

BLOOD FLOW RESTRICTED RESISTANCE TRAINING ATTENUATES MYOSTATIN GENE EXPRESSION IN A PATIENT WITH INCLUSION BODY MYOSITIS

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ABSTRACT: Inclusion body myositis is a rare idiopathic inflammatory myopathy that produces extreme muscle weakness. Blood flow restricted resistance training has been shown to improve muscle strength and muscle hypertrophy in inclusion body myositis. Objective: The aim of this study was to evaluate the effects of a resistance training programme on the expression of genes related to myostatin (MSTN) signalling in one inclusion body myositis patient. Methods: A 65-year-old man with inclusion body myositis underwent blood flow restricted resistance training for 12 weeks. The gene expression of MSTN, follistatin, follistatin-like 3, activin II B receptor, SMAD-7, MyoD, FOXO-3, and MURF-2 was quantified. Results: After 12 weeks of training, a decrease (25%) in MSTN mRNA level was observed, whereas follistatin and follistatin-like 3 gene expression increased by 40% and 70%, respectively. SMAD-7 mRNA level was augmented (20%). FOXO-3 and MURF-2 gene expression increased by 40% and 20%, respectively. No change was observed in activin II B receptor or MyoD gene expression. Conclusions: Blood flow restricted resistance training attenuated MSTN gene expression and also increased expression of myostatin endogenous inhibitors. Blood flow restricted resistance training evoked changes in the expression of genes related to MSTN signalling pathway that could in part explain the muscle hypertrophy previously observed in a patient with inclusion body myositis.

KEY WORDS: GDF-8, muscle hypertrophy, mRNA, real-time PCR

INTRODUCTION

Inclusion body myositis (IBM) is a rare idiopathic inflammatory myopathy that produces severe muscle wasting [19,20]. The prevention of muscle atrophy and weakness in IBM patients has been challenging due to low responsiveness to conventional pharmacological (i.e. glucocorticoids) and non-pharmacological (i.e. traditional resistance training) treatments [20,25]. Recently, we reported that resistance training with vascular occlusion in a patient with IBM promoted increment in muscle mass and strength [9].

Resistance training has a powerful effect on skeletal muscle structure and function. High-intensity resistance training (75-80% of 1 RM) has been recommended to promote both muscle hypertrophy and strength gain [7,23]. Alternatively, it has been recently proposed that low-intensity (i.e. 20-50% of 1 RM) blood flow restricted resistance training (BFRRT) is able to promote similar gains in strength and muscle mass when compared with traditional high-intensity training [12-14]. This type of training could be of therapeutic relevance. For instance, Yokokawa et al. [28] reported that low-intensity resistance training with vascular occlusion attenuated muscle weakness and disability in elderly individuals. However, the mecha-

nisms underlying these adaptations remain to be explored.

Over the last decade, the molecular mechanisms that modulate muscle mass as a consequence of muscle overloading conditions (e.g. resistance training) have been extensively investigated [5,18,21]. In this regard, growth and differentiation factor 8 (GDF-8; also known as myostatin [MSTN]), a member of the TGF-B superfamily, seems to play a key role in controlling muscle wasting [15,17]. For instance, it has been shown that stretching-induced longitudinal muscle growth promotes dramatic up-regulation of genes that encode MSTN endogenous inhibitors in rats [1]. Similarly, human studies have observed that a strength training programme down-regulates MSTN expression, thus increasing muscle hypertrophy [1,13,24], whereas detraining triggers type-II fibre atrophy paralleled with up-regulation of MSTN expression [11]. Altogether, these findings suggest that down-regulation of the MSTN signalling pathway may be associated with overload-induced increments in muscle hypertrophy and strength, thereby preventing muscle wasting. Considering that MSTN signalling is involved in muscle remodelling [15,17,29], muscle wasting conditions emerge as a very interesting model to investigate the role of

resistance training in MSTN gene expression and muscle protein accretion.

In the present study, we hypothesized that the expression of genes related to MSTN signalling (MSTN, follistatin, follistatin-like 3, activin IIB receptor, SMAD-7, MyoD, FOXO-3 and MURF-2) might be related to the improvement in muscle mass and strength experienced by this patient following the resistance training programme. Therefore, the purpose of this study was to investigate the effect of BFRRT programme on the expression of genes related to the MSTN signalling pathway in an IBM patient.

MATERIALS AND METHODS

All the experiments reported in the manuscript were performed in accordance with the ethical standards of the Helsinki Declaration and were approved by the School of Medicine – University of Sao Paulo – Ethical Committee (Protocol #1185/07). In order to assess the expression of genes related to MSTN signalling, muscle samples previously obtained from an IBM patient who underwent a 12-week BFRRT programme were utilized [9,10]. BFRRT was chosen because this training mode is effective in increasing muscle mass while using low exercise loads [14]. Therefore, this training seems to be suited to IBM patients, who have great difficulties in generating muscle force.

Prior to and after the intervention, muscle biopsies were obtained from a 65 year-old male patient with IBM (weight: 85 kg; height: 180 cm; VO_2 peak: $10 \text{ ml}^{-1} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) who underwent twice-a-week, 12-week BFRRT. The training protocol consisted of a brief warm-up on a treadmill. Then, the patient performed three sets of 15 RM (30 s between sets) of leg-press, knee extension, and half-squat exercises with blood flow restriction at 50% of the total vascular occlusion pressure. Two pressure cuffs were positioned near the inguinal fold region on both thighs and inflated to the training pressure. The cuff's pressure was maintained during the whole session, including intervals. Training intensity was adjusted according to the gradual increase in strength so the patient would be able to perform no more than 15 RM. All sessions were monitored by two physical trainers. The patient's characteristics, the training protocol, and the clinical outcomes were described in detail elsewhere [9,10]. Besides mild acute pain, the patient reported no adverse effects.

Muscle samples (~20 mg) were obtained from the vastus lateralis of the subject's right leg using the percutaneous biopsy technique. All biopsies were performed by a medical doctor after a 10-hour overnight fast, and the last meal was a standardized dinner. The post-intervention biopsy was conducted approximately 24 hours after the last training session. Immediately after the procedure, the muscle sample was removed from the needle and frozen in liquid nitrogen, for further storage at -80°C . The gene expression of myostatin (MSTN), follistatin (FLST), follistatin-like 3 (FL-3), activin IIB (ActIIB), SMAD-7, MyoD, FOXO-3 and MURF-2 was quantified using real-time PCR following standard procedures described elsewhere [1,16].

RESULTS

After 12 weeks of training, a moderate decrease in MSTN mRNA expression (25%) was observed, whereas FLST and FL-3 mRNAs expression increased (FLST: 40% and FL-3: 70%) (Figure 1). SMAD-7 mRNA level was slightly enhanced (20%) (Figure 2). FOXO-3 and MURF-2 gene expression increased by 40% and 20%, respectively (Figure 2). No change was observed in ActIIB and MyoD gene expression (Figure 1 and 2 respectively).

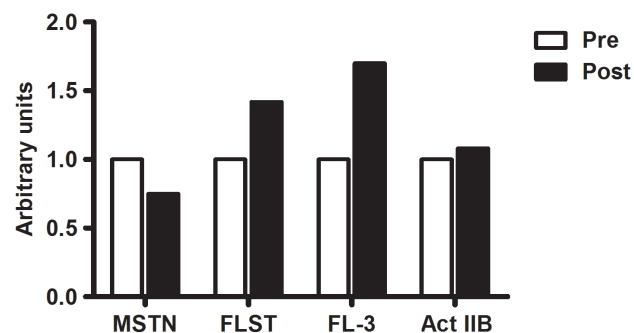


FIG. 1. THE EFFECTS OF 12-WEEK BFRRT ON MRNA EXPRESSION LEVELS OF MSTN, FLST, FL-3, AND ACTIIB IN A PATIENT WITH IBM.

Note: The post-training values are reported as fold-change relative to baseline expression at the start of the study.

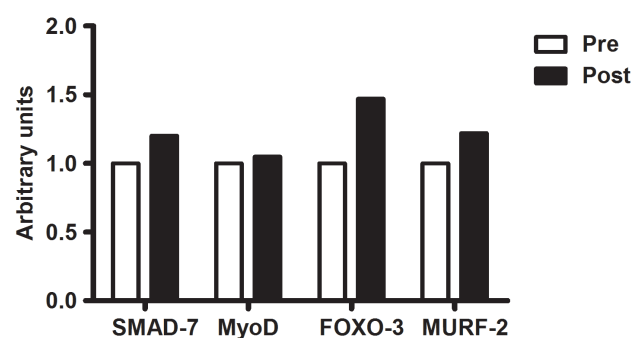


FIG. 2. THE EFFECTS OF 12-WEEK BFRRT ON MRNA EXPRESSION LEVELS OF SMAD-7, MYOD, FOXO-3, AND MURF-2 IN A PATIENT WITH IBM.

Note: The post-training values are reported as fold-change relative to baseline expression at the start of the study.

DISCUSSION

The results of this study confirmed the initial hypothesis that BFRRT could modulate MSTN signalling in an IBM patient. MSTN gene expression was depressed, whereas gene expression of the MSTN inhibitors FLST, FL-3 and SMAD-7 was up-regulated. These changes may reveal the molecular mechanisms involved in the muscle hypertrophy previously reported in the IBM patient following BFRRT [9].

IBM seems to be resistant to all proposed treatments [4], including resistance training. Spector et al. [25] examined the efficacy of a strength training programme on five IBM patients and reported no changes in the muscle cross-sectional area. Another study reported no significant alterations in fatigue or isometric peak power in seven IBM patients submitted to a home-based training programme [3]. Furthermore, BFRRT seems to improve leg press 1 RM, muscle function and thigh cross-sectional area in an IBM patient by 11.6%, 60.0%, and 4.7%, respectively [9]. These responses were paralleled by an increase in mechano-growth factor (MGF) mRNA level and a reduction in atrogin-1 mRNA level [9], indicating that this training mode might stimulate protein accretion.

Given the increase in MGF gene expression [9] and the decrease in MSTN mRNA level, it is reasonable to speculate that BFRRT favours muscle protein accretion even in a catabolic condition (i.e. IBM). Although these results corroborate this hypothesis, MSTN responses to exercise training have been controversial and need further clarification. For instance, Roth et al. [24] showed that 9-week concentric resistance training decreased MSTN gene expression, while Willoughby [26] demonstrated that muscle MSTN mRNA level, MSTN protein content and serum MSTN significantly increased after 12 weeks of resistance training.

In the present study, the mild reduction in MSTN gene expression (25%) was followed by an increase in FLST and FL-3 gene expression, which are endogenous inhibitors of activin and other TGF- β superfamily members, including MSTN [15]. Previous studies have also demonstrated that FLST mRNA level is up-regulated during muscle regeneration [2] and muscle growth [1], whereas MSTN mRNA level decreases concomitantly. More recently, Laurentino et al. [16] reported that BFRRT promoted a concomitant decrease in MSTN and increase in FLST isoforms, GASP-1, and SMAD-7 mRNA gene expression in healthy young males.

These data suggest that BFRRT increased FLST mRNA level inhibiting MSTN signalling, thereby maximizing muscle growth. Furthermore, the increase in SMAD-7 gene expression in the current

study may also explain the down-regulation of MSTN gene expression, since the SMAD-7 may attenuate MSTN expression by inhibiting its promoter activity [6,30].

FOXO-3 is a transcription factor that stimulates the expression of “atrogenes” (e.g. E3 ligases) [8] and MURF-2 is a member of the E3 ligase family which is involved in the ubiquitin proteasome degradation system [27]. Thus, the up-regulation of both FOXO-3 and MURF-2 genes suggests that the training intervention may also stimulate skeletal muscle protein degradation, which is expected in IBM patients. However, these findings cannot be interpreted independently, since the previously reported muscle hypertrophy suggests a predominance of anabolism over catabolism [22].

CONCLUSIONS

The present study provides evidence that the down-regulation of MSTN gene expression as well as the up-regulation of its endogenous inhibitors are underlying mechanisms related to the increase in muscle mass previously reported in an IBM patient submitted to BFRRT. The findings of the present study support the concept that the low-intensity BFRRT could be applied to other conditions (e.g. osteoarthritis, rheumatoid arthritis, inflammatory myopathies, and dystrophinopathies) in which 1) muscle wasting and low force production capacity are major concerns or 2) clinical limitations (e.g. chronic pain) impede the implementation of high-intensity resistance training.

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Conflict of interest: Authors declared no conflict of interest.

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