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# Acute effect of blood flow restricted resistance exercise on irisin and sex hormones

## Kan akımı kısıtlamalı direnç egzersizlerinin irisine ve cinsiyet hormonlarına akut etkisi

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**Abstract**

**Aim:** The relationship between exercise and irisin and sex hormone release is unclear and is of interest to current research. This study aimed to investigate the acute effect of blood flow restricted (BFR) resistance exercise on irisin and sex hormones. **Materials and Methods:** Healthy males (n=17) aged 20-35 years were included in the study. Participants' physical activity levels were determined using International Physical Activity Questionnaire (IPAQ)-short form. Participants underwent body composition analysis and isotonic muscle strength measurement and were randomly divided into 3 groups: low-intensity (20% of 1-RM) BFR resistance exercise (n=6), high-intensity (70% of 1-RM) non-BFR resistance exercise (n=5), and a control group (n=6). Blood samples were obtained 15 minutes post-exercise to assess acute irisin, testosterone, and estrogen responses. **Results:** The study included 17 healthy males. The mean age of participants was 26.1±2.89 years, mean physical activity was 1259.1±1003.12 MET/week, and mean 1-RM was 41.1±7.48 kg. The descriptive characteristics of the groups were homogeneously distributed (p>0.05). The acute post-intervention irisin and sex hormone levels were not statistically different between the groups (p>0.05). **Conclusion:** NThere was no difference found between the levels of irisin and sex hormones released after low-intensity BFR resistance exercise and high-intensity non-BFR resistance exercise.

**Öz**

**Amaç:** Egzersiz ile irisin ve seks hormonlarının salınımı arasındaki ilişki netlik kazanmamış, güncel bir araştırma konusu olarak ilgi çekmektedir. Bu çalışmada kan akımı kısıtlamalı direnç egzersizlerinin irisin ve cinsiyet hormonlarına akut etkisinin incelenmesi amaçlanmıştır. **Gereç ve Yöntem:** Araştırmaya, yaşı 20-35 yıl arasında olan sağlıklı erkekler (n=17) dahil edilmiştir. Katılımcıların fiziksel aktivite düzeyi Uluslararası Fiziksel Aktivite Anketi-kısa form ile belirlenmiştir. Vücut kompozisyon analizi ve izotonik kas kuvvet ölçümü yapılan bireyler kan akımı kısıtlamalı düşük şiddetli (1-Maksimum Tekrar'ın %20'sinde) direnç egzersizi (n=6), kan akımı kısıtlamasız yüksek şiddetli (1-Maksimum Tekrar'ın %70'inde) direnç egzersizi (n=5) ve kontrol grubu (n=6) olarak 3 gruba ayrılmıştır. Egzersize akut irisin, testosteron ve östrojen yanıtını değerlendirmek için egzersiz sonrası 15. dakika katılımcılardan kan alınmıştır. **Bulgular:** Araştırmaya 17 sağlıklı erkek katılmıştır. Katılımcıların yaş ortalaması 26,1±2,89 yıl, fiziksel aktivite düzeyleri 1259,1±1003,12 MET/hafta, 1-Maksimum Tekrarı ise ortalama 41,12±7,48 kg saptanmıştır. Grupların tanımlayıcı özellikleri homojen dağılmıştır (p>0,05). Akut egzersiz sonrasında irisin ve cinsiyet hormon seviyelerinde gruplar arasında istatistiksel olarak anlamlı fark bulunmamaktadır (p>0,05). **Sonuç:** Kan akımı kısıtlamalı düşük şiddetli direnç egzersizleri ile kan akımı kısıtlamasız yüksek şiddetli direnç egzersizleri sonrasında salınan irisin ve cinsiyet hormonları düzeyleri arasında fark saptanmamıştır.

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## INTRODUCTION

Acute and chronic exercise stimulates metabolic adaptations in skeletal muscles, and mitochondrial biogenesis (the formation of new mitochondria from pre-existing mitochondria) in other organs, such as adipose tissue and the liver (1). The benefits of exercise are mediated not only by metabolic and molecular remodeling of skeletal muscles, but also by the release of cytokines from muscle, termed myokines (2).

Low-intensity exercise and blood flow restriction (BFR) are combined in a method known as BFR training, which produces results that are comparable to those of high-intensity training (3, 4). Recent studies have shown that low-intensity BFR resistance exercise (LI-BFR) can produce significant hypertrophy and strength gain (3-5). Although the mechanism is not fully understood, it has been proposed that the combination of mechanical tension and the ischemic and hypoxic setting resulting from metabolic stress act as primary factors that induce a hypertrophic response in the body (3, 5). It is argued that BFR exercise results in increases in anabolic hormones, type II muscle fiber ratio, and muscle fiber volume (6). Gunderman et al. argued that BFR exercise increased protein synthesis through the activation of mTORC1 signaling and the Akt pathway (7).

Irisin may be influenced by factors like age, gender, body composition (fat ratio, muscle mass, etc.), aerobic exercise intensity, exercise type, and physical fitness (8, 9). On the other hand, it is known that testosterone levels are associated with muscle mass increase and protection from muscle atrophy (10, 11). The literature regarding the relationship between serum irisin and testosterone levels is inconclusive, with studies reporting both negative and positive associations between the two hormones (12, 13). In addition, it has been shown that BFR exercise can achieve higher testosterone levels compared to high-intensity resistance exercise, but not significantly higher (14). Considering the relationship between muscle mass and testosterone levels, it was hypothesized that there would be a positive correlation between irisin and testosterone levels (10). The literature indicates that there is a need for studies on the effect of BFR exercise on irisin levels.

We hypothesized that BFR exercise will produce different acute irisin and sex hormone responses than non-BFR exercise. This study aimed to investigate the acute effect of exercise on irisin and sex hormones.

## MATERIALS AND METHODS

The study was granted approval by the local ethics committee (date 27/11/2019 and number 247). The study included healthy non-obese (BMI ≤ 30) males (n=17) aged 20-35 years who had not performed any regular aerobic or strength exercise (≥150 minutes/week) for the past 6 months, who had no known any health problem.

All participants who met the inclusion criteria were informed about the study (Table 1) and signed consent forms between September 2020-March 2021 in the Sports Medicine Department. Participants were asked not to eat anything for 2 hours and not to exercise or consume caffeine or alcohol for 24 hours before the intervention.

International Physical Activity Questionnaire (IPAQ)-short form (15) was used to determine physical activity levels of the participants and body composition analysis (16–18) was performed. Isotonic muscle strength test was performed two days before the intervention (19).

**Table 1.** Study design

| **Day –2** (pre-intervention) | **Groups** | **Day 0** (post-intervention) |
|---|---|---|
| Informed consent<br>Anamnesis & physical examination<br>Baseline blood tests<br>IPAQ-short form<br>Body composition analysis<br>Isotonic muscle strength test (1-RM) | **BFR Group:**<br>20% of 1-RM<br>4 sets: 30, 15, 15, and 15 repetitions<br>60 seconds rest between sets<br>**Non-BFR Group:**<br>70% of 1-RM<br>4 sets: 10, 10, 10, and 10 repetitions,<br>60 second rest between sets<br>**Control Group**<br>Non-intervention | Irisin, testosterone, and estradiol tests |

1-RM=One-Repetition Maximum, IPAQ=International Physical Activity Questionnaire, BFR=Blood Flow Restricted.

### 1. Body Composition Analysis

Height, weight, thigh circumference, abdominal circumference, hip circumference of participants were measured (16) and they were underwent body composition analysis (Tanita Body Fat Analyzer, Model BC 418 and Holtain Tanner/Whitehouse Skinfold Caliper, Holtain Limited, United Kingdom) (17).

**1.1. Muscle Mass Percentage Measurement:** Muscle mass percentage was measured with the bioimpedance method (Tanita Body Fat Analyzer, Model BC 418).

**1.2. Body Fat Percentage Measurement:** Subcutaneous fat thickness was measured at three sites (chest, abdomen, and thigh) with a skinfold caliper (Holtain Tanner/Whitehouse Skinfold Caliper, Holtain Limited, United Kingdom). The Jackson-Pollock equation was used to calculate body fat ratio (20).

**1.3. Physical Fitness Tests and Pre-Workout Warm-Up Protocol:** All participants performed warm-up exercises before all tests and exercises. The warm-up included 5 minutes cyling exercise at a resistance of 75 Watt (W) and a speed of 75 revolutions per minute (rpm) using a cycle ergometer (Monark 894E, Peak Bike, Sweden).

## 2. Isotonic Muscle Strength Tests

Isotonic muscle strength was calculated by determining the maximum weight a participant could successfully lift only once (1-RM) through knee extension while sitting. For this purpose, the weights lifted, and numbers of repetitions were recorded. The results were used to calculate 1-RM for the left and right legs individually using the following equation (19): 1-RM = weight (kg) × (36/(37−repetitions)).

## 3. Characteristics of Study Groups

The participants were randomly divided into three groups using simple random sampling. The participants in the BFR group (n=6) performed bilateral low-intensity (20% of 1-RM) knee extension exercises (4 sets of 30, 15, 15, and 15 repetitions, respectively; 60 seconds of rest between each set) under BFR in a seated position on an isotonic leg extension machine (Figure 1). The participants in the non-BFR group (n=5) performed bilateral high-intensity (70% of 1-RM) knee extension exercises (4 sets of 10 repetitions; 60 seconds of rest between each set) without BFR in a seated position on an isotonic leg extension machine. To ensure that the exercises were effective, the participants were requested to complete the full range of motion in all repetitions, and cadence was monitored constantly with an audible metronome set to 0.67 Hz (18).

The participants in the control group (n=6) did not receive an exercise intervention (21).

**Figure 1.** Cuff placement and isotonic knee extension exercise with restricted blood flow

## 4. Determining the Occlusion Pressure

In order to determine the occlusion pressure for BFR exercise, the resting blood pressure of the lower extremity was measured with a sphygmomanometer for adults (Erka Perfect Aneroid, D-83646, Bad Tölz, Germany). The occlusion pressure was determined as 130% of the lower-extremity systolic blood pressure (22). During BFR exercise, blood flow was restricted with automated 10 cm wide cuffs (Smart Tool Cuff, Ohio, USA) in two different sizes (57 and 73 cm) depending on thigh circumference (Figure 2). The mean duration of BFR was 6 minutes.

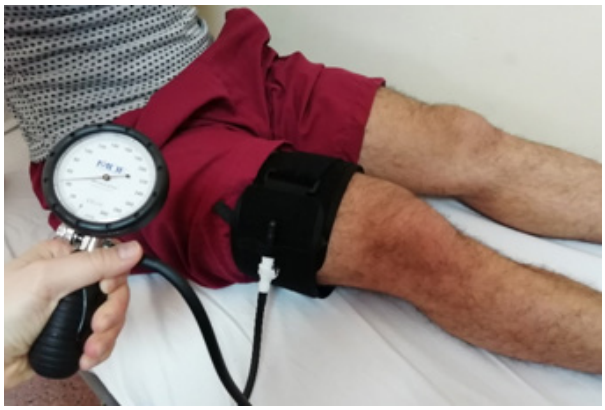

**Figure 2.** The cuff used to restrict blood flow

## 5. Biochemical Analysis

Baseline blood tests were performed to determine any participants that did not meet the inclusion criteria and included aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase(CPK), lactate dehydrogenase (LDH), glucose, urea, creatinine, lipid panel, thyroid-stimulating hormone (TSH), hemogram,

sedimentation, insulin, and high sensitive C-reactive protein (hsCRP).

The literature indicates that the half-lives of the glycosylated and de-glycosylated forms of irisin are 12 hours and 7 hours, respectively (23), and the half-life of steroid hormones is between 30-90 minutes (24). In reference to this information, we collected blood samples from all participants 15 minutes after the exercise intervention (2.30–3.30 p.m.) to measure acute irisin, testosterone, and estradiol responses.

For biochemical analysis, an experienced phlebotomist collected approximately 10 mL of venous blood from the antecubital vein. Blood was collected into serum separator tubes (SSTs) for biochemistry and, hormone, irisin measurements, into sodium citrate 3.2% containing tubes for D-dimer measurement, and into ethylenediaminetetraacetic acid (EDTA) containing tubes for sedimentation, hemogram and irisin measurements.

The blood samples were kept at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 minutes with a centrifuge device. Biochemistry tests were performed on the same day. AST, ALT, CPK, LDH, glucose, urea, creatinine lipid panel, and hsCRP were measured by biochemistry analyzer (AU 5800, Beckman Coulter, USA) using the spectrophotometric method. TSH, total testosterone, and estradiol were determined using the chemiluminescent method (Unicel DxI 800, Beckman Coulter, USA). Insulin was measured using the electrochemiluminescent method using a chemistry analyzer (Cobas 6000, Roche Diagnostics, Germany). D-dimer was determined using the immunoturbidimetric method in a coagulation device (ACL TOP 700, Instrumentation Laboratory, Italy). Sedimentation was measured using the modified Westergren method by an automated sedimentation device (Vision C, Yhlo Biotech, China). Hemogram was evaluated by a fully automatic hematology analyzer (UniCel DxH 800, Beckman Coulter, USA).

For irisin and testosterone measurement, serum and plasma samples were divided into three aliquots and stored in eppendorf tubes at –80°C until analysis. Commercial competitive enzyme-linked immunoassay (ELISA) kits were used to measure plasma irisin (Irisin, Recombinant ELISA, Phoenix Pharmaceuticals, USA; catalog no: EK-067-29) and serum free testosterone levels (Free Testosterone ELISA, DRG Instruments GmBH, Germany; catalog no: EIA-2924), in accordance with the instructions of the manufacturer.

### 6. Statistical Analysis

Data were analyzed using SPSS v 23.0 (IBM Corp., Armonk, NewYork, USA). After descriptive statistics were generated and normality of data distribution was tested with the Shapiro-Wilk test. Because of the distribution of the data is not normal, the differences between groups were analyzed using the Kruskal-Wallis test.

## RESULTS

The study included 17 adult males. The mean age was 26.1±2.89 years (range 23-33). The participants were randomly divided into three groups. The descriptive characteristics of the groups were homogeneously distributed (p>0.05), (Table 2).

**Table 2.** Descriptive characteristics

| | BFR Group | Non-BFR Group | Control Group | p value* |
|---|---|---|---|---|
| Age (years) | 26.3±3.67 | 25.4±1.95 | 26.5±3.08 | 0.825 |
| Weight (kg) | 78.7±13.77 | 81.5±18.18 | 83.6±14.33 | 0.879 |
| Height (cm) | 175.8±4.58 | 181.6±7.70 | 181.3±8.96 | 0.464 |
| BMI (kg/m2) | 25.4±4.27 | 24.4±3.69 | 25.2±2.33 | 0.945 |
| Thigh circumference (cm) | 52.3±7.00 | 48.4±5.03 | 48.8±2.64 | 0.555 |
| Abdominal circumference (cm) | 92.1±6.24 | 93.6±12.34 | 97.0±11.31 | 0.788 |
| Hip circumference (cm) | 96.3±7.96 | 98.2±7.69 | 104.0±8.53 | 0.355 |
| Body fat ratio (%) | 17.0±4.19 | 17.8±5.48 | 19.1±4.61 | 0.747 |
| Fat mass (kg) | 13.8±5.52 | 15.3±7.66 | 16.5±6.42 | 0.835 |
| Fat-free mass (kg) | 64.8±8.32 | 66.2±10.84 | 67.1±8.42 | 0.848 |

BMI=Body Mass Index. BFR=Blood Flow Restricted. BFR Group: n=6, Non-BFR Group: n=5, Control Group: n=6. *=p value is significant at the 0.05 level with determined by the Kruskal-Wallis test.

The mean physical activity of all participants was 1259.1±1003.12 MET/week. The mean 1-RM (as measured with isotonic muscle strength test) was 41.1±7.48 kg. The groups were statistically similar in terms of physical activity and isotonic muscle strength (p>0.05), (Table 3).

**Table 3.** Physical activity and isotonic leg extension muscle strength values.

| | BFR Group | Non-BFR Group | Control Group | p value* |
|---|---|---|---|---|
| **IPAQ-short form (MET/week)** | 1713.6±1525.38 | 1004.8±692.13 | 1016.6±356.09 | 0.960 |
| **1-RM (kg), mean** | 43.8±8.13 | 38.7±7.85 | 40.4±6.97 | 0.651 |
| **1-RM (kg), right leg** | 44.3±8.57 | 39.6±8.90 | 40.0±7.59 | 0.606 |
| **1-RM (kg), left leg** | 43.3±7.94 | 37.8±6.87 | 40.8±6.46 | 0.546 |

IPAQ=International Physical Activity Questionnaire, MET=Metabolic Equivalent of Task, 1-RM=One-Repetition Maximum. BFR=Blood Flow Restricted. BFR Group: n=6, Non-BFR Group: n=5, Control Group: n=6. *=p value is significant at the 0.05 level with determined by the Kruskal-Wallis test.

The acute post-exercise irisin and sex hormone levels were not statistically different between the groups (p>0.05), (Table 4).

**Table 4.** Post-intervention hormone levels

| | BFR Group | Non-BFR Group | Control Group | p value* |
|---|---|---|---|---|
| **Irisin (ng/mL)** | 16.8±0.85 | 16.3±1.04 | 16.6±1.29 | 0.778 |
| **Total testosterone (ng/dL)** | 386.1±89.09 | 377.2±114.2 | 386.1±145.03 | 0.996 |
| **Free testosterone (pg/mL)** | 14.2±2.23 | 10.5±6.15 | 12.0±5.19 | 0.511 |
| **Estradiol (pg/mL)** | 21.9±7.95 | 22.7±6.07 | 18.9±5.31 | 0.639 |

BFR=Blood Flow Restricted. BFR Group: n=6, Non-BFR Group: n=5, Control Group: n=6. *=p value is significant at the 0.05 level with determined by the Kruskal-Wallis test.

## DISCUSSION

The effect of different types of exercise on irisin and sex hormones is of interest to current research. This study investigated the acute effect of BFR exercise on circulating irisin, testosterone, and estrogen levels. There was no significant difference in irisin and sex hormone levels after low-intensity BFR and high-intensity non-BFR resistance exercise compared to the control group.

One study concerning the acute irisin response to different types of exercise (resistance exercise, endurance exercise, and combined resistance and endurance exercise) found that irisin was significantly higher one hour after exercise in the resistance exercise group compared to the remaining two groups (p<0.05) (25). Pekkala et al. reported that serum irisin levels were not statistically different aft ttter a single 1-hour exercise session on a bicycle ergometer at 50% of VO2max versus a single resistance exercise session of 5 sets of 10 repetitions in leg press (26).

There are a limited number of studies in the literature investigating the effects of BFR exercises on acute irisin and sex hormone levels. The only such study, to the best of our knowledge, evaluated acute irisin response after exercise in 8 participants and found that acute irisin levels more prominently increased after BFR exercise at 30% 1-RM compared to non-BFR exercise at 70% 1-RM (27). Unlike Kraemer et al. (27), we demonstrated that post-intervention irisin response was not statistically different between BFR and non-BFR groups.

There are relatively more studies on acute sex hormone response to BFR exercises than acute irisin response. Reeves et al. compared post-intervention growth hormone (GH), cortisol, testosterone, and free testosterone levels between eight healthy subjects under three groups: BFR exercise at 30% 1-RM, non-BFR exercise at 70% 1-RM, and BFR without exercise (21). They found that BFR exercises elicited a greater GH response, but that the groups were not statistically different in terms of testosterone, free testosterone, and cortisol levels. The authors argued that the acute GH response after BFR exercise was essential to hypertrophy, and the contribution of testosterone was negligible in the acute setting (21). Madareme et al. have found no significant difference in testosterone levels between BFR and non-BFR groups after one session of quadriceps resistance training consisting of 3 sets of 30, 15, and 15 repetitions, respectively (28). Another study by Sharifi et al. compared testosterone levels after one session of BFR training, two sessions of BFR training, one session of non-BFR resistance training, two sessions of non-BFR resistance training, and a control group, and have found no significant increase in testosterone levels in the intervention groups compared to controls (29). Fekri-Kourabbaslou et al. reported that different recovery models applied during BFR exercise have not found differences in testosterone and cortisol levels (30). Current literature suggests that acute and chronic testosterone response to BFR exercise is minimal. Despite this lack of response, these exercises produced noteworthy increases in size and strength, therefore, systemic increases of endogenous levels of testosterone may not be necessary for muscular hypertrophy (31). Similarly, we have not found any statistically significant difference between post-intervention testosterone levels of the study groups.

According to the available literature, there are no studies reporting a negative or positive relationship between serum irisin and estrogen levels. However, reduced irisin levels have been reported in amenorrheic athletes compared to eumenorrheic athletes and non-athletes (32).

Similarly, women with polycystic ovary syndrome (PCOS) were reported to have decreased levels of irisin (33). Considering the low estrogen in amenorrheic athletes and women with PCOS, it can be hypothesized that there is a positive relationship between serum irisin and estrogen levels. Considering the relationship between sex hormones and medical conditions such as PCOS, hypogonadism, and metabolic syndrome, it can be beneficial to confirm the relationship between metabolic conditions and irisin levels and treatment plan and exercise interventions accordingly. Hence, further studies are needed to elucidate the relationship between irisin and sex hormones and exercise types, duration, and intensity.

The major limitation of our study is using a case-control instead of a cross-over design, and the small number of participants in each study group.

There was no difference found between the levels of irisin and sex hormones released after low-intensity BFR resistance exercise and high-intensity non-BFR resistance exercise. Future prospective randomized controlled trials are needed to explain irisin and sex hormone responses associated with BFR exercise.

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