Comparison of acute effects of different resistance exercise protocols with and without blood flow restriction on selected hypertrophy-related hormones in competitive wrestlers

Javad Lael Sadeghi1, Hadi Habibi2, Sadegh Amani-Shalamzari1*

Abstract

The study aimed to compare the acute effects of low resistance exercises with partial and complete blood flow restriction (BFR) and heavy resistance exercise on growth hormone (GH), myostatin, testosterone, and cortisol in competitive wrestlers. Forty elite wrestlers were randomly divided into four groups (n=10); low resistance training with complete BFR (LRT+CBFR), low resistance training with partial BFR (LRT+PBFR), low resistance training (LRT), and heavy resistance training (HRT). Blood samples were collected before and after the intervention, and a specific ELISA kit measured variables. Analysis of covariance and paired t-test was performed to analyze the data. There were no significant differences in the variables between the four interventions. Intra-group results showed a significant decrease in myostatin levels in the HRT group (p=0.02), and a significant increase in GH in the LRT+CBFR (p=0.02) and LRT+PBFR (p=0.03), testosterone in the HRT group (p=0.04) and cortisol in the three groups LRT+CBFR (p=0.02), LRT+PBFR (p=0.01) and HRT (p=0.04). Despite the similarity of the changes in the four interventions, due to the percentage of changes, it seems that low resistance training with BFR could produce similar anabolic effects to high-intensity resistance training.

Key Words: Occlusion training, Wrestling, Myostatin, Growth hormone, Testosterone, Cortisol

Introduction

Coaches and athletes are always looking for new training methods that can help improve their athletic and cognitive performance. One of these models considered widely by researchers in recent decades is an exercise with limited blood flow (BFR) or Katsu exercise (Pope et al., 2013). Blood flow restriction has been proposed as a practical exercise model that improves muscle strength and endurance without producing much muscle force (Abe et al., 2006, Sudo et al., 2017). By inducing local hypoxia alone or in combination with exercise, BFR can have beneficial effects on skeletal muscle structure and function (Sudo et al., 2017). The American College of Sports Medicine suggests that for hypertrophy, resistance training should be performed at 70% of one maximum repetition (1RM), but injured individuals cannot perform this intensity of exercise; hence BFR is a practical way to promote hypertrophy with low loads. Muscle hypertrophy has been reported with the low load resistance training (20% 1RM) with BFR (Karabulut et al., 2010). Therefore, low-load resistance training with BFR seems to be a safe and effective alternative to high-intensity training (Karabulut et al., 2010), especially for injured athletes and older adults (Bigdeli et al., 2020; Loenneke et al., 2010). Although the mechanisms of hypertrophy resulting from training associated with BFR are not fully understood; cell swelling, increased metabolic stress, and increased recruitment of type 2 fibers have been suggested to stimulate anabolic signaling pathways, eventually leading to muscle hypertrophy with the release of growth factors (Jessee et al., 2018, Suga et al., 2012).

Testosterone is the most crucial androgen hormone in the human body regulating various functions such as increasing libido, metabolism, muscle development, and bone health (Wang et al., 2000). Research has shown resistance exercise leads to the release of this hormone in the blood (Vingren et al., 2010). Cortisol is the primary stress and catabolic hormone which has metabolic and anti-inflammatory effects on the body. Research has shown that circulatory cortisol level increased fol-
-lowing resistance exercise (Kraemer & Ratamess, 2005). Therefore, the testosterone to cortisol ratio appears to determine the body's anabolic or catabolic state. In addition, growth hormone and myostatin are also involved in the anabolic state. Any exercise, especially resistance exercise, increases GH levels (Kraemer & Ratamess, 2005); a substantial increase in GH level was reported following resistance training with BFR (Amani-Shalamzari et al., 2020). Kim et al. (2014) examined growth hormone and cortisol response to heavy- and low-load resistance exercise with BFR. They reported a marked increase in both without significant differences between the two interventions (Kim et al., 2014). Myostatin, an inhibitor of muscle growth, binds to actin II receptors in skeletal muscle and inhibits intracellular growth signaling pathways (Gonzalez-Cadavid et al., 1998). Myostatin also inactivates satellite cells and down-regulates the myogenic genetic factors (Rios et al., 2002). Decreased expression of the myostatin mRNA has been reported after a bout of resistance exercise (Kazemi, 2016) and regular resistance training (Bagheri et al., 2019). Therefore, it is evident that acute changes in the circulatory level of these hormones would effectively determine the anabolic state. However, the contribution of exercise intensity and BFR on the serum levels of these hormones is unclear.

The pressure of cuffs is one of the variables that affect the intensity of exercise. Research has mainly used 140 to 230 mmHg in the lower extremities. Suga et al. (2012) reported that muscle phosphate accumulation (Pi), as an indicator of fatigue, was achieved only at high pressures (230 mmHg) and moderate pressure levels (180 mmHg) (Suga et al., 2012). Overall, high cuff pressure with low intensity of exercise seems to cause muscle-level adaptations that manifest as increased muscle strength and size (Fujita et al., 2007). Given the different cuff pressure influences on adaptations and the lack of substantial scientific literature, in this study, we examined the response of hormones involved in hypertrophy (myostatin, growth hormone, testosterone, and cortisol) to a resistance exercise with different cuff pressures.

Materials and Methods

Subjects

The present research was a quasi-experimental study with pre- and post-test. Forty male elite wrestlers from Golestan province with more than 10 years of training experience, who attended a national tournament for at least a recent year, voluntarily participated in the study after completing the consent form and health and training questionnaires. One week before starting the intervention, anthropometric characteristics such as weight, height, age, body mass index, and maximum dynamic strength were measured. Subjects were already asked not to take any exercise supplements or specific medications that affect muscle protein balance for at least one month before starting the study period. The diet was monitored 24 hours before the athletes' test to prevent stimulant and caffeine intake. The anthropometric characteristics of the subjects are presented in Table 1. Maximum dynamic strength was measured according to the protocols. Randomization was performed by a third person who was not in the research group. Eligible participants were equally divided into heavy resistance training (HRT), low resistance training (LRT), low resistance training with partial BFR (LRT+PBFR), and low resistance training with complete BFR (LRT+CBFR) groups. To estimate the number of participants needed in the study, a sample size calculation was performed using G*Power Software version 3.1.9.6 for repeated measure analysis, using a rejection criterion of 0.05 and 0.8 (1-β) power, and medium effect (f=0.5). The power calculation indicated that a minimum of 40 participants was required to find such an effect.

Exercise training protocol

Warm-up includes running on a treadmill with 50-70% of maximum heart rate for 10 minutes, followed by two sets of 15 repetitions with a 40% one maximum repetition (1RM). Each repetition lasted 4 seconds, including two seconds for the eccentric phase and 2 seconds for the concentric phase. The selected movements included chest press, leg press, lat pulldown, knee flexion, elbow flexion, knee extension, elbow extension, and calf press. The formula "number of repetitions × intensity = training load" was used to equilibrium the training load intergroup. The group that performed the exercise with high load performed the exercises in three sets with 10 repetitions at 75% 1RM, and the other three groups performed the movements in four sets (15-15-15) with an intensity of 30% RM. The break between sets was 90 seconds, and there were three minutes between exercises (18). To restrict blood flow, a pneumatic cuff (5cm, width, Ghamatpooyan, Iran) was fastened at the proximal end of the thigh and arm. Pneumatic cuffs connected to gauge, after inflating and reaching to target pressure, the connection was cut off. Complete arterial occlusion pressure (AOP) was calculated using the following two formulas for the lower and upper body. Then one group trained with 50% AOP and the other group with 100% AOP (Loenneke et al., 2015).

Arterial occlusion for leg (mmHg) = 5.893 × (Thigh circumference) + 0.734 × (DBP) + 0.912 × (SBP) − 220.046

Arterial occlusion for arm (mmHg) = 0.514 × (SBP) + 0.339 × (DBP) + 1.461 × (Arm circumference) + 17.236

Cuffs remained inflated during the training period and were immediately deflated during the recovery period. A digital blood
pressure monitor measured the systolic blood pressure (SBP) and diastolic blood pressure (DBP). The thigh and arm circumferences were measured by a tape meter.

Hormonal analysis

At baseline and 20-30 min (Kraemer & Ratamess, 2005), venous blood samples were drawn for hormonal analysis immediately after the training sessions. Blood samples (5cc) were taken from an antecubital vein and collected in ethylenediaminetetraacetic (EDTA) tubes. Blood samples were centrifuged (4°C, 3000rpm) for 10 min to isolate serum and were then stored at -20°C until subsequent analysis. Serum samples were analyzed by enzyme immunoassay for free testosterone (testosterone kit IBL, RES2151, Hamburg, Germany), cortisol (cortisol kit IBL, RES2061, Hamburg, Germany), myostatin (R&D Systems, DGDF80, Inc, USA), and growth hormone (growth hormone kit IBL, MG59121, Hamburg, Germany). The inter-and intra-assay coefficients of variations (CV) of hormone measurement were 5.5% and 3.1% (testosterone), 3.4% and 2.6% (cortisol), (myostatin) 2.5% and 3.6% and 8.1% and 4.9% (growth hormone).

### Table 1. Subject’s anthropometric characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (year)</th>
<th>Body mass (kg)</th>
<th>Body fat (%)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRT+CBFR (100%)</td>
<td>24.4 ± 3.7</td>
<td>68.1 ± 4.7</td>
<td>16.8 ± 3.5</td>
<td>18.2 ± 2.2</td>
</tr>
<tr>
<td>LRT+PBFR (50%)</td>
<td>23.9 ± 2.2</td>
<td>70.0 ± 3.6</td>
<td>17.2 ± 2.3</td>
<td>19.2 ± 1.7</td>
</tr>
<tr>
<td>HRT</td>
<td>25.1 ± 3.2</td>
<td>69.2 ± 6.1</td>
<td>16.7 ± 3.1</td>
<td>18.1 ± 3.3</td>
</tr>
<tr>
<td>LRT</td>
<td>24.3 ± 3.1</td>
<td>71.9 ± 5.4</td>
<td>18.5 ± 3.2</td>
<td>21.1 ± 3.3</td>
</tr>
</tbody>
</table>

### Table 2. The value of selected hormones before and after the intervention.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Response</th>
<th>Change fold (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myostatin (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRT+CBFR</td>
<td>69.5 ± 3.7</td>
<td>67.3 ± 3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>LRT+PBFR</td>
<td>68.1 ± 4.7</td>
<td>66.1 ± 2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>HRT</td>
<td>70.5 ± 4.3</td>
<td>67.2 ± 3.3*</td>
<td>4.9*</td>
</tr>
<tr>
<td>LRT</td>
<td>68.7 ± 4.8</td>
<td>67.8 ± 4.0</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Growth hormone (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRT+CBFR</td>
<td>1.7 ± 0.5</td>
<td>2.4 ± 0.7*</td>
<td>41.2</td>
</tr>
<tr>
<td>LRT+PBFR</td>
<td>1.8 ± 0.5</td>
<td>2.6 ± 0.9*</td>
<td>44.3</td>
</tr>
<tr>
<td>HRT</td>
<td>2.2 ± 0.5</td>
<td>2.7 ± 1.3</td>
<td>22.7</td>
</tr>
<tr>
<td>LRT</td>
<td>1.9 ± 0.8</td>
<td>2.2 ± 1.1</td>
<td>15.7</td>
</tr>
<tr>
<td><strong>Testosterone (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRT+CBFR</td>
<td>7.5 ± 0.8</td>
<td>8.2 ± 0.8</td>
<td>9.6</td>
</tr>
<tr>
<td>LRT+PBFR</td>
<td>8.1 ± 1.2</td>
<td>8.6 ± 1.5</td>
<td>6.7</td>
</tr>
<tr>
<td>HRT</td>
<td>8.2 ± 1.4</td>
<td>9.5 ± 1.7*</td>
<td>15.8</td>
</tr>
<tr>
<td>LRT</td>
<td>7.8 ± 1.2</td>
<td>8.1 ± 1.5</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Cortisol (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRT+CBFR</td>
<td>319.1 ± 52.7</td>
<td>367.0 ± 56.6*</td>
<td>14.9</td>
</tr>
<tr>
<td>LRT+PBFR</td>
<td>334.5 ± 56.2</td>
<td>373.1 ± 56.1*</td>
<td>11.2</td>
</tr>
<tr>
<td>HRT</td>
<td>354.9 ± 21.3</td>
<td>386.9 ± 50.4*</td>
<td>9.0</td>
</tr>
<tr>
<td>LRT</td>
<td>304.6 ± 47.5</td>
<td>326.8 ± 41.2</td>
<td>7.3</td>
</tr>
</tbody>
</table>

* significant difference with baseline values

**Statistical analysis**

The Statistical Package of Social Sciences (SPSS, IBM, v20) was performed to analyze data. The data were presented by measuring the mean and standard deviation (SD). Shapiro-Wilk test was used to evaluate the normality of data distribution. Paired samples t-test was also used to determine to mean differences between the variables in the pretest and posttest in each group. Moreover, a one-way analysis of covariance (ANCOVA) was performed to determine differences between the groups. The level of significance was considered to be P<0.05.

**Results**

The value of selected hormones before and after the intervention are presented in Table 2. There was no significant difference between the two groups in terms of the baseline variables. The analysis of covariance showed that there was no significant difference in all measured hormones (p>0.05). Myostatin levels decreased in all four groups without any significant differences intergroup (F=0.45, p=0.71). The paired t-test showed the highest percentage of decrease (4.9%) in the HRT group (p=0.02). Also,
growth hormone increased in all four groups without any significant changes between groups (F=0.47 p=0.70), and the results of paired t-test showed a significant increase in both BFR groups (Table 2) (p<0.05). There was no significant difference between groups in testosterone level (F=1.90, p=0.15). Intragroup changes in testosterone with a 15.8% increase were significant only in the HRT group (p=0.04), and in other groups, the changes were not significant. There was no significant difference between the group in cortisol levels (F=1.69, p=0.19). Intragroup changes in cortisol were not significant only in the LRT group, and there were significant increases in the other groups. The highest percentage increase was 14.68% in the complete BFR group.

Discussion

This study aimed to compare the acute changes in testosterone, cortisol, growth hormone, and myostatin following four different resistance training protocols with and without BFR. No significant difference was observed in the measured hormone levels after the four resistance protocols. However, compared to baseline values, a marked decrease in myostatin and a substantial increase in cortisol and testosterone levels were observed in the HRT group and increased growth hormone and cortisol levels in both BFR groups. Overall, the response of these variables to a bout of resistance exercise with various intensities is similar.

All four interventions decreased myostatin levels, but the declined level was not statistically significant inter-groups. The HRT group showed the highest decrease by 4.9%, and the lowest decrease by 1.4% was observed in the LRT group. The percentage reduction in both BFR groups was moderate (3%). Myostatin is one of the critical cytokines that negatively regulates skeletal muscle growth (Ma et al., 2003), and its inhibition has been shown to increase muscle strength and mass (Whittemore et al., 2003). The previous study supported our findings and demonstrated that moderate-intensity exercise (70-60% 1RM) reduces serum myostatin levels (Roth et al., 2003). The exact mechanism of myostatin reduction has not been studied; however, Mothers against decapentaplegic homolog 7 (SMAD-7) has been reported to increase as an intracellular myostatin cascade inhibitor in response to the mechanical and metabolic stimulus (Aoki et al., 2009). Therefore, this protein is involved in reducing the myostatin expression in active muscles. Also, Lorinto et al. (2012) attributed the decreased myostatin levels to increased expression of SMAD-7 and G-protein coupled receptor-associated sorting protein-1 (GASP-1) due to metabolic stress associated with resistance training with BFR (Laurentino et al., 2012). Therefore, a bout of resistance training through mechanical and metabolic stress reduces myostatin levels.

Four resistance exercise protocols significantly increased the circulating levels of GH, and no significant difference was observed between the groups. The highest percentage increments were observed in the two BFR groups (Table 2). Elevated GH levels after a resistance exercise have been reported in many studies (Hakkinen et al., 2000; Kraemer et al., 1999). It appears that the GH response depends on the exercise intensity, so that the increase in GH was observed following heavy resistance exercise (>85% 1RM). In contrast, a slight increase was observed with low-load (Kraemer et al., 2005). Furthermore, an enormous GH response has occurred by adding BAR to low-intensity resistance training (Kim et al., 2014) and running-based training (Amani-Shalamzari et al., 2020). Hypoxia and metabolic stress induced by BFR have been reported as the causes of increased GH because there is a direct relationship between lactate and GH production (Godfrey et al., 2009). Therefore, the cause of large GH release in both BFR groups could be hypoxia and lactic acid production.

Despite the increase in circulating testosterone following four interventions, only testosterone levels rose significantly in the HRT group compared to baseline levels. There was no difference between the interventions in the testosterone response. The highest and lowest increase was observed in the HRT (15.8%) and LRT (3.2%) groups, respectively. Research has shown that high-volume, moderate-to-high loads protocols, with short break intervals and large muscle mass, increased testosterone levels more than low-volume, high-load, and long rest intervals (Kraemer & Ratamess, 2005). It may be the reason for the difference in the increasing percentage in the HRT and the LRT groups. Occlusion exercise leads to a marked increase in testosterone level by increasing the accumulation of metabolites, especially lactate, as it has been shown that lactate could increase testosterone production by activating cAMP within Leydig cells (Lu et al., 1997). Therefore, it is evident that all interventions increase testosterone production, and interventions that impose more loads on the body cause more testosterone production.

Elevated cortisol levels were observed in four interventions, and no differences were observed inter-groups. Except for the LRT group, cortisol response to the exercise interventions was significant in other groups. In general, the acute response of cortisol to exercise depends on the intensity and exercise duration. In adults, most studies reported a similar increase in cortisol levels after a resistance workout (Kraemer & Ratamess, 2005; Kon et al., 2012; Reeves et al., 2006). Consistent with our results, studies have shown that cortisol response to resistance training with BFR in adults was not significantly different from traditional resistance training (Kon et al., 2012; Reeves et al., 2006). It seems that the stress imposed on the muscles in both BFR interventions and heavy resistance exercise was slightly more than light resistance exercise.
Conclusion

Overall, although no significant differences were observed between the groups, given the percentage of the changes in the groups, the groups who received more load created a more anabolic environment. If the interventions continue, it may lead to apparent changes. Given the stress imposed in both groups of low-intensity resistance training with BFR, especially in the group with complete occlusion, it is very similar to heavy resistance training; therefore, if athletes cannot do heavy resistance training, they can use low-intensity resistance training with complete BFR.

What is already known on this subject?

Heavy resistance training causes to release more anabolic hormones.

What this study adds?

Although in this study the changes in hormones in various groups were not statistically significant, the percentage of changes was higher in the groups that endured more load. Thus, low-load resistance training with BFR creates more metabolic pressure and anabolic environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All research procedures were approved by the Ethic committees of Kharazmi University and were conducted in accordance with the Declaration of Helsinki.

Informed consent Participants signed a written informed consent form that was approved by the ethical committee.

Author contributions


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