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Original Article

Effect of Resistance Training with Blood Flow Restriction on Follistatin to Myostatin Ratio, Body Composition and Anaerobic Power of Trained-Volleyball Players

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ABSTRACT

Background and objectives: The present study was designed to determine the effect of blood flow restriction training (BFRT) on follistatin to myostatin ratio, body composition and anaerobic power of trained volleyball players.

Methods: Eighteen trained volleyball players were randomly assigned into two study groups: resistance training with blood flow restriction (BFRT; n=9) and resistance training without blood flow restriction (WBFRT; n=9). The subjects performed trainings three sessions a week, for eight weeks. In each session, barbell squat, leg extension, leg curl and dumbbell lunges were performed in three sets of 15 repetitions, with rest intervals of 30 seconds. Serum follistatin and myostatin concentrations, body composition and anaerobic power were assessed before and after the study. Data were analyzed using the paired sample t-test, Wilcoxon test, independent sample t-test and Mann-Whitney U test. All statistical analyses were done in SPSS (version 22), and a P-value of less than 0.05 was considered statistically significant.

Results: Follistatin levels increased significantly (P=0.001), while myostatin levels decreased significantly in both groups (P=0.001). Follistatin to myostatin ratio increased significantly in both groups (P=0.001). Although body fat percentage decreased in both groups, it did not differ significantly between the two groups (P=0.28). Moreover, anaerobic power increased significantly in both groups (P=0.001), but this increase was more profound in the BFRT group (P=0.001).

Conclusion: Based on our findings, blood flow restriction can be applied as remarkable approach to boost body adaptation responses to resistance training.

KEYWORDS: Resistance training, Myostatin, Follistatin, Blood flow restriction.

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INTRODUCTION

Volleyball is a complex sport that requires functioning of both the aerobic and anaerobic energy systems. Vertical jump is one of the most intense muscular movements that demands particular emphasize on development of muscular power in trainings (1). Physical trainers recommend different training methods to prevent common injuries in athletes. In blood flow restriction training (BFRT), a cuff is wrapped around the proximal portion of the thigh or arm (2). In this type of training, athletes exercise at lower percentage of one-repetition maximum (1RM), which has more positive effects compared to traditional (without cuffs) resistance training (RT). It also reduces pressure on joints, ligaments and tendons. BFRT produces an anabolic response through numerous pathways, including recruitment of type II muscle fibers, which have a greater potential for growth compared to type I muscle fibers (3). Evidence suggests that BFRT may change the level of some proteins including myostatin (MSTN), a member of the transforming growth factor-beta (TGF-β) superfamily that regulates muscle growth (4). MSTN is produced in skeletal muscle cells and acts as a muscle growth inhibitor that circulates through the bloodstream and ultimately binds to activin type II receptors (5). BFRT changes MSTN levels due to oxygen deficiency and metabolite accumulation, resulting in muscle hypertrophy (6). Follistatin (FLST) is a glycoprotein that can block MSTN (7) by preventing the of binding of MSTN to its receptor, thus preventing muscle atrophy and increasing lean body mass (8). RT also reduces MSTN expression, which in turn increases muscle hypertrophy. Santos et al. examined effect of different RT programs on MSTN signaling pathway in 29 physically active men with a minimum of two-year experience in RT. They reported that the RT programs significantly increased expression of MSTN signaling pathway inhibitors (9). In a study by Attarzadeh et al., FLST to MSTN (F: M) ratio increased dramatically in 24 sedentary young women, in response to eight weeks of high-intensity RT (10). Moreover, RT with BFR has a more profound effect on the myokine levels compared to traditional RT. In Iran, BFRT is rarely incorporated in the athletes training programs. Owing to the importance of muscular power and BFRT for volleyball players, this study investigated the effect of eight weeks of BFRT on F: M ratio, body composition and anaerobic power in trained volleyball players.

MATERIAL AND METHODS

Trained volleyball players (aged 20-25 years) were recruited according to the following inclusion criteria: absence of cardiovascular disease, diabetes and hypertension or any other condition that requires medical attention. The volunteers completed a baseline medical form and gave written consent before enrollment in the study. Eighteen eligible individuals were recruited and then randomly divided into two RT groups: blood flow restriction training (BFRT; n=9) and without blood flow restriction training (WBFRT, n=9). Body weight was measured on emptied bladder, using a digital scale (Lumbar, China) with accuracy of 0.1 Kg. Height was measured by a wall-mount measuring tape with accuracy of 0.1 cm. Body mass index (BMI), body fat percentage (BFP) and skeletal muscle mass (SMM) were evaluated using a multi-frequency bioelectrical impedance analyzer (Inbody 720, South Korea). Before blood sampling, the participants fasted for 12 hours (overnight fasting) and refrained from physical activity for 36 hours. Blood samples (5 ml) were taken from the cubital vein 48 hours before the first training session and 48 hours after the last training session. Serum MSTN and FLST concentrations were measured using human MSTN and FLST ELISA kits (CK-E11241 and CK-E10682).

Maximal strength was measured 24 hours after the body composition measurement. The 1RM starting point was determined in a preliminary assessment, and muscle strength was measured using the following formula (11):

$1RM = Weight \div (1.0278 - 0.0278 \times reps)$.

After the 1RM strength test, the participants performed RT three sessions a week, for eight weeks. The two RT programs specifically targeted the lower limb. Each session started with ten minutes of general warm-up (slow running, stretching and light RT), followed by specific warm-up with two sets of 20 repetitions at 20% of 1RM in the first session. The subjects rested for 30 seconds between each set. The exercises included barbell squat, leg extension, leg curls and dumbbell lunges. The first session stared with three sets of

fifteen repetitions, which were increased by one set every two week until it reached six sets and fifteen repetitions. The training was performed at 20% of 1RM in the first four weeks, which was raised to 30% of 1RM in the second four weeks. The repetitions were performed as fast as possible (2).

The cuff inflation pressure was set to 160 mmHg in the first session and increased by 10 mmHg in the following sessions, until it reached 240 mmHg (12).

Data analysis was performed using SPSS (version 22). Mean values and standard deviation (SD) were calculated after testing the normality of data using the Shapiro-Wilk test. The Levene's test was used to ensure the homogeneity of variances. Considering the non-normal distribution of some data, the Wilcoxon test and Mann-Whitney U test were used for intragroup and intergroup comparisons, respectively. The independent t-test and paired sample t-test were used for assessment of intragroup and inter group changes for normal data, respectively.

RESULTS

FLST levels increased significantly (P=0.001), while MSTN levels decreased significantly in both groups (P=0.001). The F: M ratio increased significantly in both groups (P=0.001) and differed significantly between the two groups (P=0.001). The anaerobic power increased significantly in both training groups (P=0.001).

Anaerobic power differed significantly between the two study groups (P=0.001). BMI did not change significantly in the two study groups (Table 1).

Table 1- Comparison of intergroup and intragroup variations of some variables

Variable	Group	Mean ± SD		Intragroup changes			Intergroup changes	
		Pre-training	Post-training	Mean difference	t	P-value	F	P-value
F:M ratio	WBFRT	0.098±0.01	0.089±0.009	-0.008	8.56	0.001	3.68	0.001
	BFRT	0.094±0.012	0.12±0.27	-0.03	-3.11	0.014		
Anaerobic power (W)	WBFRT	639.95±82.65	657.40±84.76	-17.44	-6.47	0.001	0.8	0.001
	BFRT	693.55±58.11	738.82±59.84	-45.27	-17.88	0.001		
BMI (Kg/m^2)	WBFRT	22.27±2.39	22.22±2.35	0.05	0.31	0.758	0.05	0.28
	BFRT	22.10±2.71	22.50±2.66	-0.22	-1.24	0.247		
SMM (Kg)	WBFRT	34.70±5.10	35.35±5.17	-0.65	-5.34	0.001	0.24	0.63
	BFRT	38.78±4.99	40.78±5.27	-2.00	-10.56	0.00		
PBF (%)	WBFRT	14.65±5.97	13.48±5.5	1.16	2.03	0.07	0.07	0.79
	BFRT	11.84±3.84	9.88±3.78	1.95	3.38	0.01		

Table 2- Results of the Mann-Whitney U test for comparison of variable between the WBFRT and BFRT groups

Variable	Z	P-value
MSTN (Ng/ L)	-3.576	0.001
FLST (Ng/ ml)	-3.578	0.001

Table 3- Results of the Wilcoxon test for comparison of variable between the WBFRT and BFRT groups

Variable	Group	Z	P-value
MSTN (Ng/ L)	WBFRT	-2.66	0.08
	BFRT	-2.66	0.08
FLST (Ng/ ml)	WBFRT	-1.95	0.05
	BFRT	-2.66	0.08

DISCUSSION

This study investigated the effects of two different eight-week RT protocols on F: M ratio, body composition and anaerobic power in trained volleyball players. The serum FLST levels increased significantly in response to both RT programs, but the increase was greater in the BFRT group. The F: M ratio increased significantly and MSTN decreased slightly in the BFRT group. The anaerobic power was significantly higher in the BFRT group. MSTN is a catabolic factor found in skeletal muscles and an anabolic agent in adipose tissue, which can have a major role in muscular hypertrophy. Roth et al. reported a 37% reduction in MSTN mRNA expression in men and women in response to nine weeks of RT (13). Santos et al. reported a 25% decrease in MSTN levels of 65-year-old male patients with muscle tissue inflammation in response to BFRT (14). However, Hulmi et al. showed MSTN mRNA overexpression after 12 weeks of RT in elderly men (15). MSTN is a member of the TGF-ß family and a negative regulator of muscle growth through Smad 2/3 phosphorylation (16). Theoretically, the reduction in MSTN expression exacerbates muscle hypertrophy, which can be abated with BFRT (17). The reduction in MSTN levels in response to BFRT can be attributed to the activation of mammalian/mechanistic target of rapamycin (mTOR) signaling pathway. MSTN levels in muscle cells decrease following mechanical loading as well as BFRT. Limited blood flow causes oxygen deficiency and accumulation of metabolites, which leads to activation of the mTOR signaling pathway and eventually muscle hypertrophy (18). BFRT reduces MSTN levels through protein kinase B and forkhead box O signaling pathways (19, 20).

BFRT at intensity of 20-30% of 1RM can induce muscle hypertrophy. We observed a substantial increase in serum FLST levels in both study groups, particularly in the BFRT group. In a study by Attarzadeh et al., F: M ratio increased dramatically in response to eight weeks of high-intensity RT in 24 sedentary young women (10). Inconsistent with our findings, Jensky et al. found no significant change in the level of FLST after seven sessions of eccentric exercise and concentric exercise, which might be due to the short-term study period (21).

We found no significant difference between the two groups in terms of body composition. In a study by Monikh et al., six weeks of RT did not reduce BFP significant, which may be attributed to the low-intensity and short duration of the RT program (22). Kwon et al. showed that low-intensity RT in diabetic women decreases BFP (23). Since RT focuses on the core muscles, it increases adipose tissue blood flow, which can consequently increase adipose tissue metabolism.

BMI has a direct correlation with the volume and intensity of RT. It seems that training with intensity of 70-75% of heart rate reverse increases both adipose tissue blood flow and fat oxidation, and is therefore appropriate for fat loss (24). The of lack of BMI reduction in the present study could be due to the low volume and intensity of the trainings and to the increase in PPAR-γ and sterol regulatory element-binding protein-1 activity (25, 26), which in turn increases mTORC1 activity.

In recent years, many studies have focused on the effect of RT on the power of athletes, especially in volleyball players. We found a significant difference in the anaerobic power between the two study groups. Park et al. found that two weeks of walking with BFR increases anaerobic power (27). Abdolmaleki et al. stated that RT with or without BFR has no significant impact on anaerobic power and explosive power in athletes and non-athlete participants (28). This could be related to use of a low-pressure cuff. BFRT might increase muscle hypertrophy and subsequently muscular strength by activating the mTOR signaling pathway (29). Owing to the high repetitions as well as the high-intensity of the BFRT program, the athletes could achieve both muscle hypertrophy and strength, which may be also associated with the increase in anaerobic power.

In our study, the F: M ratio differed significantly between the two study groups. BFRT generally increases activity of anabolic signaling pathways through motor unit recruitment (30), lactate accumulation (31), mTOR signaling pathway, heat shock proteins (32), cell swelling (33) and nitric oxide secretion (34), all of which ultimately decrease the catabolic factors. Nevertheless, the superiority of BFRT over WBFRT elucidates that these mechanisms can be ascribed to increase in anabolic factors (FLST and F: M ratio) and decrease in catabolic factors

(MSTN). Because of the assorted dietary restrictions and reconciling responses to RT, lack of management of sleep hours and the waking up of participants, additionally as individual variations are confronted.

CONCLUSION

Based on our findings, blood flow restriction can be applied as remarkable approach to boost body adaptation responses to resistance training.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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