

Effect of Blood Flow Restricted Exercise on Acute Systemic Irisin, Myostatin and Decorin Levels

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The aim of the study is to measure and compare acute systemic irisin, myostatin and decorin levels after a single session of blood flow restricted resistance exercise and resistance exercise without blood flow restriction in healthy, trained male participants aged 18-35 years. For this purpose, a total of 22 people will be included in the study. Participants will be randomly allocated to 2 exercise groups as resistance exercise with blood flow restriction (BFR-RE) and

resistance exercise without blood flow restriction (HL-RE) and will be subjected to cross-over. In the HL-RE intervention, the exercise will be performed with a loading of 80% of 1 RM, with 4 sets x 7 repetitions, with 60 seconds of rest between sets. In the BFR-RE intervention, the exercise will be done as a set of 30 repetitions with a loading of 30% of 1 RM and an additional 3 sets x 15 repetitions, with 30 seconds rest between sets. Total exercise volumes were tried to be equalized and skeletal muscle hypertrophy was selected in accordance with exercise guidelines. In both groups, bilateral leg extension exercise will be performed using the leg extension tool for resistance exercise. In the blood flow restriction group (BFR-RE), the cuff will be placed in the proximal region of the thigh bilaterally, inflated to a pressure equivalent to 50% of the estimated arterial occlusion pressure (AOP), and leg extension exercise will be performed under this condition. In the BFR-RE group, the blood flow restriction time will be between 5-10 minutes. Exercise sessions will be conducted under supervision. Venous blood samples will be collected from the arm antecubital region of the participants just before the exercise session, immediately after the exercise, and 1 hour after the exercise. Plasma irisin, myostatin and decorin levels will be measured from the samples taken. It is well known that resistance exercise is important in maintaining and increasing muscle mass (hypertrophy). Studies have shown the involvement of certain myokines in skeletal muscle hypertrophy, although few studies have been conducted on the systemic response of myokines to BFR-RE that may play a potential role in hypertrophy. Therefore, the planned study aimed to reveal the similarities or differences in the systemic myokine response between BFR-RE and HL-RE.

22 male participants between the ages of 18-35 will be included in the study. Participants must have been doing resistance exercises for at least one year. In the study, plasma irisin, myostatin and decorin myokine levels will be measured from venous blood samples to be taken from the participants. Sample size was analyzed using 2-group repeated measures (three time points for each period) crossover design, single-factor, repeated measure methodology (repeated measure anova). A sample of 19 athletes measured at 6 time points reaches 80% power to detect differences between averages using the F-Test at a significance level of 0.05. The effect size was set as $f = 0.25$ (medium) and the correlation between measurements was taken as 0.5. The accepted sample size for equal group size is 20. Considering loss of follow-up or non-compliance with the experiment, the final sample size was 22 with a 10% attrition rate.

A randomization algorithm (Maximum Allowed % Deviation = 10%) was applied using PASS software 11.0 (NCSS LLC, Kaysville, UT) to generate a randomization list that would allow participants to be assigned to two groups of 11 each.

The parameters to be examined in statistical analyzes are plasma irisin, myostatin and decorin levels. Statistical analyzes IBM SPSS Statistics 25.0 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) package program and R 3.5.3 software (R software, version 3.5.3, R Foundation for Statistical Computing, Vienna, Austria ; <http://r-project.org>). Significance level was determined as 0.05 in all analyzes. Time group interaction will be analyzed at a significance level of 0.1. In the study, numerical data will be summarized with mean, standard deviation, median, minimum and maximum values, and categorical data will be summarized using frequency and ratio values. The assumption of normality in numerical variables will be checked with the Shapiro Wilk test at each group and time point, and in parallel with the results, the time-dependent change in the groups will be analyzed

parametrically with the linear mixed model (linear mixed model) or non-parametrically with the Brunner-Langer model. If the interaction is found to be significant, subgroup analyses will be made, both within and between groups. Bonferroni correction will be used for multiple comparisons. Qualitative variables will be analyzed with Pearson Chi-square test, while all quantitative demographic data between groups will be analyzed with independent group ANOVA or Kruskal-Wallis test.

Study Protocol:

- Informing the participants about the study and obtaining their consent.
- Recording the demographic information of the participants.
- Making anthropometric measurements before the interventions, filling out the EGZ-A+ questionnaire, calculating HRR, making 1RM and AOP measurements.
- Exercise interventions will be done at the same times of the day and at 1 week intervals.
- Before the exercise interventions, warm-up on the bicycle ergometer at 50% of the HRR for 10 minutes, followed by lower extremity stretches.
- In the HL-RE intervention, the exercise will be performed with 4 sets x 7 repetitions, with a 60-second rest between sets, with the load on the knee extension device being 80% of 1 RM. Venous blood samples will be taken immediately before exercise, immediately after exercise, and 1 hour after exercise.
- In the BFR-RE intervention, the cuff will be placed in the proximal thigh region and inflated with the help of the cuff to the pressure corresponding to 50% of the AOP, and the exercise will be performed under this condition. The exercise will be performed as a set of 30 repetitions with a load of 30% of 1 RM on the knee extension device and an additional 3 sets x 15 repetitions, with 30 seconds rest between sets. Venous blood samples will be taken immediately before exercise, immediately after exercise, and 1 hour after exercise.
- In order to increase the comfort of the participant, blood samples will be collected by experienced people through a vein to be opened in the arm antecubital region.