

Research article

## Hormone Responses to an Acute Bout of Low Intensity Blood Flow Restricted Resistance Exercise in College-Aged Females

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### Abstract

The purpose of this study was to determine whether the acute hormone response to exercise differed between low intensity blood flow restricted resistance exercise and traditional high-intensity resistance exercise in college-aged women. A total of 13 healthy women (aged 18-25 yrs), who were taking oral contraceptives, volunteered for this randomized crossover study. Subjects performed a session of low intensity blood flow restricted resistance exercise (BFR) (20% of 1-RM, 1 set 30 reps, 2 sets 15 reps) and a session of traditional high intensity resistance exercise without blood flow restriction (HI) (3 sets of 10 repetitions at 80% of 1-RM) on separate days. Fasting serum cortisol and growth hormone (GH) and blood lactate responses were measured in the morning pre and post exercise sessions. GH (Change: HI:  $6.34 \pm 1.72$ ; BFR:  $4.22 \pm 1.40$  ng·mL<sup>-1</sup>) and cortisol (Change: HI:  $4.46 \pm 1.53$ ; BFR:  $8.10 \pm 2.30$  ug·dL<sup>-1</sup>) significantly ( $p < 0.05$ ) increased immediately post exercise for both protocols compared to baseline and there were no significant differences between the protocols for these responses. In contrast, blood lactate levels (HI:  $7.35 \pm 0.45$ ; BFR:  $4.02 \pm 0.33$  mmol·L<sup>-1</sup>) and ratings of perceived exertion were significantly ( $p < 0.01$ ) higher for the HI protocol. In conclusion, acute BFR restricted resistance exercise stimulated similar increases in anabolic and catabolic hormone responses in young women.

**Key words:** Growth Hormone, Cortisol, blood flow restriction.

### Introduction

High intensity resistance exercise ( $\geq 70\%$  maximal strength) generally is recommended as an optimal stimulus for muscle hypertrophy and muscular strength (ACSM, 2009). It is important to consider, however, that there are individuals, such as the very sedentary, frail, elderly, and others with injury concerns, for whom high intensity resistance exercise may not be appropriate or may be contraindicated. A new type of exercise that involves blood flow restriction (BFR) performed in conjunction with resistance or walking exercise at low intensities has potential to be a useful modality for improving neuromuscular function in such populations. BFR exercise involves restricting arterial blood flow to the exercising muscle and occluding venous return resulting in blood pooling in the lower limbs (Manini and Clark, 2009). The intensity for BFR resistance exercise protocols in the literature varies from 15-30% of maximal strength, although many studies have utilized 20% of one repetition maximum (1-RM) as the low intensity stimulus (Fahs et

al., 2012; Loenneke et al., 2012). One advantage of this type of low intensity resistance exercise is the reduction in mechanical stress that is placed on the joints of the body (Sato et al., 2005).

Recent evidence supports the use of low intensity resistance exercise with blood flow restriction as an effective exercise mode to enhance muscular hypertrophy and muscular strength gains (Loenneke et al., 2012; Madarame et al., 2010; Takarada et al., 2000; Yasuda et al., 2010). Several mechanisms have been postulated for the muscular adaptations with low intensity BFR exercise, including a reduction in oxygen content (Tanimoto et al., 2005) and increases in lactate (Kawada, 2005; Takarada et al., 2000), which stimulates growth hormone (GH) release and the early recruitment of high threshold muscle fibers.

It is well-documented that resistance exercise is a stimulus for both acute and chronic hormone responses (see Crewther et al., 2011; Kraemer and Ratamess, 2005 for reviews). GH and cortisol are two hormones that have been studied extensively because of their potential role in muscular adaptations to resistance exercise (Kraemer and Ratamess, 2005). Previous literature suggests that the magnitude of the acute endocrine response is influenced by the intensity and volume of the resistance exercise, with high volume, moderate to high intensity protocols eliciting greater GH and cortisol responses (Kraemer and Ratamess, 2005). In addition, there are reported sex differences in resting GH levels and in acute GH responses to exercise (Kraemer et al., 1998). Similar to traditional high intensity protocols, low intensity BFR resistance exercise has been shown to acutely increase serum hormone levels and blood lactate in men (Sato et al., 2005; Takano et al., 2005; Tanimoto et al., 2005; Takarada et al., 2000). To date, there have been no studies conducted on acute hormone responses to BFR resistance exercise in women.

The purpose of this study was to determine whether the acute hormone response to exercise differed between low intensity BFR resistance exercise and traditional high-intensity resistance exercise in college-aged women. We hypothesized that low intensity BFR resistance exercise would elicit similar endocrine responses as a traditional high intensity resistance exercise session.

### Methods

#### Subjects

Thirteen healthy, recreationally active females aged 18-25 years who were taking oral contraceptives volunteered for this study. The sample size was based on a power analysis from previously published BFR hormone studies in men showing large effect sizes ( $> 3.0$ ) (Fujita et al., 2007; Sato et al., 2005; Takano et al., 2005), thus, a sample size of 13 was adequate to ensure a statistical power greater than .80 at a probability of  $p \leq 0.05$ . Subjects were screened with a health status questionnaire prior to participation in this study. They also completed a menstrual history and physical activity questionnaire to provide information about oral contraceptive use, menstrual cycle characteristics prior to OC use, and exercise participation history. None of the women had been engaged in a regular resistance training program for the previous four months prior to the study. Women were excluded if they had any orthopedic conditions that prevented them from exercising, if they had been diagnosed with any endocrine-related disorders, or if they had a BMI greater than  $40 \text{ kg}\cdot\text{m}^{-2}$ . The subjects gave written informed consent before participation. The Institutional Review Board at the University of Oklahoma approved this study.

### Research design

This study employed a randomized, repeated measures crossover design where subjects performed two resistance exercise protocols: 1. A low intensity blood flow restricted resistance exercise session (20% 1-RM, 1 set 30 reps, 2 sets 15 reps) (BFR); and 2. A high intensity resistance exercise (80% 1-RM, 3 sets 10 reps) (HI). The protocols were designed to differ in total workload, such that the HI total workload ( $\sim 4017 \text{ kg}$ ) would be approximately double the BFR total workload ( $\sim 2008 \text{ kg}$ ). Each protocol involved isotonic knee extension and leg press resistance exercises and the sessions were separated by 4 days based on previous responses from similar protocols in our laboratory.

This study required 3 visits to the Neuromuscular Laboratory. During the first visit, subjects gave informed consent, completed questionnaires, underwent total body scans for body composition assessment, performed muscular strength testing, and finally, were familiarized with the blood flow restriction cuffs and procedures. The resistance exercise protocols were completed in random order in the second and third visits, and were completed at the same time of the morning within subject. Morning (7-9 a.m.) fasting blood draws were collected at rest and immediately after each exercise protocol for hormone, lactate, and hematocrit measurements.

All subjects were instructed to eat a good meal the night prior to the intervention at about 5:30 or 6:00 pm. It was recommended that the meal contain about 400-600 grams of carbohydrate (examples were provided) and they were also told to consume a light snack around 8:00 pm. Explicit instructions were also given to drink plenty of water, to fast from 11:00 pm until the end of the testing session the following morning and to refrain from alcohol consumption for 24 hours prior to testing.

### Muscular strength testing

Muscular strength for the leg press and knee extension

isotonic exercises (Cybex International, Inc., Medway, MA) was assessed using standardized 1-RM testing procedures (Kraemer et al., 2006). After a 5 minute warm up on a cycle ergometer, each subject performed 8-10 repetitions at a light load (about 50% estimated 1RM) for the resistance exercise. After 1 minute rest, the subject then lifted a load about 80% of 1RM through the full range of motion. The load was increased after each successful lift (i.e., a full range of motion, proper form, and correct cadence) until a failed attempt occurred. One minute rest was given between attempts and the 1RM was achieved within 5 attempts. After a 5 minute rest period, the subject then performed the same procedure for the second resistance exercise. This testing procedure has been found to be reliable for both exercises (intraclass correlation coefficients  $> 0.99$ ) in young women in our laboratory (Seo et al., 2012).

### Body composition

Body composition was assessed by Dual Energy X-Ray Absorptiometry (GE Medical Systems, Lunar Prodigy encore software version 10.50.086, Madison, WI). All subjects underwent a total body scan while wearing light clothing in order to obtain % body fat, fat mass and fat-free mass values. The scan mode was set according to the truncal thickness (thick  $> 25 \text{ cm}$ , standard 13-25 cm, thin  $< 13 \text{ cm}$ ) and all scans were performed by a single qualified technician. In our laboratory, the coefficients of variation (CV %) for fat mass, % body fat, and fat free mass are 2.74%, 2.5% and 1.24%, respectively.

### Exercise protocols

Each exercise session began with a 5 minute warm up on a cycle ergometer. The HI protocol required subjects to perform two resistance exercises (knee extension, then leg press) at 80% 1RM, 3 sets x 10 repetitions with 1 minute rest between sets and exercises. For the BFR condition, subjects performed the exercises (knee extension then leg press) while wearing specialized elastic cuffs (50 mm width, KAATSU Master, Sato Sports Plaza, Tokyo, Japan) on the proximal thighs. Two muscle groups were selected for testing in order to achieve a blood flow restriction time of about 10-15 minutes, similar to other previously published studies (Abe et al., 2005; Fahs et al., 2012; Karabulut et al., 2010). The initial cuff pressure was set between 40 and 60 mm Hg, then inflated to 120 mm Hg for 30 seconds then released. The cuff pressure was increased by 20 mm Hg incrementally until the target pressure of 200 Hg was reached as described by previously published studies (Abe et al., 2006; Yasuda et al., 2006). The subject then performed the knee extension exercise at 20% 1RM, 1 set of 30 repetitions followed by 2 sets of 15 repetitions (cadence 1.5 seconds concentric and 1.5 seconds eccentric) with 1 minute rest between sets. After 1 minute rest, the same protocol was performed for the leg press. The cuff was deflated and removed after the completion of the two lower body exercises and the total time of vascular restriction was about 10 minutes. Subjects were asked to give their ratings of perceived exertion (RPE) on the original Borg scale (6-20) after each set for each exercise (Borg, 1982).

### Blood sampling and hormone assays

Pre- and immediately post-exercise blood samples were obtained following an 8 hour fast in the morning by venipuncture. Venous blood was collected in serum separator tubes from an antecubital vein. Immediately after the venipuncture, blood lactate was measured and two capillary tubes were filled, then the samples were allowed to clot for approximately 15-20 minutes. Hematocrit was measured in duplicate by a micro-hematocrit centrifuge and a digital hematocrit reader (Stat Spin Inc., Norwood, MA). The percent changes in plasma volume were calculated by the following formula: % change plasma volume =  $(100 / (100 - \text{Hct pre})) \times 100 ((\text{Hct post}) / \text{Hct post})$  (Van Beaumont, 1972). Blood lactate concentrations were measured using an Accusport Lactate Analyzer (Boehringer Mannheim, Indianapolis, IN). Blood samples were centrifuged and the serum transferred into microtubes and stored in a freezer at -70 °C until the hormone assays were performed.

All serum samples for each subject were measured in the same assay. GH concentrations in serum samples and controls were measured in duplicate using immunoradiometric assay kits (Active Growth Hormone IRMA DSL-1900, Diagnostic Systems Laboratories Inc., Webster, TX). The intra assay CVs were 1-8% and inter assay CVs were 5.5-12.8%. Cortisol concentrations in serum samples and controls were determined in duplicate by radioimmunoassay kits (Active Cortisol RIA DSL-2100, Diagnostic Systems Laboratories Inc., Webster, TX). Intra assay and inter assay CVs ranged from 8.9-11.7% and 6.5-10.4%, respectively.

### Statistical analyses

Descriptive data are presented as means  $\pm$  SE. The Kolmogorov-Smirnov procedure was used to test the normality of the dependent variables. All variables were normally distributed. Two way repeated measures ANOVA (exercise condition  $\times$  time) was used to compare the effects of the two exercise protocols on RPE, lactate, hematocrit and hormone concentrations. Paired t-tests were used as post hoc tests when a significant exercise condition  $\times$  time interaction was found. Pearson product moment correlation coefficients were computed to determine relationships between pre-exercise lactate and hormone levels and absolute changes between time points in GH and cortisol. Statistical significance was set at  $p \leq 0.05$  and analyses were performed using SPSS version 19.0 software (SPSS Inc., Chicago, IL).

## Results

### Subjects

The physical characteristics of the subjects are shown in Table 1.

**Table 1. Subject Characteristics (n = 13).**

Variable	Mean ( $\pm$ SE)
Age (yrs)	21.5 (.6)
Height (m)	1.68 (.02)
Body fat (%)	30.2 (1.7)
Fat mass (kg)	19.6 (2.2)
Fat free mass (kg)	44.4 (1.4)
Recreational Activity (hrs/wk)	6.5 (1.2)
Knee Extension 1-RM (kg)	67.9 (4.2)
Leg Press 1-RM (kg)	99.4 (6.0)

1-RM – 1 Repetition Maximum

### Lactate and hematocrit responses

Table 2 shows the lactate and hematocrit responses to the BFR and HI protocols. There were significant main effects for condition and time ( $p < 0.001$ ) and a significant condition  $\times$  time interaction effect ( $p < 0.001$ ) for lactate responses. Lactate increased after both exercise protocols, but there was a greater increase in lactate in response to the HI protocol. Hematocrit significantly increased ( $p = 0.012$ ) from pre to post exercise for both exercise protocols, but there was no significant condition or condition  $\times$  time interaction effects. The percent changes in plasma volume were not significantly different between the two exercise protocols ( $-4.1 \pm 4.5\%$  for BFR;  $-9.4 \pm 1.7\%$  for HI). Participants reported significantly higher RPE scores for the HI protocol, except for after the first set of the knee extension exercise (Table 3).

**Table 3. Ratings of Perceived Exertion (RPE) During Exercise (n = 13). Values are mean ( $\pm$ SE).**

		BFR	HI
Knee Extension	Set 1	15.7 (.3)	16.6 (.7)
	Set 2	16.2 (.5)	17.9 (.5) **
	Set 3	16.9 (.6)	18.6 (.4) **
Leg Press	Set 1	13.2 (.6)	15.5 (.6) **
	Set 2	13.5 (.5)	16.9 (.6) **
	Set 3	13.9 (.6)	17.0 (.8) **

BFR – Blood Flow Restricted Resistance Exercise; HI - High-Intensity Resistance Exercise. \*\*  $p \leq 0.01$  significant difference between protocols.

### Hormone responses

Baseline GH and cortisol concentrations were not significantly different between the two exercise sessions (Table 2). There was a significant time main effect for cortisol ( $p < 0.001$ ) and GH ( $p < 0.001$ ), where both hormone concentrations increased; however, no significant exercise condition or condition  $\times$  time interaction effects were detected. Thus, GH and cortisol showed similar responses to the BFR and HI resistance exercise protocols. Absolute changes (pre to post exercise) in GH and cortisol

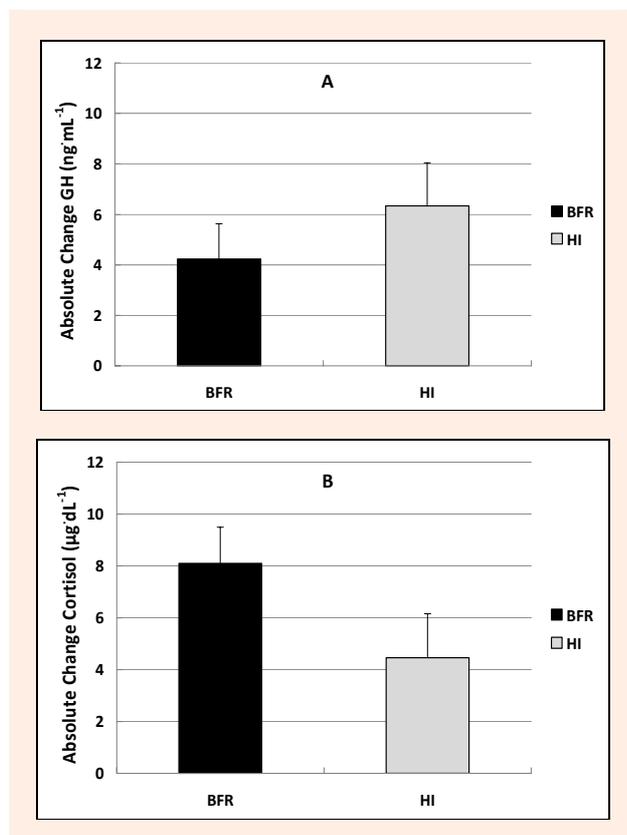
**Table 2. Blood Lactate, Hematocrit, and Hormone Responses Before (Pre) and Immediately Post (Post) Resistance Exercise (n = 13). Values are mean ( $\pm$  SE).**

Variable	BFR		HI	
	Pre	Post	Pre	Post
Hematocrit (%)	43.2 (1.0)	44.5 (.7) *	41.3 (1.6)	43.8 (.6) *
Lactate (mmol·L <sup>-1</sup> )	1.46 (.21)	4.02 (.33) **	1.33 (.20)	7.35 (.45) **†
Cortisol (µg·dL <sup>-1</sup> )	34.93 (3.15)	43.03 (2.51) **	35.16 (2.43)	39.62 (2.76) **
Growth Hormone (ng·mL <sup>-1</sup> )	2.00 (.82)	6.23 (1.40) **	2.09 (.87)	8.43 (1.62) **

\* $p < 0.05$  \*\*  $p < 0.01$  vs. Pre; †  $p < 0.01$  vs. BFR at same time point.

BFR – Blood Flow Restricted Resistance Exercise; HI - High-Intensity Resistance Exercise

were not significantly different between exercise conditions (Figure 1). There were no significant correlations between lactate and hormone levels at any time point. However, pre exercise cortisol was negatively related ( $r = -0.61$ ;  $p < 0.05$ ) to the absolute change (pre to post exercise) in cortisol for the BFR session.



**Figure 1.** Absolute Changes in Growth Hormone (A) and Cortisol (B) Levels in Response to Blood Flow Restricted (BFR) and Traditional High Intensity (HI) Resistance Exercise ( $n=13$ ). Values are Means  $\pm$  SE

## Discussion

To our knowledge, this study is the first investigation to assess acute hormone responses to blood flow restricted resistance exercise in young women. The major findings were that the BFR and HI protocols elicited similar GH and cortisol responses; however, RPE and lactate levels were significantly higher for the HI protocol. Our GH and cortisol response patterns agree with other investigations of hormone responses to traditional resistance exercise conducted in women (Hymer et al., 2001; Kraemer et al., 1993; 1998; 2008).

Comparisons of our data with previous BFR resistance exercise studies in men are complicated by protocol differences, such as the intensity, fixed number of sets/reps or reps performed to failure, number/type of exercises, the intensity of the comparative protocol and timing of blood sampling. Our GH findings are in general agreement with male studies that found low intensity BFR resistance exercise elicited similar GH responses to high intensity resistance exercise (Manini et al., 2012; Tanimoto et al., 2005). In contrast, higher GH responses to

BFR resistance exercise have been reported compared to low intensity (Fujita et al., 2007; Takarda et al., 2000) or moderate intensity ( $\sim 70\%$  1RM) (Reeves et al., 2006) traditional resistance exercise bouts. Fujita et al. (2007) found higher cortisol responses to BFR protocol compared to the low intensity control session but the protocols used by Reeves et al. (2006) did not elicit significant cortisol responses.

There are some differences in female responses to BFR resistance exercise compared to previous reports in males. The magnitude of the GH responses in our study seem slightly higher than the maximal GH responses in young men reported by Manini et al. (2012). This is not unexpected because women generally have higher resting GH levels than men and have been shown to have higher peak GH responses to exercise, particularly if they are taking oral contraceptives (Giannoulis et al., 2005; Kraemer et al., 1998; 2008). The women in our study reported higher RPE for the HI protocol. In contrast, young men reported similar RPE for BFR and high intensity resistance exercise protocols that were performed to fatigue (Hollander et al., 2010; Manini et al. 2012). We found that women had greater increases in lactate concentration during the HI protocol, whereas similar lactate responses between BFR and moderate to high intensity resistance exercise had been reported previously in young men (Reeves et al., 2006; Tanimoto et al., 2005). The higher RPE and lactate responses in our study are not surprising given the HI protocol was designed to have a higher total workload than the BFR protocol. The lactate response is important because blood lactate levels and acidosis are associated with higher GH and cortisol responses to resistance exercise (Kraemer and Ratamess, 2005). Another possible explanation for our RPE and lactate findings compared to previous reports in men is that our protocols did not require subjects to lift to volitional fatigue.

Concerning the timing of the responses, Manini et al. (2012) found that blood lactate levels were significantly higher initially after the high load protocol (peak lactate at 20 minutes after the onset of exercise), whereas a similar magnitude peak lactate for the BFR protocol occurred later in the protocol (30 minutes). Several other studies found that blood lactate was highest immediately post BFR exercise (Fujita et al., 2007; Reeves et al., 2006). Therefore, the observation of later peak lactate with BFR reported by Manini et al. (2012) may be more related to the blood sampling timing in their study rather than a delay in the lactate release into the blood because of venous pooling in the lower extremity.

There are several limitations to this study. We did not measure hydration status of the women before or during the exercise protocols, but we did give them explicit pre-testing dietary and exercise instructions. Since we included only women who were OC users, the findings of this study may not necessarily be applicable to women not taking OCs. We selected OC users to control for variations in growth hormone levels during the phases of the menstrual cycle (Hornum et al., 1997). However, it is important to consider that OC use has been associated with greater GH responses to acute high intensity resis-

tance exercise protocols than those observed during the follicular phase of the menstrual cycle (Kraemer et al., 2008).

## Conclusion

In conclusion, acute low intensity blood flow restricted resistance exercise was effective for stimulating increases in GH and cortisol in young women. More importantly, the magnitude of the hormone responses to the BFR protocol was similar to those elicited by the higher total workload HI protocol. In addition, the patterns of hormone responses were consistent with previous blood flow restriction studies conducted in young men.

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### Key points

- Growth hormone and cortisol levels significantly increased after a single bout of low intensity blood flow restricted resistance exercise in young women.
- There were no significant differences in hormone responses between the low intensity blood flow restricted protocol and the traditional high intensity higher total workload protocol.
- Low intensity blood flow restricted resistance exercise provides a sufficient stimulus to elicit anabolic and catabolic hormone responses in young women.

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