are associated with oxidative pressure and such condition may influence the enzyme response. There are reports that show with the increase in body fat, oxidative pressure is increased (17). The increase in oxidative stress and production of free radicals may result in lipid peroxidation of cell membrane and cause the secretion of CK and LDH enzymes into the cell plasma and increase of their activity there (18).

The result indicated that the strength increased significantly in all exercises in SLM, LSM and control group, while there was no significant difference in the three groups. It is unlikely that the increase in the strength of muscles occurred due to the hypertrophy of the involved muscle since the exercise program lasted only six weeks and this length of training is insufficient to lead to hypertrophy, thus the increase of muscle strength may be attributed to neuromuscular adaptation (19). It has been reported that one to two months of RT leads to neuromuscular adaptation by recruitment of more motor units into actions and coordination of recruitment. Learning effect and activity of the large muscles involved result in neuromuscular adaptation (20). The result of this research is in agreement with the findings of Spinetti et al. (2010) who examined the effect of exercise order of RT for 12 weeks and reported that the strength of all the muscles for bench press, lat pull down, triceps and biceps increased significantly while there was no significant difference in the order of exercise with large and small muscle groups except the bench press exercise (6). Simao et al. (2010, 2013) also showed that an RT program performed twice per week for 12 consecutive weeks with the order of small to large and vice versa arrangement resulted in increase of 1RM strength of all the exercises involved in the training program except the curved arm barbell. It needs to be mentioned that researchers reported that exercise order is not a significant determinant of hypertrophy and strength (4, 5). Despite the agreement of the results of these researches, it needs to be noted that the increase in strength of all muscles was observed. It seems like the training experiments of the participants in this research contributed to these contradictory findings. In the present study subjects were untrained females with no experience of resistance training. Therefore, it may be assumed that the starting strength of the participants in the present study has been low and their response to the RT has been high whereas the participants in the aforementioned researches were untrained but benefited from a relatively

desirable physical fitness condition.

The result of the present research showed that the level of CK enzyme activity did not change significantly after the end of RT and the CK levels were not significantly different in the three groups. In a limited number of researches conducted to examine the effect of RT on baseline activity of CK and LDH, da Silva and et al. (2010) reported that performing RT in the form of delorme and with oxford methods for four weeks, significant increase was observed in creatine kinase and lactate dehydrogenase enzyme (14). It seems like the presence of the difference in the findings are due to the duration, type of the protocol and intensity of the training program (21, 22). The activity of CK may be interpreted as an indication of suitability of prescribed exercise program. High level of activity of CK indicates that the stress and training load was too high to induce muscle cell injury, Conversely, the low level of these enzymes shows that the training program does not lead to adaptation (23). In the present study CK levels did not change significantly after RT in all groups. Therefore, the resistance training protocol of present study is probably safe to counsel athletes with suspected muscle damage to continue to undertake RT. In this research, CK and LDH has been in the suitable range, so that it can be said that intensity of resistance training is safe and it is according to the recommended values (23).

In the present research, it was observed that regardless of exercise order, the level of CK enzyme did not change significantly. The level of change in CK enzyme depends on many factors including age, muscle mass, the level of physical activity, climate and gender (11).

In the present study, our subjects are young active untrained women. It seems gender hormones may have an effect on CK enzyme changes. There are reports that show estrogen is a significant factor in cell membrane integrity and prevents CK secretion from the damaged muscle cells (24). In the present research, it was found that the basal changes in CK and LDH after RT did not show susceptibility to the exercise order. It seems lack of differences (baseline CK and LDH) between these two RT protocols is related to response of CK and LDH to exercise order of resistance exercise, since different responses eventually lead to different adaptations.

However, the response of enzymes due to the exercise order of RT was not examined in the present research. But the CK and LDH responses to exercise order of resistance exercise were examined by Chaves et al. (2013). These authors examined the changes in CK of 10 young men with experience of two years of resistance exercise performed at 80% with 1RM including 6 exercises by upper and lower extremities. They reported that there were no significant differences in changes in CK enzyme of the two exercise or-

der of resistance exercise (12). These findings indicate that applying different orders of exercise in resistance exercise program is not a significant factor (stimulus) that may lead to the cell adaptation against muscle damage; thus it has no significant effect on CK and LDH enzymes of baseline serum.

However, there is some limitation in results of the present study. We could not control dietary habits of subjects accurately. Also, some variables such as age, gender, race, muscle mass, physical activity and climatic condition can affect the CK changes. Therefore the present results of study should be considered regarding these limitations.

### 5.1. Conclusion

The result of this research indicated that the increase in muscle strength and changes in muscle damage indices is not dependent on the exercise order of resistance training and the exercise order of resistance training is not a significant factor in the occurrence of micro trauma. According to the result of this research, it may be safe to advise the coaches and athletes not to be concerned about the order of exercise in resistance training in regard to muscle damages and muscular strength development; and follow their resistance training program based on their goals and designs.

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### Footnotes

**Authors' Contribution:** Mohammad Ali Azarbayjani developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and is guarantor. Maydeye Nazari and Kamal Azizbeigi contributed to the development of the protocol, abstracted data, and prepared the manuscript.

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