Effects of Short Term Low Intensity Resistance Training with Blood Flow Restriction on Bone Markers and Muscle Cross-Sectional Area in Young Men

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ABSTRACT

Int J Exerc Sci 5(2): 136-147, 2012. This study compared the effects of short term resistance training with and without blood flow restriction (BFR) on bone turnover markers and muscle cross-sectional area (MCSA) in young men (18-35 yrs). Subjects were randomly assigned to a BFR (20% 1RM) resistance training group (BFRT, n=10), a high intensity (80% 1RM) resistance training group (RT, n=10), or a BFR only group (BFR, n=10). Both BFRT and RT trained 3 days per week (2 sets, 10 repetitions) for 3 weeks for leg press, knee extension, and knee flexion isotonic exercises. BFR underwent the BFR procedure without the exercise protocol for 10 minutes 3 days per week. Body composition (DXA) and thigh MCSA (pQCT) were measured. Fasting bone formation (Bone ALP) and resorption (CTX) markers were assessed in the morning pre and post training. All groups significantly (p < 0.05) improved MCSA, but RT (3.48 ± 0.68 %) had a greater increase compared to BFR (1.15 ± 0.54 %). RT also showed a significant increase (p < 0.01) in Bone ALP after training (50.91 ± 12.77 %). In conclusion, low intensity resistance training with BFR was less effective than high intensity resistance training for eliciting bone formation and muscle hypertrophy responses.

KEY WORDS: Bone remodeling, vascular restriction, skeletal muscle

INTRODUCTION

It is well established that weight bearing physical activities involving high impact loading, such as tennis, jumping, and weight lifting, are important for the maintenance of bone health (17). In addition to its beneficial effects on bone mineral density (BMD), both acute (5) and chronic resistance exercise (11) have been shown to alter bone biomarkers. Bone remodeling is a dynamic process that is responsible for the maintenance of BMD and architecture in bone tissue. Biochemical markers of bone turnover provide a dynamic measurement of skeletal status (19). For example, bone alkaline phosphatase (Bone ALP), a marker of bone formation, and C-terminal cross-linking telopeptide of Type I collagen (CTX), a marker of bone resorption, have been used to measure bone metabolism and monitor bone remodeling rates (27). High levels of bone turnover markers are related to an
increased risk of fracture (24), whereas reduced bone turnover is associated with therapeutic efficacy of bone resorption inhibitors (10). Therefore, it is important to measure the ratio of bone formation to bone resorption for bone turnover imbalance as well as absolute value changes. Few studies, however, have investigated the responses of bone markers to different intensity exercises in healthy populations. Vincent and Braith (36) found that 6 months of high intensity (80% 1 repetition maximum (1RM)) or moderate intensity resistance exercise (50% 1RM) significantly increased Osteocalcin (OC), a bone formation marker, in both training groups, whereas only the high intensity group demonstrated a significant increase in Bone ALP in older adults. Similarly, Fujimura et al. (11) found that 4 months of high intensity resistance training increased Bone ALP, while it transiently suppressed OC in young men. These findings suggest that high intensity training imposes high levels of mechanical stress on the bone causing changes in bone remodeling rates.

Recent evidence indicates that low intensity high volume resistance exercise (e.g. 20% 1RM, 4 sets of 30-15-15-15 repetitions) combined with blood flow restriction (BFR) can increase muscle strength and hypertrophy (2, 4, 29, 32) as well as anabolic hormones (4, 30). This type of exercise involves the acute application of pressure to the upper thighs with specially designed belts, resulting in partial occlusion of blood flow to the lower legs. In a recent study (12), 6 days of twice daily low intensity (20% 1RM) resistance exercise with BFR produced significant muscle hypertrophy (+3.5%) and muscular strength (+7%). Also, BFR resistance exercise has been shown to elicit greater electromyographic activity in the contracting muscle than low intensity resistance exercise without occlusion (30). Low intensity resistance exercise with BFR may be an alternative mode of training for older adults and clinical populations who are unable to perform high intensity resistance training (14). Previous long term high intensity resistance training studies have shown higher increases in Bone ALP (11,36). On the other hand, its effects on bone metabolism with BFR have not been well established as there have been only two published studies to date on this topic. Beekley et al. (6) reported that 3 weeks of low intensity BFR walk training significantly increased serum Bone ALP, muscle cross-sectional area, and muscle strength in young men. Karabulut et al. (15) found that 6 weeks of low intensity resistance training with BFR significantly increased Bone ALP in older men to a similar magnitude as traditional high intensity resistance training. Although BFR resistance training does not impose high external loads to the bone, it causes changes in muscle oxygenation levels, which show large decreases (~80% reduction from resting levels) during the exercise bout and large increases (~40% increase above resting levels) after the exercise (34). Recently, Schipani et al. (25) suggested that hypoxia may stimulate the signaling pathway that couples angiogenesis and bone formation; thus, hypothetically, the transient hypoxia that occurs during BFR resistance exercise is a potential mechanism for increasing bone formation rate.

The purpose of this study was to compare the effects of a 3 week low intensity resistance exercise with blood flow restriction and high intensity resistance exercise on bone turnover markers and muscle cross-sectional area in healthy
young untrained males. We matched the training volumes of these two resistance training protocols to test the hypothesis that transient hypoxia associated with the BFR resistance training would stimulate similar bone and muscle adaptations as high intensity resistance exercise. Specifically, we expected significant increases in serum Bone ALP levels, decreases in serum CTX and increases in muscle size with both protocols.

METHODS

Participants
Thirty healthy males between the ages of 18 and 35 years were recruited from the University of Oklahoma and the surrounding Oklahoma City metro area. The subjects had not engaged in a resistance training program for at least 4 months prior to the study. This study was approved by the University Institutional Review Board for Human Subjects, and written informed consent was obtained from each subject.

Protocol
All subjects visited the Neuromuscular Laboratory at the University of Oklahoma prior to the first day of physiological testing to complete the informed consent, health status, calcium intake and Physical Activity Readiness Questionnaire (PAR-Q) forms. The subjects were randomly assigned to either the low intensity resistance group with BFR (BFRT, n=10), high intensity resistance group without BFR (RT, n=10) or to BFR only group (BFR, n=10). A sample size of 9 per group was needed for 80% statistical power for an effect size of 1.67 calculated from the Bone ALP findings reported by Beekley et al. (6). This experimental study involved two total body scans for regional body composition using Dual Energy X-Ray Absorptiometry (DXA). Muscle cross-sectional area (MCSA) and bone characteristics were measured using peripheral Quantitative Computed Tomography (pQCT) at baseline and after testing. On the first day of testing, the subjects in BFRT and RT groups completed strength (1RM) testing for leg press (LP), knee flexion (KF), and knee extension (KE) exercises to determine the training workloads. For BFRT and BFR groups, each subject had a familiarization session for training with BFR. Subjects were instructed to maintain their normal daily activities during the training program, which should not include any resistance exercise. All subjects were trained individually by project staff for their specific protocol and the compliance was 100%. The training protocols were conducted three days per week for about 20~30 minutes for 3 weeks. Both BFRT and RT groups performed the resistance training protocol consisting of a 5-10 minute warm-up (cycling) followed by 2 sets of 10 repetitions at an intensity workload (80% 1RM for RT and 20% 1RM for BFRT) for LP, KE, and KF exercises. BFRT and BFR groups underwent BFR procedures. Each subject in BFRT group wore BFR cuffs (KAATSU-Master, Sato Sports Plaza, Tokyo, Japan), which were placed around the upper thigh of each leg. Once the cuff pressure was inflated for each subject, they completed LP, KE, and KF followed by 2 sets of 10 repetitions at 20% of 1RM. The rest period was required for 2 minutes between exercises and for 1 minute between sets. RT group followed the same exercise protocol without BFR at 80% 1RM. BFR group underwent the BFR procedures without exercise protocol for 10 minutes. The pressure cuffs remained inflated throughout the training session, which lasted less than 15 minutes, and then pressure was released at the end of the
session. The participants were correctly instructed by an expert trainer on how to perform each exercise correctly.

Dual Energy X-Ray Absorptiometry (DXA) (enCORE 2002 version 10.50.086 software, GE Lunar Medical Systems, Madison WI) was used to measure body composition (%fat, fat mass, bone free lean body mass, fat free mass) of the subjects from total body scans conducted pre and post training. After having the subject remove footwear, the subject’s height and weight was obtained and all metal, plastic objects or other high density objects associated with the subject’s clothes were removed. Subjects were placed in a supine position, centered on the DXA table with Velcro straps placed around the knees and ankles. The scan mode was set based on the subject’s trunkal thickness as follows: Thick, >25 cm; Standard, 13-25 cm; and Thin, <13 cm. Quality assurance and spine phantom calibration procedures were performed each testing day prior to subject scans. One qualified technician performed all total body scans. In our laboratory, the coefficients of variation (CV%) for % fat, fat mass, and bone free lean body mass (BFLBM) variables are 2.5%, 2.7% and 1.4 %, respectively.

Muscle cross-sectional area (MCSA) at 50% of the right femur length was determined by pQCT (XCT 3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) by a trained pQCT technician at baseline and post training. The pQCT is used to determine the muscle area around the cross section of the diaphysis as well as separate measures of cortical and trabecular bone (8). In order to measure the scans, the subject was seated in the scanning chair with the right leg in the support straps, positioned in the center of the gantry, and participant was asked to remain still and to breathe normally during the scan acquisition. Scans were acquired with a voxel size of 0.4 mm, a slice thickness of 2.2 mm, and a scan speed of 20 mm/sec. The CalcBd function of the software is used to obtain results for total cross-sectional area in millimeters squared. Obtaining MCSA values requires two ‘CalcBd’ analyses to separate muscle, fat and bone. Scan analyses for MCSA used a threshold driven contour detection (Mode 1) and Peel (Mode 2). The thresholds used in analysis 1 were -100 and 40, and the thresholds used in analysis 2 were 710 and 40. A noise filter (F01F06U01) was applied to all scans. MCSA is derived by subtracting the ‘subcortical area’ of analysis 2 from ‘subcortical area’ of analysis 1. The same technician performed all scans. In our laboratory, the precision value (CV%) for mid thigh MCSA is 1.6%.

Muscular strength for the LP, KE, and KF resistance exercises was assessed by one repetition maximum (1RM) procedures at baseline and after the training period. After familiarization with the resistance machines, the subjects performed LP, KE, and KF at 8-10 at a light load (~ 50% of predicted 1RM) as a warm up. Following a 1 minute rest period, the load was increased until the subject was unable to lift the load through the full range of motion for a single repetition. The 1RM was achieved within 5 attempts. If a subject was able to lift the entire weight stack on a machine, then a multiple repetition maximum was used to predict the 1RM. The multiple repetition maximum was calculated using the following equations of Mayhew et al. (20).
%1RM = 52.2 + 41.9 x e^{(-0.055\text{reps})}

1RM = repetition weight (kg) / (predicted percent 1RM / 100)

The strength results are reported in a previous publication (16).

After a 5-10 minute warm up on a Monark bicycle ergometer, each subject in the BFRT and BFR groups wore specially designed elastic cuffs (50 mm width, BFR Master, Sato Sports Plaza, Tokyo, Japan) around both thighs at 1-2 cm distal to the inguinal folds. Resting systolic blood pressure (SBP) of the arm was measured using to estimate leg systolic blood pressure. Normal resting SBP of the legs is about 20% higher than the upper arm. In this present study, the final BFR pressure was 20 % higher than the estimated leg SBP. The cuff pressure used to restrict blood flow was determined with the following equation:

\[ \text{BFR pressure (mmHg)} = (\text{SBP} \times 1.2) \times 1.2. \]

After the cuffs were placed around the upper thigh, subjects were seated in a chair for the cuff inflation. The initial pressure of the cuffs was set at about 50 mmHg, then pressure was increased by 20 mmHg (starting at 120 mmHg) and held for 30 s, then released for 10 s until the final target pressure was reached. For the BFRT group, restrictive pressure was maintained throughout the period of resistance exercise, which lasted less than 15 minutes, including the rest periods and the pressure was released at the end of the session (figure 1). The BFR subjects remained seated in a chair with the inflated cuffs on for about 10 minutes, then the pressure was released.

Blood samples (approximately 6 ml) were obtained by a phlebotomist in the morning following an 8 hour overnight fast at baseline and 2-3 days after the last training session to control for last bout effects. Once the blood sample was collected from the antecubital vein, the sample was centrifuged in order to separate the serum from the red blood cells. The serum was aliquoted into microtubes and stored in a -84°C Celsius freezer until the bone marker assays were performed. The enzyme-linked immunosorbent assay (Serum CrossLaps ELISA) (Nordic Bioscience Diagnostics, Denmark) was used to measure the concentration of serum CTX. The range of intra assay and inter assay CVs were 1.96 - 2.37% and 0.59 - 11.52%, respectively. The Metra BAP enzyme immunoassay (Quidel Corporation, Mountain view, CA) was used to measure the serum concentrations of Bone ALP. The range of intra assay and inter assay coefficients of variation were 4.99 - 5.17% and 0.26 - 2.11%, respectively.
All serum samples were run in duplicate and all samples for a given subject were analyzed within the same assay.

**Statistical analysis**

All descriptive data are presented as the Mean ± SE for the dependent variables. Group differences in baseline values for the dependent variables were determined by one-way analysis of variance (ANOVA). A two-way ANOVA with repeated measures [Group (BFRT vs. RT vs. BFR) × Time (pre vs. post)] compared the responses over time between groups. If significant group × time interactions occurred, paired t-tests were used as post-hoc tests to determine significant time differences within each group. The individual percent changes in serum Bone ALP, CTX, Bone ALP/CTX ratio and MCSA were calculated \( %\Delta = \left( \frac{\text{post} - \text{pre}}{\text{pre}} \right) \times 100 \) for each subject. One-way ANOVA with the Bonferroni post hoc procedure was used to examine significant group differences in percent change bone marker and MCSA variables. All statistical procedures were performed by SPSS for Windows 18.0 version (Chicago, IL). The level of significance was set at \( p \leq 0.05 \).

**RESULTS**

Table 1 shows the means ± SE for the physical characteristics and pre and post training body composition variables for each group. There were no significant group differences at baseline for any of these variables (\( p > 0.05 \)). Body weight did not significantly change after the intervention, however, there were significant (\( p < 0.05 \)) time main effects for percent body fat, which showed a small decrease, and for BFLBM, which showed a small increase after training for all groups.

Table 1 shows MCSA changes after 3 weeks of training for each group. There were no significant group differences at baseline (\( p > 0.05 \)). There was a significant time main effect (\( p < 0.01 \)) for MCSA, which increased from pre to post training. There was a trend for a group × time interaction effect (\( p = 0.069 \)). One-way ANOVA detected significant group differences (\( p < 0.05 \)) in percent change in MCSA with RT (3.48 ± 0.68%) exhibiting greater percent increases than BFR (1.15 ± 0.54%) (figure 2).

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**Table 1. Body Composition and Muscle Cross-sectional Area**

<table>
<thead>
<tr>
<th>Variables</th>
<th>BFRT (n=10)</th>
<th>RT (n=10)</th>
<th>BFR (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>25.95 ± 1.62</td>
<td>--</td>
<td>22.35 ± 0.84</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.77 ± 1.98</td>
<td>--</td>
<td>177.68 ± 1.56</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.55 ± 5.00</td>
<td>85.05 ± 5.08</td>
<td>78.65 ± 4.40</td>
</tr>
<tr>
<td>% BF</td>
<td>26.53 ± 2.20</td>
<td>26.31 ± 2.15’</td>
<td>20.09 ± 3.15</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>23.08 ± 2.85</td>
<td>22.99 ± 2.76</td>
<td>16.26 ± 3.19</td>
</tr>
<tr>
<td>BFLBM (kg)</td>
<td>57.81 ± 2.17</td>
<td>58.42 ± 2.65’</td>
<td>55.92 ± 1.51</td>
</tr>
<tr>
<td>MCSA (mm²)</td>
<td>17348.66 ± 889.01</td>
<td>17758.13 ± 912.78”</td>
<td>15192.18 ± 558.15</td>
</tr>
</tbody>
</table>

Values are mean ± SE; BFRT, Low intensity resistance training with blood flow restriction group; RT, High intensity resistance training group; BFR, Blood flow restriction only; %BF, %Body Fat; FM, Fat Mass; BFLBM, Bone Free Lean Body Mass; MCSA, thigh muscle cross-sectional area.

No significant group differences at baseline for any of these variables (\( p > 0.05 \)). Significant time main effects for %BF (\( * p < 0.05 \)), BFLBM (\( * p < 0.05 \)) and MCSA (\( * p < 0.01 \)).
Table 2 shows the serum bone marker responses after 3 weeks of intervention training for the training groups. Baseline mean values for bone marker variables did not differ among the 3 groups (P > 0.05).

There was a significant group x time interaction effect for Bone ALP (p < 0.001), which significantly increased only in RT group. In addition, significant (p < 0.001) group differences in percent changes in serum Bone ALP concentrations were found (figure 3) as RT had a greater percent increase (50.91 ± 12.77 %) in Bone ALP compared to BFRT (6.73 ± 6.66 %, p < 0.01) and BFR (-6.00 ± 6.86 %, p < 0.001). There were no significant effects for serum CTX concentrations or significant group differences in percent changes in serum CTX (BFRT 17.47 ± 12.40 %; RT 2.22 ± 9.29 %; BFR 2.83 ± 10.78 %). The Bone ALP/CTX ratio had a significant group x time interaction effect (p < 0.01) and there was a trend (p = 0.058) for a group main effect (figure 4). The RT group showed a significant (p < 0.01) increase in the Bone ALP/CTX ratio from pre to post training and the percent increase for this group was significantly greater than that of the BFRT or BFR groups (RT 58.69 ± 19.83 %; BFRT -2.24 ± 10.59 %; BFR 3.46 ± 14.66 %).
DISCUSSION

Our primary finding was that low intensity resistance training (20% 1RM) with blood flow restriction was not as effective as traditional high intensity resistance training (80% 1RM) for stimulating Bone ALP responses for this short term intervention. We also found significant increases in mid thigh muscle cross-sectional area, which were greater for the traditional high intensity resistance training group than for the low intensity blood flow restriction group. As previously reported, the strength changes showed a similar pattern, as RT had a significantly greater percent increase (29.19 ± 3.97 %) in knee extension strength than BFRT (13.41 ± 2.98 %) and BFR (8.76 ± 3.66 %) groups (16).

In our first blood flow restriction study, we utilized a randomized crossover design where young men performed both acute resistance exercise protocols at a low intensity (20% 1RM) with and without blood flow restriction (7). We reported that the acute bout of BFR resistance training resulted in a significant decrease in the bone resorption marker (serum N-terminal cross-linking telopeptide of type I collagen) but did not affect the bone formation marker, Bone ALP. In the current study, we found a different pattern for chronic bone marker responses as Bone ALP increased in the high intensity resistance training group, but CTX did not change after any of the 3 week training protocols. This Bone ALP response, in conjunction with data from longer duration resistance training studies (11,28,36), supports the concept that high intensity exercise provides an osteogenic stimulus to the skeleton (35). Few investigations have been conducted on bone marker responses to short term exercise interventions. Lester et al. (18) examined the effects of different modes of exercise (aerobic, resistance, or combined aerobic and resistance) with a control group on bone markers after eight weeks of training. They found that the combined group and resistance exercise group had significant increases in serum Bone ALP concentrations post training. On the other hand, CTX concentrations were similar in all four groups and remained stable throughout the training programs. Although our training protocols differed from those used by Lester et al. (18), we had similar results for both Bone ALP and CTX concentrations.

To date, there has been only one study that examined the chronic effects of BFR resistance training on bone metabolism. In their 6 week intervention in older men, Karabulut et al. (15) found that the bone formation marker (Bone ALP) and the bone turnover marker ratio (Bone ALP/CTX) significantly improved with both the low intensity blood flow restriction resistance training and high intensity resistance training protocols compared to the non-
Since we also found significant Bone ALP responses to the high intensity resistance training protocol in only 3 weeks, the primary explanation for our lack of responses in the BFR resistance training group can be attributed to the volume of the BFR training protocol. Karabulut et al. (15) used 1 set of 30 repetitions, followed by 2 sets of 15 repetitions for their low intensity blood flow restriction training, whereas we used 2 sets of 10 repetitions for both resistance training protocols. We designed our study specifically to directly compare the intensities of the two training protocols (80% 1RM vs. 20% 1RM plus BFR), thus, we kept the number of repetitions and sets constant for the two protocols. It is evident from our findings that the higher training volume (sets × reps) typically used with BFR training is essential for stimulating bone marker responses, therefore, our data do not support the transient hypoxia mechanism for stimulating bone metabolism (25).

Muscular hypertrophy induced by resistance training (13,21,26) results from an increased rate of protein synthesis, decreased rate of protein degradation, or both (9). In this study, we evaluated MCSA using pQCT and documented a significant improvement, as the traditional high intensity group increased MCSA 3.48 ± 0.68% compared to 2.37 ± 0.64% for BFRT and 1.15 ± 0.54% for BFR. It should be noted that the BFR response was lower than precision (CV% 1.6%) for this measurement, thus, it is not considered a real change. Recent studies using short term low intensity resistance exercise (20 – 50% 1RM) with blood flow restriction have also found significant improvements in muscle strength and muscle size (3, 31,33). Abe et al. (4) reported that two weeks of twice daily low intensity resistance with BFR resulted in skeletal muscle size increases (8.5%) that were similar in magnitude to those reported in traditional high intensity training of 3-4 months (1). Based on the muscle hypertrophy findings of these short duration training studies, Fujita et al. (12) suggested higher training frequency (> 3sessions/week) for a shortened training period (< 5weeks) is a more efficient training program for increasing muscle mass and muscular strength. In contrast, other studies (23) reported that muscle hypertrophy becomes evident by 6-7 weeks of training, although changes in protein quality (9), fiber types (9) and protein synthesis rates (23) occur much earlier. There are several possible mechanisms for muscle hypertrophy during blood flow restriction resistance exercise. Previous studies have documented significant increases in anabolic hormones, such as serum GH and IGF-1concentrations in response to BFR exercise (4, 30). Also, resistance exercise with a cuff belt causes venous pooling and significant cell/muscle swelling, which may increase muscle CSA/volume (4). In terms of strength changes, both RT and BFRT had significant gains in knee extension strength but leg press and knee flexion did not significant increase pre to post training (16). Again, these findings may be attributed to the lower training volume in our study compared to the blood flow restriction resistance exercise protocol used by Abe et al. (4).

There are several limitations that may have affected the results of this study. Bone turnover marker responses may have been affected by hormonal or dietary factors that were not measured in this study. Although
we controlled for food intake and circadian rhythm, we did not assess or control vitamin D status, which influences bone metabolism (27). However, the intervention was conducted entirely in the summer months, thus, minimizing the seasonal influence of vitamin D on bone markers.

In conclusion, the traditional high intensity (80% 1RM) resistance exercise intervention was more effective for inducing bone formation marker responses compared to low intensity (20% 1RM) resistance exercise with blood flow restriction matched for training volume. Future studies should implement a high volume low intensity blood flow restriction resistance training protocol when examining bone responses.

ACKNOWLEDGEMENTS

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