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Original Research

The acute and chronic effects of resistance training with blood flow restriction on hormonal responses in untrained young men: A comparison of frequency

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Abstract: The present study aimed to determine the effect of low-intensity training with blood flow restriction (BFR) on the response rate of anabolic hormones. Forty healthy and untrained young men, aged 18 to 25 years old, were randomly divided into five groups: one session of BFR training (BFR1), two sessions of BFR training (BFR2), one session of resistance training without BFR (WBFR1), two sessions of resistance training without BFR (WBFR2), and the control group (without training). BFR groups had three sets of 20 repetitions with 20-30% 1RM, and none-BFR groups had three sets of 10 repetitions with 70-80% 1RM for six weeks. Both BFR1 and WBFR1 groups trained 3day a week (1 session in a day and three sessions a week), BFR2 and WBFR2 groups trained three days a week (but two sessions a day and six sessions in a week) and Control group did not perform any training. The mean changes in growth hormone(GH), testosterone(TS), and vascular endothelial growth factor (VEGF) hormones were determined by ELISA technique before, after a first training session and after six weeks of the training program. To the analysis of data, two way repeated measures ANOVA at a significant level of $P<0.05$ also were used. The results showed a significant increase in GH levels in each of the four training groups as compared with the pre-test and the control group after a first training session and after six weeks of the training program ($P<0.05$). There was no significant increase in TS levels in each of the four training groups, as compared with the pre-test and the control group in both acute and chronic TS response ($P>0.05$). Only the WBFR1 group did not significantly increase in VEGF levels after the first training session ($P>0.05$). In chronic VEGF response, there were no significant changes observed in all training groups as compared with the control group($P>0.05$). Despite the effectiveness of low-intensity BFR training, such as high-intensity resistance training on hormonal responses, two sessions per day training with the same volume does not necessarily result in larger responses in all hormones than one session per day training.

Key words: Resistance training with BFR; Growth hormone; Testosterone; Vascular endothelial growth factor.

Introduction

Strength and endurance, considered as functions of skeletal muscles, are essential parts of overall health. The American College of Sports Medicine (ACSM) pointed out that individuals reach muscular hypertrophy and benefits of resistance training by exercise with 70%of one repetition maximum or more (1). Moreover, heavy resistance training is considered as a strong stimulant for muscle growth, hypertrophy, improving muscle strength, and finally increasing anabolic hormones, such as growth hormone (GH) and insulin-like growth factor (IGF-1) (2, 3). On the other hand, performing physical activity with 10-20% of maximum exercise intensity will rarely increase GH concentration, and consequently, hypertrophy will not be observed (4). Also, the exercise of any intensity, less than 70% of repetition maximum (RM), can rarely lead to significant hypertrophy (5). However, high-intensity training increases the risk of injury in persons susceptible to injury (6). Heavy exercises are not appropriate and recommended for a specific group of people, such as women, patients, elderly, patients with arthritis or osteoporosis, and, as a general rule, they are reluctant to do such exercises (7). In determining a solution to minimize the problems in performing high-intensity resistance training, researchers have provided a form of resistance training with fewer performance limitations than high-intensity resistance training (8). These exercises called exercises with blood flow restriction (BFR), cause hypertrophy over a short period and increase muscle strength, with a high degree of compatibility consistent with high-intensity exercises (1, 9). Exercises with BFR involve using a type of wrapping device, typically a pneumatic cuff, fastened on the proximal vessels of the muscles exercised. As a result, the venous blood flow to the exercised muscle, which is called occlusion and the arterial blood flow, is restricted (venous pooling, not complete occlusion) (10). In this regard, numerous studies carried out to address low-intensity training with BFR have shown that muscle hypertrophy occurs at low intensity of 20% of repetition maximum and a pressure of about 160 mm Hg. These training can be useful to athletes, patients in post-operative rehabilitation (especially with ACL injuries), rehabilitation of cardiac patients, and elderly (11). Also, recent research that sought to address the poten-

tial safety issues of these types of training concluded that training with BFR did not pose any risk beyond the traditional resistance training. Therefore, many studies have focused on the effect of training with BFR on the amount of hypertrophy and muscle strength, as well as the effects of these training on hormonal responses, especially anabolic hormones. For example, in their study, Takarada *et al.* (2000) investigated the rapid hormonal response to training with BFR and showed that in the group with BFR, lactate increased and the concentration of GH reached 290 times the resting time (12). In another study by Pullinen *et al.* (2002) on the hormonal response of training with and without restriction, it was shown that training with and without restriction increased lactate, but the growth hormone only increases significantly in training with restriction (4). Also, there was no difference between testosterone and cortisol in both groups (4). In a study by Abe *et al.* (2006), it was shown that BFR training, on arm and leg muscles, increased the secretion of GH and the level of lactic acid, but exercises on the lower trunk muscles released more noradrenaline (NA), as compared with exercises on the arm (6). In another study, Abe *et al.* (2005) and Scarth *et al.* (2006) measured the cross-sectional increase in muscle fibers in response to resistance training of twice daily and with BFR, the group with BFR had a higher increase in 1repetition maximum(1RM) in squat practices, as well as more muscular hypertrophy than the traditional training group (13, 14) . Also, Yasuda *et al.* (2010) investigated four sets of chest press (15-15-15-30) twice a day for two weeks, and similar to Abe *et al.* (2006), showed that hypertrophy and muscle strength increased independent of changes in testosterone levels (15). Also, Lakrin *et al.* (2012), who worked on angiogenesis gene response, reported the highest increase in response to training with restriction as compared with other angiogenic agents belonging to the vascular endothelial growth factor (VEGF) (7). Finally, in a study conducted by Patterson *et al.* (2013) on the effect of training with BFR on hormonal responses and cytochrome, there was a significant increase in the secretion of the two hormones of VEGF and GH after training (9). Based on what has been discussed so far, only a few studies have been conducted on BFR training two sessions per day, as most research has focused on one session per day BFR training. Although it has been suggested that two sessions per day training with BFR are associated with more benefits of muscle hypertrophy (7), but research in this area is very limited and few have addressed hormonal responses. Of course, most of these researches have focused on exercising one muscle group (upper or lower trunk) two sessions a day. The present study tends to proffer an answer to this question whether upper and lower trunk separate training once a day can be a more effective long-lasting method than once a day separate training on hormonal responses which probably can be affective on angiogenesis and muscle hypertrophy.

Materials and Methods

The research method is an experimental pretest-post test design with the control group. The study population consisted of 100 healthy and untrained young people who volunteered to participate, of which 40 were randomly selected. The inclusion criteria for participation in the study included: none of the subjects had a history of hypertension, musculoskeletal disorders, bone fractures, regular resistance training in the last six months, smoking, overweight, use of nerve stimulant drugs or the central part of adrenal glands, and low-calorie diets. All of these were examined by a specialist physician. Before the start of the main training, the main objective and method of the study were explained to the subjects, and then the subjects completed the consent forms and medical records. One week before the training, the main anthropometric parameters, including height, weight, age, BMI, systolic, and diastolic blood pressure, were measured (Table 1).

Training protocol

All subjects in this study attended the gym one week before the beginning of the main program to learn the main experimental protocol. Also, 48 h before the beginning of the training program, the maximum power of subjects was tested using the indirect BS (Brzycki) method (16). The formula for indirect estimation of maximum power is shown in Equation 1.

$$1\text{RM} = \frac{\text{The value of the weight moved (W)}}{[1.0278 - (\textit{Number until repetition fatigue} \times 0.278)]}$$

The subjects were randomly divided into five groups: one session of resistance training with BFR, two ses-

Table 1. Descriptive characteristics of the participants. (BFR1) one session of BFR training, (BFR2) two sessions of BFR training, (WBFR1)one session of resistance training without BFR, (WBFR2) two sessions of resistance training without BFR, and the control group

Variable/group	Age (year)	Weight (kg)	Height (cm)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Heart rate (beat per minute)	BMI (kg/cm^2)
BFR2	18.75±1.63	68±4.23	174±5.78	12±1.13	6.6±0.82	61±5.23	22.4±2.26
WBFR2	20.12±2.19	75±5.42	177±4.53	12.7±0.44	7.1±0.62	65±4.31	21.5±1.32
BFR1	21.25±1.9	70±4.55	173±4.11	11.4±0.71	7±0.41	66±4.65	23.3±3.71
WBFR1	18.75±1.46	69±5.31	177±5.59	13.2±1.17	7.7±1.11	68±4.72	22±2.33
Control	22/69±0.69	73±4.15	178±4.72	12±0.31	7±0.59	67±4.88	23±1.88

Table 2. The principle of overload for training groups with blood flow restriction.

Week	1	2	3	4	5	6
Exercise intensity (1RM)	20% 1RM	20% 1RM	25% 1RM	25% 1RM	30% 1RM	30% 1RM
Cuff pressure for arm (mmHg)	110	120	130	140	150	160
Cuff pressure for leg (mmHg)	180	190	200	210	220	230

Table 3. Resistance training protocol with blood flow restriction.

Exercises/ Set	Leg press with machine	Leg hug with machine	Leg extension with machine	5 minutes rest	Chest press with barbell	Barbell biceps curl	Dumbbells biceps curl
Set1	20reps	20reps	20reps	-	20reps	20reps	20reps
rest	45 seconds	45 seconds	45 seconds	-	45 seconds	45 seconds	45 seconds
Set2	20reps	20reps	20reps	-	20reps	20reps	20reps
rest	45 seconds	45 seconds	45 seconds	-	45 seconds	45 seconds	45 seconds
Set3	20reps	20reps	20reps	-	20reps	20reps	20reps

sions of resistance training with BFR, one session of simple resistance training, two sessions of simple resistance training, and a control group. Both BFR1 and WBFR1 groups trained 3day a week (1 session in a day and three sessions a week), BFR2 and WBFR2 groups trained three days a week (but two sessions a day and six sessions in a week) and Control group did not perform any training. Both groups of trainings with BFR performed leg press, leg hug, leg extension, chest press, barbell biceps curl and dumbbells biceps curl (Table 2) with intensity of 20-30% 1RM and 3 sets of 20 repetitions not till fatigue (Table 3), while the groups of simple training performed the same training with intensity of 70-80% 1RM (Table 4), and 3 sets of 10 repetitions not till fatigue (Table 5). The rest time between sets and exercises for groups with BFR was 45s (Table 3) and for simple resistance groups was1 min (Table 5). The time of exercise in the two sessions training was 11:30 am (lower trunk) and 4:30 pm (upper trunk). However, groups of one session per day training (lower and upper body) performed it at 4 pm so that complete blood sampling (post-test) will be taken from all groups at a constant time. The exercise duration for two sessions training was approximately 30 min, 8 min warm-up, 8 min of cooling down, and 15 min of major exercises in their schedule.

On the other hand, the exercise duration for groups of one session training was 50 min, which included 8 min of warming up, 8 min of cooling down, 5 min of rest between upper and lower body training, and about 30 of main exercises. The exercise intensity for simple resistance training groups with 1 and two sessions per day were both 75% 1RM (Table 4). This intensity in training groups with BFR was 25% 1RM. Also, the cuff pressure for the arms and feet was 110 and 180 mmHg, respectively (Table 2). To determine the serum concentrations of TS, GH and VEGF hormones, the first blood samples were taken in fasting state between 8:00 and 8:30 am and the second was immediately after the first training session at about 5:00 pm and the third was 48 h after the last session of 6 weeks of training program like the first and second blood sampling times. For matching the subjects, all subjects were given the same breakfast and lunch. Subjects were allowed to discontinue training if they did not want to cooperate with the study or felt pain.

Table 4. The principle of overload for training groups without blood flow restriction.

Week	1	2	3	4	5	6
Exercise intensity (1RM)	70%	70%	75%	75%	80%	80%

Blood sampling and analysis

Blood samples for plasma were placed on ice for approximately 30 min, before centrifugation at 4000 rpm for 10 min at 4 C. The plasma was then frozen and stored at -80 C until further analysis. Haematocrit was determined from whole-blood in triplicate, using the micro-capillary technique. Hemoglobin concentration was measured in duplicate using a commercially available kit (Randox, Co Antrim, UK). Plasma volume changes were estimated using the method described by Dill and Costill (1974) and presented data are corrected for any changes in plasma volume from rest. The serum levels of GH were measured by the human hormonal kit, Monobind Company, made in Germany. The following day, the plates were washed and blocked with 5 % bovine serum albumin (BSA; Probumin, Millipore, Illinois, USA) in Tris-buffered saline (TBS). The plates were incubated for one h at room temperature after which they were washed, and samples or standards were added to the wells. Samples were diluted 1:5 in TBS with 10 % fetal calf serum. Plates were incubated for further one h before being washed. The enzyme streptavidin alkaline phosphatase was diluted 1:2,000 in TBS with 1 % BSA and 100 lL was added per well. Plates were then incubated for 45 min. After washing, an ELISA amplification system was used (Invitrogen, Paisley, UK). The reaction was stopped with 10 % sulphuric acid, and the absorbance of the wells was read at 490 nm with a correction of 690 nm (Varioskan Flash, Thermo Scientific, Vantaa, Finland). Samples were analyzed in duplicate with an

Table 5. Resistance training protocol without blood flow restriction.

Exercises /Set	Leg press with machine	Leg hug with machine	Leg extension with machine	5 min rest	Chest press with barbell	Barbell biceps curl	Dumbbells biceps curl
Set1	10reps	10reps	10reps	-	10reps	10reps	10reps
rest	60second	60second	60second	-	60second	60second	60second
Set2	10reps	10reps	10reps	-	10reps	10reps	10reps
rest	60second	60second	60second	-	60second	60second	60second
Set3	10reps	10reps	10reps	-	10reps	10reps	10reps

Table 6. The results of maximum power test before and after the training protocol. (BFR1) one session of BFR training, (BFR2) two sessions of BFR training, (WBFR1)one session of resistance training without BFR, (WBFR2) two sessions of resistance training without BFR, and the control group

Study variables	Leg press with the machine (kg)	Chest press with a barbell (kg)
Training groups	pre-test, post-test	pre-test, post-test
BFR2	190±20.12 ,170±14.82 ↑*#	80±7.66 , 68±7.44 ↑*#
WBFR2	210±16.86 , 175±17.01 ↑*#	66±6.37 ,59±6.45 ↑*#
BFR1	205±14.44 , 185±20.11 ↑*#	65±6.23 , 57±7.52 ↑*#
WBFR1	220±0.33 , 195±19.11 ↑*#	78±6.07 , 69±7.88 ↑*#
Control	180±18.32 , 180 ±18.51 -	75±6.20± , 75±6.44 -

*Significant difference with pre-test, #Significant difference with the control group, ↑Increase, ↓Decrease,-Unchanged.

inter-assay coefficient of 7.4 %. This assay measures total IL-6 content and do not distinguish between free and receptor-bound IL-6. Plasma VEGF was determined in duplicate by ELISA (Bendermedsystems, Vienna, Austria). The mean intra- and inter-assay coefficient of variation was 6.2 and 4.3 %, respectively. Plasma cortisol was measured in duplicate by ELISA (DRG Instruments, Germany). The mean intra- and inter-assay coefficient of variation was 5.6 and 6.6 %, respectively. The serum TS level by the human hormonal kit, DRG company, made in Germany, and the serum VEGF level by human hormonal kit, Bioscience Company, made in the USA, all measured by ELISA.

Statistical method

Descriptive statistics were used to describe the subjects 'characteristics and covariance analysis test to eliminate the differences in the pre-test. Also, for comparing the difference between the means of the variables', two-way variance analysis with repeated measurements was used. In the case of significant differences, Bonferroni post hoc test was used. The significance level in all tests was considered P<0.05, and for statistical analysis, SPSS version 21 was used.

Results

The results of the data analysis showed that after six weeks of training (post-test2), within-group comparison showed a significant increase in maximal power in all research groups. The maximum power variations, based on comparison of pre-test with post-test after 6 weeks, were from 185 ± 20.11 kg in BFR1 group to 205 ± 14.44 kg in leg press test, and from 57 ± 7.52 to 65 ± 6.23 kg in chest press test; in WBFR1 group from 195 ± 19.11 to 220 ± 0.33 kg in leg press test and from 69 ± 7.88 to 78± 6.07 kg; in BFR2 group from 170 ± 14.82 to 190 ± 20.12 kg in leg press test, from 68 ± 7.44 to 80 ± 7.66 kg in chest press test; and in WBFR2 group from 175 ± 17.01 to 210 ± 86.66 kg in leg press test and from 59 ± 6.45 to 66.6 ± 6.37 kg in chest press test. These were significant in all groups (P<0.05). The summary of the results is shown in Table 6.

The study results of the within-group comparison showed a significant increase in serum GH levels in all training groups "Figure 1" (P<0.05). Changes in serum GH level from pre-test to post-test1 (first training session) and post-test2 (after 6-weeks) in BFR2 group, were respectively, 0.031±0.01ng/ml to 1.44±0.52 ng/ml and 0.21±0.06 ng/ml ; in WBFR2 group were 0.031±0.01ng/ml to 3.38±0.86ng/ml and 0.31±0.12 ng/ml; in BFR1 group were 0.026±0.01 ng/ml to 1.98±1.44 ng/ml and 0.19±0.05 ng/ml ; and in WBFR1 group were 0.026±0.01 ng/ml to 1.36±0.51 ng/ml and 0.29±0.45 ng/ml. Also, the between-group comparison showed that all groups were significantly different from the control group, and this difference was significant "Figure 1" (P<0.05).The result of the Bonferroni post hoc test also revealed that there was a significant difference from post-test-1 to post-test-2 comparison in all training groups "Figure 1" (P<0.05). The interactive effects

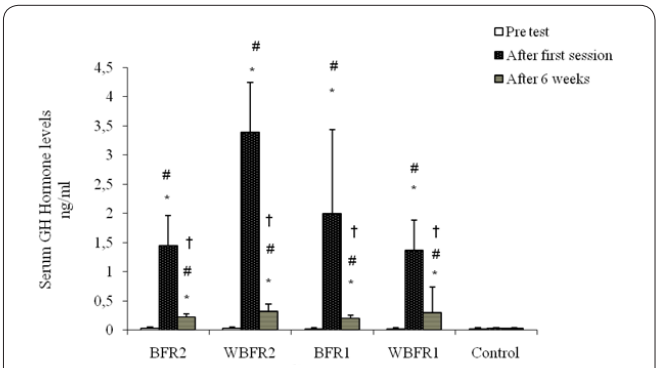

Figure 1. Acute and chronic changes in serum levels of GH in the experimental and control groups. *Significant difference with pre-test (P<0.05). #Significant difference with the control group (P<0.05).†Significant difference with the first training session(P<0.05).

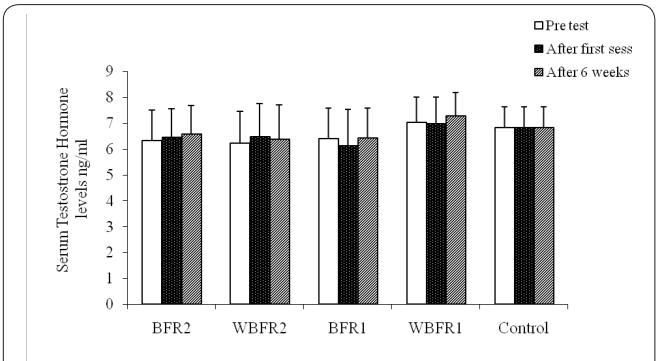

Figure 2. Acute and chronic changes in serum levels of TS in the experimental and control groups.

of group and time on GH levels were also significant (P<0.05).

Furthermore, the results of the study of within-group comparison of pre-test to post-test1 and post-test2 in serum testosterone levels showed that the changes were not significant in all training groups "Figure 2" (P > 0.05). The pre-test testosterone levels as compared with the post-test-1 and post-test-2 showed that testosterone hormone levels in BFR2 group, were respectively, 6.31±1.19 ng/ml to 6.45±1.1 ng/ml and 6.55±1.13ng/ml; in WBFR2 group were 6.21±1.23 ng/ml to 6.48±1.26 ng/ml and 6.37±1.31ng/ml; in BFR1 group were 6.39±1.18 ng/ml to 6.12±1.39

ng/ml and 6.40±1.17 ng/ml; and in WBFR1 group were 7.01±0.98 ng/ml to 6.96±1.03 ng/ml and 7.26±.92 ng/ml. On the other hand, the between-group comparison showed that there was no significant difference between all training groups and the control group "Figure 2" (p> 0.05).

Also, the results of the within-group comparison of pre-test to post-test1 and post-test2 of VEGF levels showed that these changes were significant "Figure 3" (P<0.05). The pre-test VEGF changes as compared with the post-test1 and post-test2 showed that serum VEGF levels in BFR2 group, were respectively, 25.2±20.1pg/ml to 54.9±40.5 pg/ml and 19.88±17.54pg/ml; in WBFR2 group were 25.1 ±10.9 pg/ml to 57.6±31.7 pg/ml and 21.6±10.6 pg/ml; in BFR1 group were 18.8±12.9 pg/ml to 34.5±27.8 pg/ml and 18.5±13.3 pg/ml; and in WBFR1group were 15.5±9.7 pg/ml to 19.1±13.6 pg/ml and 15.4±9.8 pg/ml. Also, the between-group comparison showed no significant difference between all training groups and the control group "Figure 3" (p> 0.05). The result of the Bonferroni post hoc test also revealed that only WBFR1 did not have a significant difference from post-test1 to post-test2 comparison "Figure 3" (p> 0.05). The rest of the training groups were significantly different from post-test1 to post-test2 comparison "Figure 3" (P<0.05). However, the interaction effects of group and time were significant in VEGF levels (P> 0.05). The summary of the results is shown in Table 7.

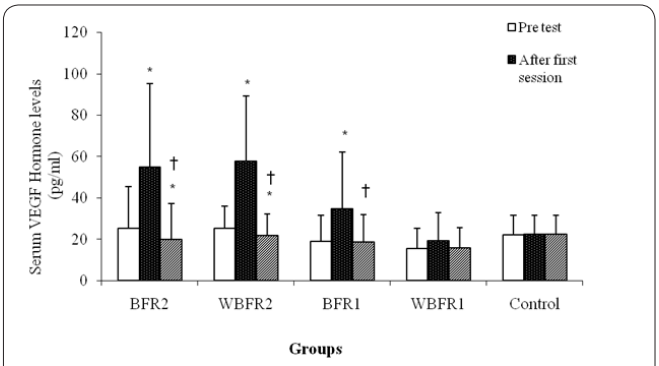

Figure 3. Acute and chronic changes in serum levels of VEGF in the experimental and control groups. *Significant difference with a pre-test in each group (P<0.05). #Significant difference with the control group (P<0.05). †Significant difference with the first training session (P<0.05).

Discussion

The present study was carried out to determine the effects of 6 weeks of BFR exercises with different volumes and frequencies on the response of anabolic hormones: TS, GH, and VEGF. For this purpose, four different training protocols, including one session of BFR training (BFR1), two sessions of BFR (BFR2), one session of resistance training without BFR (WBFR1), two sessions of resistance training without BFR (WBFR2), and a control group were designed for six weeks. The main objective of this study, which distinguished it from previous studies, was to measure VEGF hormone using the method of low-intensity resistance training with BFR two sessions per day. Also, the exercises on both upper and lower body muscles were performed on young untrained people to determine further clear responses than those trained, whose intervening effects of their exercises could mislead one from achieving more accurate results

One of the most important findings of this study was that both low and high-intensity exercise with BFR significantly increased the amount of GH immediately and after six weeks of resistance training. The results of the present study showed that the interaction effect of time and group was significant in the GH level "Figure 1". The greatest and largest acute and chronic responses were related to WBFR2 and WBFR1 groups, respectively suggesting that it would be more effective to perform upper and lower body exercises individually

Table 7. Results of the research variables after applying different training protocols.

Study variables	Growth hormone (ng/ml)			Testosterone hormone (ng/ml)			Vascular endothelial growth factor (pg/ml)		
Training groups	pre-test	After fist session	After 6th week	pre-test	After fist session	After 6th week	pre-test	After fist session	After 6th week
BFR2	0.031±0.01	1.44±0.52 #*↑	0.21±0.06 †#*↑	6.31±1.19	6.45±1.1 ↑	6.55±1.13 ↑	25.2±20.1	54.9±40.5 †*↑	19.88±17.54 *↓
WBFR2	0.035±0.01	3.38±0.86 #*↑	0.31±0.12 †#*↑	6.21±1.23	6.48±1.26 ↓	6.37±1.31 ↑	25.1 ±10.9	57.6±31.7 †*↑	21.6±10.6 *↓
BFR1	0.026±0.01	1.98±1.44 #*↑	0.19±0.05 †#*↑	6.39±1.18	6.12±1.39 ↓	6.40±1.17 ↑	18.8±12.9	34.5±27.8 †*↑	18.5±13.3 ↓
WBFR1	0.026±0.01	1.36±0.51 #*↑	0.29±0.45 †#*↑	7.01±0.98	6.96±1.03 ↓	7.26±.92 ↑	15.5±9.7	19.1±13.6 ↑	15.4±9.8 ↑
control	0.025±0.01	0.024±0.01 -	0.024±0.01 -	6.81±0.32	6.85±0.5 -	6.83±.07 -	22.2±9.3	22.2±9.5 -	22.58±9.63 -

*Significant difference with pre-test, #Significant differences with the control group, †Significant difference with a first training session, ↑Increase, ↓Decrease, -Unchanged.

and two sessions per day about the response of anabolic hormones "Figure 1". GH response to exercise was influenced by calendar age, muscle mass, type of muscle activity (concentric and eccentric), exercise status, exercise volume, and rest between each set. Interestingly, neither simple resistance training with low intensity nor BFR alone can increase GH concentration (17). This suggests that the GH concentration will be increased only if these two are combined, which means that a large GH response requires ischemic conditions and physical activity (18). Also, the most important finding of this study was that two sessions per day training caused the accumulation of GH, which in addition to the previous factors (the effect of the training frequency) caused a larger response. The proposed mechanism for increasing GH after resistance training with BFR was increasing muscle lactate activation. It has been shown that GH is further stimulated in the muscles'acidified environment. In particular, metabolic acids are involved in lactate accumulation, which increases GH. Also, various research results showed that low-intensity exercises with BFR produce more pain in the pain receptors than simple high-intensity exercises. Although this mechanism is not completely clear, reducing intravenous blood flow to exit the limb under training by restricting blood flow stimulates proton pump receptors (19). Acute pain is known to act as a regulator of GH secretion by stimulating opiate receptors. For example, Greisen *et al.* (2001) reported a significant GH response to electrical stimulation-induced to the abdominal skin (20). Researchers believe that perceived pain during muscle contractions leads to an increase in GH responses, and it seems that more pain in low -intensity exercises with BFR, as compared with simple high-intensity exercise with low pain, leads to more GH responses (21). The result of Bonferroni post hoc test also revealed that there was a significant difference from post-test-1 to post-test-2 comparison in all training groups indicating that chronic resistance training with BFR lead to an increase in GH responses, however, GH responses were reduced after six weeks of resistance training in compared with acute GH responses. These results are in line with the results of Takarada *et al.* (2000), Anabastani (2014) and Mohammadi *et al.* (2015), indicating that training with BFR increases GH immediately after the exercise (12, 22, 23). On the other hand, the findings of these researchers on GH suggest that these results are generally in line with various studies on the effect of training with BFR on GH, and these results are similar and in agreement with those of Manini *et al.* (2012) and Tanimoto *et al.* (2005), that investigated GH response to resistance trainings with BFR and simple high intensity exercises (21, 24). Also, in a study by Kim *et al.* (2014) on the measurement of acute hormone responses in women (aged 18-25 years old), the resistance training with BFR exercises were compared with simple resistance training (25). The results of this study showed a significant increase in GH levels before and after simple resistance training and resistance training with BFR, but no significant difference were observed between the two groups based on the amount of this hormone. These results are in line with the present research. In another study, Manini *et al.* (2012) compared hormone responses in young and old men in two training conditions with BFR and simple resistance training (21). The results showed a larger GH response in young men than the elderly. Thus this difference in the resistance training group with BFR was higher than that of the simple resistance group, both in the elderly and in the young men groups. However, in this research, the GH response of older men to resistance training with BFR was lower than other previous studies (24). In a study by Patterson *et al.* (2013), it was shown that GH did not significantly increase immediately after exercise in resistance training with BFR, but this hormone increased significantly 30 min after exercise and had higher levels as compared with resistance training without BFR (9).

Another finding of the present study was that no significant increase observed in testosterone levels immediately and after six weeks of resistance training in all training groups and under different conditions "Figure 2". The results of the study showed that the effect of time and group and subsequently, the effect of interaction were not significant in testosterone levels "Figure 2". Different research findings showed that simple resistance training (without BFR) greatly increases the concentration of total testosterone in men; however, some studies have shown that this is not the case in women. Although testosterone appears to increase significantly in high-intensity exercises, testosterone response to training with BFR is less recognized. The possible mechanism behind this acute increase in testosterone with low-intensity training with BFR may be due to increased lactate and concentration of catecholamines, which usually increase during training (10). Animal research has shown that lactate increases the cAMP production that stimulates testicular Leydig cells to increase testosterone (26). Also, some animal studies have shown that increased catecholamine can stimulate Leydig cells by stimulating β2-adrenergic receptors to produce testosterone (27). The change in plasma volume, previously observed in BFR exercises, can explain any increase in testosterone hormone by exercising with BFR (10). It has been shown that intense resistance training changes the androgen receptors in the pathway of skeletal muscle and, with this in mind, the possible explanation why low-intensity training does not increase testosterone levels at rest is likely due to the low intensity of performances in training. It is acceptable that if endurance exercise continues until fatigue, it can increase testosterone levels (28). In general, and according to the literature available so far, the acute and chronic responses of testosterone to training with BFR were minimal, although strength increased and hypertrophy occurred in these exercises. The present study suggests that increased muscle hypertrophy in training with BFR does not depend on increased testosterone levels. These results require a deeper analysis, as testosterone levels were measured 15 min again after exercise, which revealed no significant increase in this hormone (29). The results of this research on testosterone hormone are in agreement with the results of studies by Fujita *et al.* (2007), Anabasti *et al.* (2014) and Mohammadi *et al.* (2015) (17, 22, 23). Most studies on acute testosterone response to resistance training with BFR have shown that these exercises do not result in a significant increase in this hormone.

Contrary to the findings of the researchers mentio-

ned above, Madarame *et al.* (2008) showed that training with BFR, if done on the lower body muscles (extension and flexion of the legs), can acutely increase the testosterone levels (29). 15 min after exercise, testosterone levels were measured again, and this time, no significant increase was observed in this hormone. The difference in the results of the present study with Madarame'sresearch (2008) can be due to continued sets until fatigue and the higher production and accumulation of lactate (10, 29).

On the other hand, VEGF responses significantly increased immediately after the first training session in all training groups but only in WBFR1 group was not significant in within-group comparison Also, the results showed that VEGF responses reduced after six weeks in all training groups and were not significant in both within and between groups comparison" Figure3". Also, the results showed that only the effect of time and interaction were significant" Figure3". The regulation of VEGF expression in response to hypoxic conditions is largely regulated by HIF-1α factor. VEGF gene contains an upstream sequence that increases the expression of VEGF mRNA when bound to HIF-1α. Under normal conditions, HIF-1α (ubiquitinated) consequently decreases in less than five minutes. However, under hypoxic conditions, HIF-1α is stimulated to stabilize and express VEGF and stimulate more than one hundred other genes, not involved in angiogenesis, erythrocytes, and glucose metabolism (7). Endothelial dysfunction is improved by angiogenesis stimulated by endothelial vascular growth factors. Also, it has been well established that the presence of VEGF is critical to stimulating angiogenesis and arteriogenesis. The effective mechanism in this phenomenon can be attributed to localized ischemia induced by training with BFR. The secretion and production of VEGF occur under hypoxic conditions or lack of oxygen in the muscle during the exercise (30). VEGF may increase due to the increased availability of nitric oxide (NO), which may have a beneficial effect on endothelial function (31). The result of the Bonferroni post hoc test also revealed that only WBFR1 did not have a significant difference from post-test-1 to post-test-2 comparison "Figure 3".The rest of the training groups were significantly different from post-test-1 to post-test-2 comparison "Figure 3". To confirm the findings of the present study, Patterson *et al.* (2013), in a study on older men with mean age of 70±6.5 years, showed that performing one session of resistance training with BFR immediately after exercise did not result in a significant increase in VEGF values, but these values significantly increased at 30, 60 and 90 min after exercise (9).

Therefore, the results of the research conducted by Patterson are in agreement with the results of the present study on groups of two sessions of BFR2 and WBFR2, as both studies did not show a significant increase in VEGF hormone immediately after exercise (9). Takano *et al*. (2005) measured the acute effect of resistance training with BFR on hemodynamic and hormonal responses, after the bilateral hip extension of up to 30 repetitions with 20% 1RM and then three sets until fatigue (32). It was shown that the acute levels of VEGF significantly increased and in Line with the findings of this study, Lakrin *et al*. (2012) showed that 6 adult males (about 22 years) had no significant increase in VEGF concentration after 120 repetitions of one-sided knee extension as compared with the control group (7). Although this increase in interaction (group×conditions) was observed in VEGF hormone, the reason for this difference could be attributed to the sampling time in the research conducted by Lakrin *et al.* (2012), which was 4, and also to 24 h after the exercise (7).

Nevertheless, in a recent study conducted by Behjat *et al*. (2015) in which the effect of exercise with BFR on the chronic response of the VEGF hormone was measured, it was shown that after eight weeks of resistance training with BFR on elderly women, these exercises reduced the rest levels of VEGF hormone (33). Thus, the results obtained are exactly in agreement with the results of the present study on a two-session training group with and without BFR. But in one session per day training group with and without BFR, these changes were not significant. Therefore, the results of the mentioned study are similar to the present study. In general, despite the effectiveness of low-intensity training with BFR, such as simple resistance training with high hormonal responses, two sessions per day training with the same volume does not necessarily lead to larger responses in all hormones as compared with one session per day training.

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