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Effects of Blood Flow Restriction Training on Follistatin Concentrations and Physical Performance in Elderly Females

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ABSTRACT

Background and objectives: Blood flow restriction training (BFRT) has gained substantial interest due to the lower required intensity, which may be beneficial for individuals who are not able to lift heavy weights. Therefore, we aimed at evaluating effects of 12 weeks of resistance training with and without blood flow restriction on follistatin (FST) concentrations and physical performance in elderly females.

Methods: Thirty elderly female were randomly assigned into the following groups: resistance training with blood flow restriction (BFRT; n=10), resistance training without blood flow restriction (WBFRT; n=10) and control (n=10). The resistance training was carried out three session a week for 12 weeks. Serum concentrations of FST, muscular endurance and dynamic balance were assessed at baseline and after the 12week intervention.

Results: Significant main effects of time were observed for FST ($p = 0.03$, $\eta 2 = 0.15$), muscular endurance ($p = 0.00$, $\eta 2 = 0.59$) and dynamic balance ($p=0.00$, $\eta 2 = 0.57$). FST [BFRT= 1.4 ng/ml (effect size Cohen's {d} = -0.8) significantly increased only in BFRT group. However, muscular endurance [BFRT= 95 (d= -4.1) and WBFRT = 32 (d= -0.9)] significantly increased in both intervention groups ($P<0.05$). In addition, dynamic balance [BFRT= -0.5 seconds (d= 2.2)] significantly increased only in the BFRT group ($P<0.05$).

Conclusion: BFRT was able to increase FST concentrations. Due to its mechanistic role in muscle mass alterations, elderly females can incorporate our BFRT protocol to improve anabolic conditions for muscular adaptations.

Keywords: Resistance training, Follistatin, Aged, Female.

INTRODUCTION

The onset of the aging process, a natural biological course of change, occurs during the life cycle (1). Throughout aging, numerous bodily functions begin to progressively deteriorate. Sarcopenia is delineated as deterioration of muscle mass, power and strength. Muscle mass has been indicated to be degraded by 40% between the ages of 60 and 80 years (2). Resistance training (RT) has been reported to be an efficient method to prevent the loss of muscle mass, and the beginning of sarcopenia (3, 4). Consequently, consistent RT in the elderly increases muscle mass, strength and power, thereby improving the quality of life (5, 6).

Lately, blood flow restriction training (BFRT) has been recognized as an advantageous method to boost muscle mass even at low intensities in comparison to high intensity traditional RT (7). Blood flow restriction (BFR) activates numerous cellular mechanisms such as boosting mechanistic target of rapamycin (mTOR) signaling, ribosomal protein S6 kinase beta-1 (S6K) as well as decrements of forkhead box O (FOXO) signaling, which results in maximizing muscle mass (8). In this regard, it has been proven that BFRT leads to increase in muscle mass greater than without BFRT (7). Evidence suggests that BFRT may modulate some myokines (9). Follistatin (FST) is a glycosylated protein that is a member of the transforming growth factor-beta (TGF-β) family and contributes to the development of muscle mass (10). It increases muscle mass by downregulating myostatin binding to its receptor, thereby preventing the atrophic actions of myostatin (11). Various investigations have examined FST to characterize their response to different exercise modalities. For instance, Bagheri et al. (2019) indicated considerable increments in FST concentrations following eight weeks of combined lower limb and upper limb RT in middle aged men (3). In addition, they have also shown that FST concentrations increase during eight weeks of concurrent training in sarcopenic elderly men (4). In their previous study, they reported that FST concentrations increase after eight weeks of lower limb RT in trained volleyball players (12).

Overall, RT is recommended to increase FST concentrations. Since BFRT is performed with lower intensities, there is a lower risk of skeletal injuries, especially in older adults Accordingly, this study was performed to evaluate effects of 12 weeks of BFRT following RT on FST, muscular endurance and dynamic balance in older females. We hypothesized that BFRT would increase FST concentrations in the elderly females.

MATERIALS AND METHODS

Sample size calculation was conducted using the G*Power analysis software (15). Our rationale for sample size was based on a previous study, which evaluated changes in serum concentrations of FST after eight weeks of RT in 30 elderly men (4). The results of this study showed significant increase in FST concentrations (4). Based on $\alpha = 0.05$, a power (1- β) of 0.80, the analysis revealed a sample size of 30 participants (n=10 per group) was needed to have sufficient power to detect significant changes in FST concentrations between the study groups. Therefore, 30 elderly females participated in the present study. Inclusion criteria were age of >60 years, confirmed sarcopenia (diagnosed based on a hand grip strength of less than 26-30 kg, speed of four meters walking less than 0.8 m/s and muscle mass index of less than two standard deviations from the average young population), sedentary life style and no history of disease including Parkinson's disease, cardiovascular disease, diabetes, hypertension, obesity and kidney disease. Exclusion criteria included smoking, participation in hormonal/mental therapy, engagement in regular physical activities within the past year, alcohol consumption, using medications and taking supplements. The mentioned criteria were closely assessed by a specialist physician using the PAR-Q and the medical health/history survey. Informed consent was taken from all subjects prior to participation and all procedures was in accordance with the Declaration of Helsinki.

Before baseline measurements and after the participants were familiarized with the study tests and procedures, the subjects were randomly assigned into three groups of blood flow restriction training (BFRT; n=10), without blood flow restriction training (WBFRT; n= 10) or control (C; n=10). Measurements were done at baseline and after eight weeks (48-72 hours after the last training session) during the same time of the day (~1 hour) and under the same environmental conditions (~20 °C and ~55% humidity). The subjects were instructed not to alter their regular lifestyle and dietary habits during the study.

Testing sessions were performed at the same time of day for each participant under the same environmental condition (~20°C and ~55% humidity). Height and weight of the subjects were measured with precision of 1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m^2).

To assess lower and upper limb maximal strength, one-repetition maximum (1-RM) prediction equation was used to estimate the 1-RM based on the load and repetitions recorded as follows: 1-RM= (L) / [1.0278- (R × 0.0278)] where L is the external load (kg) and R is the number of repetitions performed (3).

At first, all subjects performed a week of concurrent training, consisting of three exercise sessions, for familiarization with proper lifting technique and all exercises and equipment.

Following the preparatory phase, RT began with 20% of 1-RM for the 1st week to the 3rd. Then, the intensity increased to25% of 1-RM in the 4th to 7th week and further increased to 30% of 1-RM during 8th to 12th week. The exercises were performed with 15-30 repetitions. In addition, there were 30 to 60 seconds rest between each set and 60 to 120 seconds rest between exercise rest intervals. The trainings were carried out three days a week. The pressure of cuff was 140 to 190 mmHg for lower body exercises and 90 to 140 mmHg for upper body exercises. The exercises included leg press, leg extension, leg curl, calf raises, lateral raise, chest press and lat pulldown. Subjects in the BFRT group performed RT with a cuff tightened to the proximal portion of arm and femur (3, 7).

Blood samples (5 ml) were taken after 12 hours of overnight fasting and 36 hours after the last training session.

The samples were taken from the cubital vein using standard procedures and then were clotted for 15 min at room temperature before being centrifuged at 3000 rpm for 15 minutes. Serum was removed and frozen at -70 °C for later analysis. The serum was used in duplicate to measure the concentration of FST (ZB-OEH4717815 Zellbio, Germany). Intra-assay and inter-assay for serum FST concentrations were less than 10% and 12%, respectively. In addition, sensitivity of FST was 2.5 ng/mL.

A six-minute walk test was used to estimate muscular endurance performance. The test was performed in a 60 m long, circular corridor. The participants were asked to walk as fast as possible to cover the longest distance possible within 6 min. after the end of the test, the participants had to have the impression that they could not have gone any more (13).

Timed up and go test (TUGT) was used to determine participants' dynamic balance performance as previously described. The TUGT assesses the number of seconds needed for a participant to stand up from a chair, walk 3 meter at their usual pace past a line on the floor, turn around, walk back to the chair, and sit down again with the back against the chair (14).

Results were reported as mean ± standard deviation. Normality of data was assessed using the Shapiro-Wilk test. Group differences at baseline were examined using one-way analysis of variance (ANOVA). A two (time: before vs. after) x three (groups: BFRT, WBFRT, C) repeated measures ANOVA was used to determine intergroup differences over time. Significant differences were followed up by the Bonferroni post hoc test. All analyses were performed using SPSS (version 24.0, IBM; Chicago, IL) and at statistical significance of 0.05.

RESULTS

Mean age of the participants was 62.7 ± 1.5 years. There was no significant difference between the groups at baseline (Table 1). Training compliance was 100% for both the BFRT and WBFRT groups. There was no change observed in the variables over time for the control group. Time had a significant main effect on FST (Figure 1A, $p = 0.03$, $\eta 2 = 0.15$), muscular endurance (Figure 2B, $p = 0.00$, $\eta 2 = 0.59$) and dynamic balance (Figure 1C, $p=0.00$, $\eta 2 = 0.57$). FST [BFRT= 1.4 ng/ml (effect size Cohen's {d} = -0.8) significantly increased only in the BFRT group. However, muscular endurance [BFRT= 95 (d= -4.1) and WBFRT = 32 (d= -0.9)] significantly increased in both intervention groups ($P<0.05$). In addition, dynamic balance [BFRT= -0.5 seconds (d= 2.2)] significantly increased only in the BFRT group. There was no significant difference between the groups in terms of the study variables.

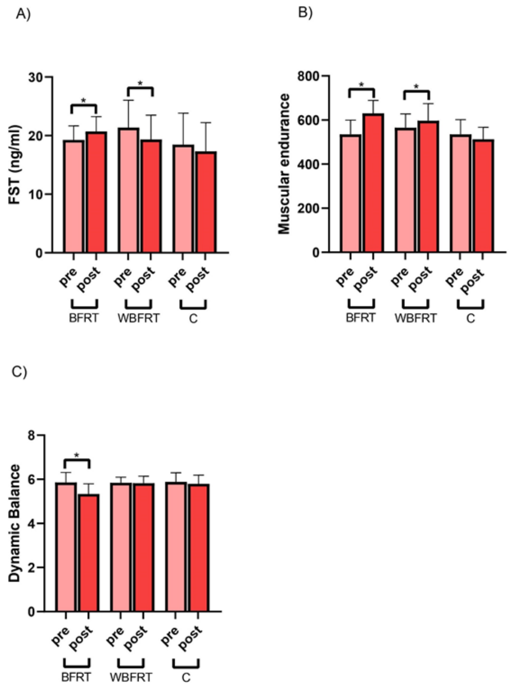

Figure 1- Comparison of pretest and posttest values of FST (A), muscular endurance (B) and dynamic balance (C) between the study groups. * indicates statistically significant difference from baseline.

Table 1- Physiological characteristics of the participants at baseline and after the 12-week training intervention

Groups	BFRT		WBFRT		Control	
Variable	Baseline	After 12 weeks	Baseline	After 12 weeks	Baseline	After 12 weeks
Age (kg)	63.4 ± 1.5	------	62 ± 1.2	------	62.9 ± 1.5	------
Height (cm)	157.4 ± 3.1	------	157.7 ± 3.1	------	155.8 ± 3.9	------
Weight (kg)	65.5 ± 1.8	71.5 ± 9.7	67.3 ± 1.6	72.8 ± 6.5	68.4 ± 1.4	71.5 ± 9.5

DISCUSSION

We examined the effects of 12 weeks of RT with and without BFR on FST concentrations and physical performance in elderly females. We found that BFRT increased FST concentrations and improved dynamic balance. However, these variables did not change significantly in the WBFRT and control groups. In addition, muscular endurance was improved after both BFRT and WBFRT. The single-chain polypeptide FST is a member of the TGF superfamily (16), which is ubiquitously expressed in the human body, including in the skeletal muscle, and has both paracrine and autocrine effects. Former studies suggested that FST has both anabolic and catabolic effects on skeletal muscles (17) via FST's ability to bind and inactivate myostatin, a known negative regulator of muscle mass (18). Consequently, by inactivating myostatin signaling through FST increments, muscle mass will be increased due to the anabolic condition of the intracellular space.

Consistent with our results, a previous study showed that eight weeks of high-intensity RT increased serum FST concentrations in sedentary females and middle-aged men (19). Similarly, FST concentrations increased following 3x/week supervised eight weeks of whole-body RT in middle-aged men (3). In addition, FST concentrations significantly increased following eight weeks of lower body BFRT in trained-volleyball players, which suggest an anabolic environment (12). Research has shown that when combined with BFRT, RT could elicit numerous metabolic effects leading to increased muscle mass. The main mechanisms that explain these changes in FST concentrations are upregulation of mTORC1, S6K protein, heat shock proteins as well as downregulation of FOXO and Tuberous Sclerosis 2 (TSC2) signaling pathway (8, 20). Furthermore, TSC1 signaling pathway is regulated by inactivation of AMP-activated protein kinase (AMPK) through proper fuels derived from carbohydrates and fats (8).

Thus, FOXO and TSC2 signaling are inhibitors of muscle mass growth. In addition, metabolic acid increments during BFRT has a considerable role in regulating muscular adaptations as well (20). Although most previous BFRT studies have focused on myostatin evaluations, FST and myostatin have a strict connection in modulating muscular responses to physical activity (4). Accordingly, myostatin blockage would lead to higher increments of FST concentrations, which in turn causes higher muscle mass quality. This is in line with our findings. To the best of our knowledge, no study has evaluated the effects of BFRT on muscular endurance and dynamic balance in elderly females. However, numerous studies have shown the positive correlation of muscle mass with muscular endurance and strength (21). Thus, it could be argued that increment of FST and subsequent muscle adaptation increase muscular endurance. In our study, we measured muscular endurance with 6-minute walking. Indeed, improvements of muscle quality have increased the number of performed steps of our participants.

Regarding the dynamic balance performance, it has been indicated that lower muscle mass is associated with poor structural parameters of bone and balance in elderly men (22). Therefore, to avoid these decrements in muscular performance, older adults are recommended to perform regular RT. Undeniably, performing BFRT increases dynamic balance which in turn, would increase the quality of life of the elderly.

CONCLUSION

Our findings show that 12 weeks of BFRT significantly increased FST concentrations, improved muscular endurance and dynamic balance in elderly females. Consequently, BRFT could be suggested as a beneficial training intervention to combat age-related decrements of muscle mass, which leads to numerous functional performances in this cohort.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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