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Effects of upper-body, lower-body, or combined resistance training on the ratio of follistatin and myostatin in middle-aged men

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Short title: Follistatin and Myostatin changes through resistance training

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Abstract

Purpose: Due to the mechanistic role of myostatin and follistatin in modulating muscle mass, shifts in the follistatin to myostatin ratio (F: M) may help explain changes in muscular size in response to resistance training (RT). The present study examined whether differential responses in follistatin and myostatin occur based on the amount of active musculature in a RT program in middle-aged men.

Methods: Forty middle-aged men (age= 46.5±3.1 years) were randomly assigned to one of 4 groups, upper-body RT (UB; n=10), lower-body RT (LB; n=10), combined RT (UB+LB; n=10) or control (C; n=10). The training protocol consisted of 3 exercise sessions per week for 8 weeks. Blood samples were obtained at baseline and 48 hours after the final session of the training program.

Results: Muscle mass significantly increased ($p<0.05$) following UB= 0.76 ± 0.46 kg, LB= 0.90 ± 0.29 kg, UB+LB= 1.38 ± 0.70 kg, compared to no changes after control. Serum follistatin increased in the LB= 0.24 ± 0.06 $ng.mL^{-1}$, UB= 0.27 ± 0.17 $ng.mL^{-1}$, UB+LB= 0.50 ± 0.18 $ng.mL^{-1}$, while serum myostatin decreased in the LB= -0.11 ± 0.08 $ng.mL^{-1}$ and UB+LB= -0.34 ± 0.23 $ng.mL^{-1}$, but not UB= 0.07 ± 0.16 $ng.mL^{-1}$. Further, change in concentration following training was larger between UB+LB and either LB or UB alone for both follistatin and myostatin.

Conclusions: Both UB and LB increase muscle mass and alter the F: M ratio, however the change in these endocrine markers is approximately twice as large if UB and LB is combined. The endocrine response to RT of myostatin and follistatin may depend on the volume of muscle mass activated during training.

Keywords: Follistatin, Myostatin, Resistance training, Follistatin to Myostatin ratio.

Abbreviations:

RT= Resistance training

F: M= Follistatin to myostatin ratio

SkMM= Skeletal muscle mass

PBF= Percent body fat

BMI=Body mass index

LBM=Lean body mass

FM=Fat mass

Introduction

Increasing age is associated with gradual, progressive and spontaneous erosive changes in most physiological systems and functions of the body. It has been well demonstrated that a substantial decrease in muscle strength occurs with aging, which decreases the ability to perform daily activities in middle-age and older adults (Viitasalo et al. 1985). To combat these decrements in muscular performance and functional ability, it is recommended that middle-aged and older adults perform regular resistance training (RT) (Hakkinen et al. 2000). It is well established that RT is an effective modality for increasing muscular strength, hypertrophy, and power (Frontera et al. 1988). Based on available evidence, regular RT in middle-aged adults induces muscle hypertrophy, which positively influences muscular strength and quality of life, both in the immediate period, and ideally into older age (Law et al. 2016). Recently, the influence of RT on myokines and adipo-myokines involved in the stimulation and inhibition of the muscular hypertrophic response has been explored. One notable myokine is myostatin, a powerful negative regulator of muscle size (Elliott et al. 2012). Myostatin circulates in the bloodstream, binds to Activin Type II receptors in the muscle, thereby instigating intercellular signaling pathways that inhibit muscle growth (Gonzalez-Cadavid et al. 1998). Whilst a logical hypothesis would be for an increase in serum myostatin with advancing age, there is currently mixed evidence for this. Indeed, while early work suggested that serum myostatin levels increase with advancing age (Yarasheski et al. 2002) , more recent examinations have suggested no difference in circulating myostatin concentration in younger vs older men with and without sarcopenia (Ratkevicius et al. 2011) nor in a cross-sectional study of individuals spanning 18-68 years of age (Barrios- Silva et al. 2018). RT can reduce the expression of myostatin at the mRNA or protein level (Hulmi et al. 2007), leading to exercise-induced muscle hypertrophy in the healthy humans. In fact, many studies have shown that RT can generate a significant decrease in myostatin levels (Allen et al. 2011; Laurentino et al. 2012). For instance, It has revealed that serum myostatin levels decreased 10% by enzyme-linked immunosorbent assay (ELISA) after 12-week arm and leg press RT (Saremi et al. 2010). Also, It has been reported reduced myostatin mRNA expression in young and old men and women in response to 9 weeks of RT (Roth et al. 2003). The mechanism responsible for the RT-induced blunting of myostatin may involve the myostatin-inhibitor follistatin. This glycoprotein, blocks the myostatin receptor, thus decreases myostatin's effect and increasing muscle mass (Tortoriello et al. 2001). In the presence of follistatin, myostatin is unable to bind to its own receptor, and its atrophic actions are inhibited. In a study, the authors demonstrated that 12-week high-intensity RT increased serum follistatin-like related gene levels, a homolog of follistatin in certain physiological mechanisms, including myostatin inhibition (Willoughby 2004). Thus, the ratio of follistatin to myostatin (F:M) has become a commonly reported important factor in studies examining body composition (i.e. lean mass and fat mass) and the alterations of muscle strength that occur with chronic training (Rodgers and Garikipati 2008; Tortoriello et al. 2001). It was investigated the effect of eight weeks of two different intensities of RT (low or high) on F: M ratio in 21 sedentary young women. They showed that F:M ratio increased dramatically in high-intensity group (Attarzadeh Hosseini et al. 2017). Additionally, it has observed an increase of F:M ratio after 8 weeks of blood flow restriction (KAATSU) training in active men (Laurentino et al. 2012). However, although several studies report an increase in the F:M ratio with RT, such changes have not been found

in all investigations (de Souza et al. 2014; Schiffer et al. 2011). This discrepancy might be related to different variables within a RT program, including the amount of active musculature involved. It is likely that the total amount of active musculature plays a role in adaptations to RT, and current recommendations advocate the training of the entire body's musculature (Medicine 2009). However, limited information is available concerning the impact of the quantity of active musculature on the F: M ratio. Relative to reports in younger adults, less work has examined the effect of training on myostatin and follistatin in older cohorts. The Vienna Active Ageing cohort reported no change in myostatin following 6 months of RT in a cohort of older women (65 – 92 years of age). RT was performed twice a week and consisted of various elastic band exercises for the major muscle groups. Intensity was progressed by increasing the resistance of elastic band. (Hofmann et al. 2016). Whilst chronically trained masters athletes show similar concentrations of circulating serum myostatin as age matched untrained controls (Elliott et al. 2017) . As myostatin appears unresponsive to acute and chronic training in an older cohort, early and sustained (e.g. young-adult to middle aged) RT interventions may be needed for functional maintenance of muscle mass and strength during ageing. Therefore, the purpose of the present investigation was to determine the responses in follistatin and myostatin following RT of differing muscle volumes in middle-aged men. It was hypothesized that the RT program involving the greatest quantity of muscle mass (i.e. full-body training) would elicit greater increases in follistatin and decreases in myostatin as compared to programs involving only select portions of the body's musculature (i.e. upper body or lower body training only).

Methods

Participants

Forty middle-aged men (40-53 years) participated on the present study. Exclusion criteria included cardiovascular diseases, diabetes, hypertension or other risk factors based on a doctor's examination. All men were sedentary, and self-reported performing less than 1 hour of exercise per week in the previous year. Participants were not taking any supplements or medications, including non-steroidal anti-inflammatory drugs. All participants gave written informed consent before their inclusion in the study. The study protocol was approved by the Institutional Human Subject committee and carried out in accordance with the Declaration of Helsinki.

Study Design

Before baseline measurements, participants were familiarized with the study tests and procedures and were randomly assigned to into one of four groups: upper body RT (UB; n=10), lower body RT (LB; n=10), combined upper and lower body RT (UB+LB; n=10) or control (C; n=10) groups. Allocation was stratified by BMI (<25.0 or ≥25.0 kg/m^2), and the sequence was randomized by a computer. Measurements were collected at baseline and after 8 weeks during the same time of day (–1 hour). Participants were instructed not to alter their regular lifestyle and dietary habits during the study.

Anthropometry and Body Composition

Upon entering the laboratory, participants were asked to urinate (void) completely within 30 min of the test and then had their body weight measured with a digital scale (lumbar, China) to the nearest 0.1 kg. Participant's height was measured with a stadiometer (Race industrialization, China) to the nearest 0.1 cm.

BMI, PBF, SkMM and LBM were evaluated by multi-frequency bioelectrical impedance device (Inbody 720, South Korea; Table 1). We instructed the participants to fast for 12 hours (an overnight fast, with at least 8 hours of sleep) and refrain from physical activity for the previous 36 hours before the test. The participants were also instructed to avoid exercising consuming alcohol for 48 hours before the test.

Blood Sampling and Analysis

Fasting blood samples (5 mL) were obtained from the cubital vein using standard procedures. The initial collection occurred 48 hours before the baseline training session. After clotting occurred, blood samples were centrifuged at 3000 RPM for 10 minutes. Spun serum was removed from the centrifuge and frozen at –70°C for later analysis. Serum myostatin (bound) (human myostatin, Glory Science Co, Del Rio, TX, USA) and follistatin (human follistatin, Glory Science Co, Del Rio, TX, USA) concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions. The intraassay and interassay coefficient of variation were 8.1% and 4.5%, 8.5% and 5.4% for myostatin and follistatin, respectively.

Strength Testing

1RM testing

Maximal strength testing took place 24 hours after the body composition measurement. 1RM testing was performed to determine training intensity for our resistance training protocol. Before the beginning of the test, authors explained all the purpose, attendant risks, and discomforts, responsibilities of the participant, benefits, inquiries and freedom of consent for all of them in Ferdowsi university of Mashhad. They were instructed to refrain from alcohol for 48 hours, caffeinated drinks for 12 hours and food intake for 2 hours before the testing session; however, water consumption was allowed. Participants warmed up their bodies for 10 minutes of general (5 minutes slow running on treadmill; 3 to 5 km speed, or elliptical; with 5 to 10 level) and specific warm-up activities (5 minutes, e.g. medicine ball twist 1×10, medicine ball wood chops 1×10, straddled toe touch 2×5, Dynamic quadriceps stretch 1 x 5, Medicine ball squat 1 x 5 to 8) before the test. The participants performed 2 attempts and their highest lifted weight and number of repetitions was recorded. The number of repetitions to fatigue did not exceed 10. There was a 3 to 5 minutes between attempts. After the testing session, the participant's maximal strength was predicted using the following formula:

1RM= weight/ (1.0278–0.0278×reps) (Murach and Bagley 2016):

Isometric quadriceps strength test

The participants stood on the body of the device and bent the knees to 130 to 140 degrees and held the trunk upright. The participant grasped the dynamometer handle with the pronation position and adjusted the length of the chain so that the handle of the dynamometer was placed on the thigh. The existing belt attached to the participants shoulders and attached it to each side of the handle of the dynamometer. This allowed the direct quantification of quadriceps isometric force. The participants performed the test 3 times each time and rested for 3 to 5 minutes between each attempt to reduce measurement error due to fatigue. All three attempts were recorded, while the highest was used for the analysis.

Isometric handgrip strength test

The participants held the dynamometer in the hand to be tested, with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer was adjusted as required, with the base resting on first metacarpal (heel of palm), while the handle rested on the middle phalange of the four fingers. When ready the participant contracted the dynamometer with maximum isometric effort, which was maintained for approximately 5 seconds. No other body movement was allowed. The participant was strongly encouraged to give a maximum effort. All three attempts were recorded, while the highest was used for the analysis (Roberts et al. 2011).

Resistance Training Protocol

Preparatory phase:

All the participants performed 1 week of RT, consisting of 3 exercise sessions, for familiarization before the main training intervention. This phase allowed for supervised instruction of proper lifting technique, familiarization with all exercises, and ensured that the participants initiated the study with a comparable training base**.** The adaptation phase included a total of 6 exercises (Table 3). The preparatory phase program was adapted from previous literature in middle-age men (Church et al. 2016).

Training Phase

Following the preparatory phase, participants in the UB, LB and UB+LB completed the supervised training 3 times a week, separated by at least 48 hours for 8 weeks. Before each training session, the first 10 minutes included general and specific warm-up activities (slow running, stretching and light RT). After the general warm-up, participants completed a specific warm-up of 2 sets of 20 repetitions with 30% of 1RM with a 30 seconds between sets. Following the specific warm-up, training included 3 sets per exercise in weeks 1-4 and 4 sets per exercise in weeks 5-8. The rest interval between sets was 30 seconds in the first and second week, 60 seconds in the third and fourth week, 75 seconds in fifth and sixth weeks, and 90 seconds in seventh and eighth weeks (table 4). They rest interval between exercises was 2 minutes throughout the training period.

In each RT program, all exercises included 3 sets with the intensity of 50% to 80% 1RM (Table 4). To verify the principle of overload, the following formula was used to predict the 1RM and then determine the exercise load before the start of the training period, the fourth week, and at the end of the eighth week:

1RM = W/ [1.0278(0.0278.r)] (Murach and Bagley 2016)

The exercises (in order) included in the UB training program were lateral pulldown, chest press, barbell shoulder press, lateral raise, standing barbell biceps curl, cable triceps pushdown. The exercises included in the LB training program were barbell squat, hack squat, lunges, leg extension, lying leg curls and standing calf raises. The exercises included in the UB+LB program were lateral pulldown, barbell squat, chest press, lunges, lateral raise and standing calf raise. All training sessions were completed under cautious supervision from certified trainers and researchers. The periodized RT programs were adapted from previous literature (Simão et al. 2013), following recommendations by the National Strength and Conditioning Association (Haff and Triplett 2015).

Nutrient intake and dietary analysis

Participants were instructed not to alter their dietary habits during the study. To minimize dietary variability, the participants were required to submit 3-day (2 weekdays and 1 weekend) food records at baseline and at 8 weeks of the assigned intervention. Each dietary item was entered into the program Diet Analysis Plus version 10 (Cengage, Boston, MA, USA) and total energy consumption, and the amount of energy derived from proteins, fats, and carbohydrates was assessed, with paired sample t-tests used to confirm a lack of change in dietary factors at pre- and post-time points (table 5).

Statistical analysis

Normality was confirmed by the Shapiro–Wilk test. Based on previous data (Negaresh et al. 2017; Saremi et al. 2010). It was calculated that 10 participants per group would provide 80% power (2-sided $\alpha = 0.05$) to detect 9% and 7% changes in myostatin and follistatin, respectively. Differences between phenotypic measures, strength measures and endocrine markers were compared by a 4× 2 ANOVA with repeated measures (group (control x LB x UB x UB+UB) x time (pre x post)). One-way ANOVA was used for 'change in' (Δ) scores between times (pre, post) where appropriate. Post hoc testing was performed by Bonferroni corrected t-tests. SPSS (Version 24, IBM) was used for all statistical analysis, and all figures were prepared in GraphPad Prism (Version 5.03, GraphPad Software).

Results

RT alters phenotype in a training model independent manner

Baseline parameters between the two groups were not significantly different (p>0.05). A significant group x time interaction was noted for SkMM ($p < 0.001$) and for FM ($p < 0.001$; Figure 1A & C, respectively). SkMM had a significantly greater increase [UB= 0.76 kg (95% confidence interval {CI}, 0.48 to 1.05); LB= 0.90 kg (95% CI, 0.72 to 1.08) and UB+LB= 1.38 kg (95% CI, 0.95 to 1.81)] and FM a decreased [UB= -1.61 kg (95% CI, -1.91 to -1.29); LB= -1.32 kg (95% CI, -1.57 to -1.07) and UB+LB= -2.26 kg (95% CI, -3.31, to -1.21)] post-training than did the control ($p < 0.05$ in training groups, $p > 0.05$ control group). However, no difference in absolute mass of SkMM gained or FM lost was noted between UB+LB and either UB or LB alone ($p > 0.05$; Figure 1B & D).

Whole-body RT alters endocrine markers to a greater degree than upper or lower body RT alone

A significant group x time interaction was noted for serum concentration of myostatin, follistatin and the myostatin:follistatin concentration ratio (myostatin $p < 0.001$, follistatin $p < 0.001$, myostatin: follistatin ratio $p < 0.001$). Myostatin concentration was shown to be decreased ($p < 0.05$) in LB [-0.11 $ng.mL^{-1}$ (95% CI, -0.16 to -0.06)] and UB+LB [-0.34 $ng.mL^{-1}$ (95% CI, -0.48 to -0.19)] groups, but not UB or control ($p > 0.05$; figure 2C & D). Whilst follistatin was increased post-training [UB= 0.27 $ng.mL^{-1}$ (95% CI, 0.16 to 0.38); LB= 0.24 $ng.mL^{-1}$ (95% CI, 0.20 to 0.28) and UB+LB= 0.50 $ng.mL^{-1}$ (95% CI, 0.39 to 0.61)] relative to control groups ($p < 0.05$ in training groups; Figure 2A), of more interest was the observation that UB+LB increased follistatin to a greater extent than UB or LB alone ($p < 0.05$; Figure 2B). Finally, the follistatin:myostatin ratio was increased in all training groups [UB= 0.07 (95% CI, 0.04 to 0.10); LB= 0.07 $ng.mL^{-1}$ (95% CI, 0.06 to 0.09) and UB+LB= 0.18 (95% CI, 0.12 to 0.24)], relative to both the control group ($p < 0.05$), and to a greater degree in UB+LB than UB or LB alone ($p < 0.05$; Figure 2E & F).

RT improves isometric strength

A significant group x time interaction ($p < 0.001$, table 2) was noted for isometric quadriceps which significant increased ($p < 0.05$) in all training groups [UB= 2.4 kg (95% CI, 2.2 to 2.6); LB= 4.6 kg (95% CI, 4.3 to 5.0) and UB+LB= 3.6 kg (95% CI, 3.2 to 3.9)] compared to no changes after the control group. The LB increased isometric quadriceps strength to a greater extent than UB+LB or LB alone ($p < 0.05$). There were also significant increases ($p < 0.05$) in right [UB= 4.6 kg (95% CI, 4.3 to 5.1); LB= 2.4 kg (95% CI, 2.1 to 2.6) and UB+LB= 2.7 kg (95% CI, 2.4 to 2.9)] and left [UB= 3.2 kg (95% CI, 2.8 to 3.6); LB= 1.5 kg (95% CI, 1.2 to 1.8) and UB+LB= 2.3 kg (95% CI, 2.1 to 2.6)] isometric handgrip strength in all training groups but no changes after control. The UB increased right and left isometric handgrip strength to a greater extent than UB+LB or LB alone ($p < 0.05$)

Discussion

This study aimed to identify the effect of three 8-week RT protocols, each activating different volumes of muscle mass (LB, UB or UB+LB) on follistatin, myostatin and their ratio in middle-aged men. The key finding we report is that the response of follistatin and myostatin to muscle mass building RT is proportional to the volume of muscle mass activated. In response to each RT program (UB+LB, UB, and LB), serum follistatin levels increased significantly, and the greatest effect was observed in the group performing both upper- and lower-body RT (i.e. UB+LB). The F: M ratio also increased significantly in all three groups, with the greatest effect observed in UB+LB. Myostatin decreased similarly in all training groups, although not significantly in the UB group following training. As would be expected, all forms of RT resulted in increased muscle mass gain. Indeed, it is well described in the literature that RT results in increases in muscle mass in most healthy individuals (Grgic et al. 2018), and that greater muscle gain results from higher volumes of training (Schoenfeld et al. 2016), higher number of sets completed (Krieger 2010), and indeed specificity of training response to the muscle mass utilized in training (Taniguchi 1997; Taniguchi 1998) . The role of myostatin and follistatin in response to RT has been reported prior to this paper, and it is noteworthy that our results here mirror those of others. In particular, Walker et al. (2004) showed that plasma myostatin protein levels decreased by approximately 20 % in participants after 10 weeks of twice weekly bouts of RT, in a manner similar to that shown by others (Saremi et al. 2010). Both younger and older men show decreased circulating myostatin and increased follistatin concentration in response to 8 week of RT, in a highly similar manner to the results we report here (Negaresh et al. 2017). However, it is noteworthy, that when challenged with UB+LB, our participants show an endocrine response of myostatin and follistatin that is approximately twice what is seen if UB or LB alone is performed. This novel finding suggests that the volume of muscle mass activated is a key factor in the endocrine portion of the anabolic response to RT. Indeed, RT studies that have examined volume of training report lower volumes produce a lesser endocrine response of anabolic hormones such as growth hormone and testosterone (Goto et al. 2004; Gotshalk et al. 1997) . In contrast to these results and the findings we report here, Walker et al, (2004) did not show a difference in plasma myostatin response between a whole body vs a UB training protocol similar to our own, when semi-quantitatively examined by Western blot. We would speculate that this may be due to the measurement technique used, and that current ELISA based protocols would be more sensitive to changes in concentration than Western blot approaches; however, this has not be objectively tested by ourselves to date. Alternatively, it was examined a relatively light training workload (twice weekly) and muscle mass gain relatively to the load used in our middle-aged men, which may underlie the differences in myostatin response by muscle volume trained (UB vs LB vs LB+UB)(Walker et al. 2004). The single-chain polypeptide follistatin is a member of the larger family of the Transforming growth factors (Görgens et al. 2013), that is ubiquitously expressed in all tissues of the human body, including skeletal muscle, and has paracrine and autocrine effects. Previous reports showed that follistatin has anabolic and catabolic effects in skeletal muscle and adipose tissue, respectively (Gilson et al. 2009; Rodino- Klapac et al. 2009), likely via follistatin's ability to bind and inactivate myostatin (Amthor et al. 2004). Most studies examining RT and follistatin have found increased circulating follistatin concentrations in their research, which is in line with the results we report here (Hofmann et al. 2016; Jang et al. 2016; Negaresh et al. 2017).

The increase in F: M ratio after our UB+LB as related to a change in the balanced disturbance of the muscle growth regulators towards positive regulators. In normal conditions, to maintain muscle fiber size, there is a homeostatic balance between positive (such as follistatin and IGF-1) and negative (such as myostatin) regulators of muscle growth; but this balance, when the muscle is atrophic, leads to a dominance in negative regulators; hence, loading the muscle through RT, may lead to the dominance positive regulators. Although the mechanism of communication between these regulators is not completely clear, this connection seems to be made through a very complex negative feedback loop (Gonzalez-Cadavid et al. 1998). Furthermore, has been shown that the release rate of myokines and regulatory factors for muscle growth is largely dependent on the intensity and volume of muscles involved in the activity (Motevalli et al. 2015). It appears that the volume of muscle activated is a key variable in RT, as this promotes anabolic pathways in a larger quantity of tissue (Burd et al. 2010; Terzis et al. 2010). Accordingly, the combined exercise protocol of this study, compared with the upper and lower trunk exercise protocols, showed an increase in the F: M ratio in middle-aged men due to the involvement of more muscles in RT. However, at least within the time frames reported here, this did not result in a significant difference in the gains in whole body muscle mass or decrease in fat mass between training groups. This may be at least partially explained by the short duration of the training protocol. It is generally held that neural adaptations predominate first, while muscle hypertrophy comes to being evident after approximately 8 weeks of training (Haff and Triplett 2015). Therefore, our 8-week intervention might not have warranted enough time for a significant difference in muscle mass gains between training groups (although there was a non-significant tendency for the UB+LB to have higher improvements than the other groups). Additionally, somewhat similar training volume was used in all 3 groups [6 exercises (3 for each the upper and lower half) in the UB+LB compared to 6 exercises for the UB and LB groups], which potentially affected the myostatin and follistatin response and hence the muscle mass gain and fat loss in the UB+LB. Myostatin can both inhibit or promote adipogenesis (Deng et al. 2017) which may create some confusion as far as its effects on fat loss in our study. Nonetheless, previous results in rats show that the reduction in fat mass in myostatin deficient rats is influenced by metabolic changes in skeletal muscle (Guo et al. 2009). Therefore, the changes in fat mass in the UB+LB that tended to be greater (although non-significant) than the LB and UB, might be influenced by greater improvement in muscle mass in the UB+LB group. However, future RT studies in different populations are needed to validate our claim.

The interesting findings of our investigation should be understood in the context of the following limitations. We did not measure training motivation and fiber type, which would have strengthened the matching of participants and hence our study design. We were not able to document training data, such as loads and changes in work volume, that might have helped explaining some of our findings. This study is also limited by short duration and low-moderate intensity (50-80% of 1RM) of our intervention; which warrants further investigations with longer interventions and heavier loads with a focus on understanding the relationship between changes in SkMM and FM, and myostatin as well as follistatin responses after combined upper- and lower-body RT.

In conclusion, the findings of this study show that the use of combined upper- and lower-body RT increases the serum ratio of F: M in middle-aged men over upper or lower-body training along, suggesting that

volume of muscle mass activated may influence the magnitude of the serum myostatin and follistatin response. Future research might examine the volume of muscle activated vs the total amount of work completed, as this research suggests one of these two variables underlies the endocrine anabolic response of follistatin and myostatin.

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Conflict of interest

The authors declare no conflict of interest.

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Figures Legends

Figure 1: RT increases skeletal muscle mass and decreases fat mass independent of training modality. A) Skeletal muscle mass (SkMM; kg) by group pre and post training. B) Change in (Δ) SkMM (kg) from pre to post training. C) Fat mass (FM: kg) by group pre and post training. D) ΔFM (kg) from pre to post training. Error bars represent standard deviation. Individual data points as shown. * indicates differences from groups as shown, ^ indicates differences from control. Upper body (UB), lower body (LB), upper body and lower body combined (UB+LB).

Figure 2: RT alters endocrine factors myostatin and follistatin as a function of the volume of muscle mass trained. A) Follistatin ($ng.mL^{-1}$). B) Change in (Δ) follistatin pre to post ($ng.mL^{-1}$). C) Myostatin ($ng.mL^{-1}$). D) Δ myostatin ($ng.mL^{-1}$). E) follistatin:myostatin (F:M) ratio. F) ΔF: M ($ng.mL^{-1}$). Error bars represent standard deviation. Individual data points as shown. * indicates differences from groups as shown, ^ indicates differences from control. Upper body (UB), lower body (LB), upper body and lower body combined (UB+LB).

Figure

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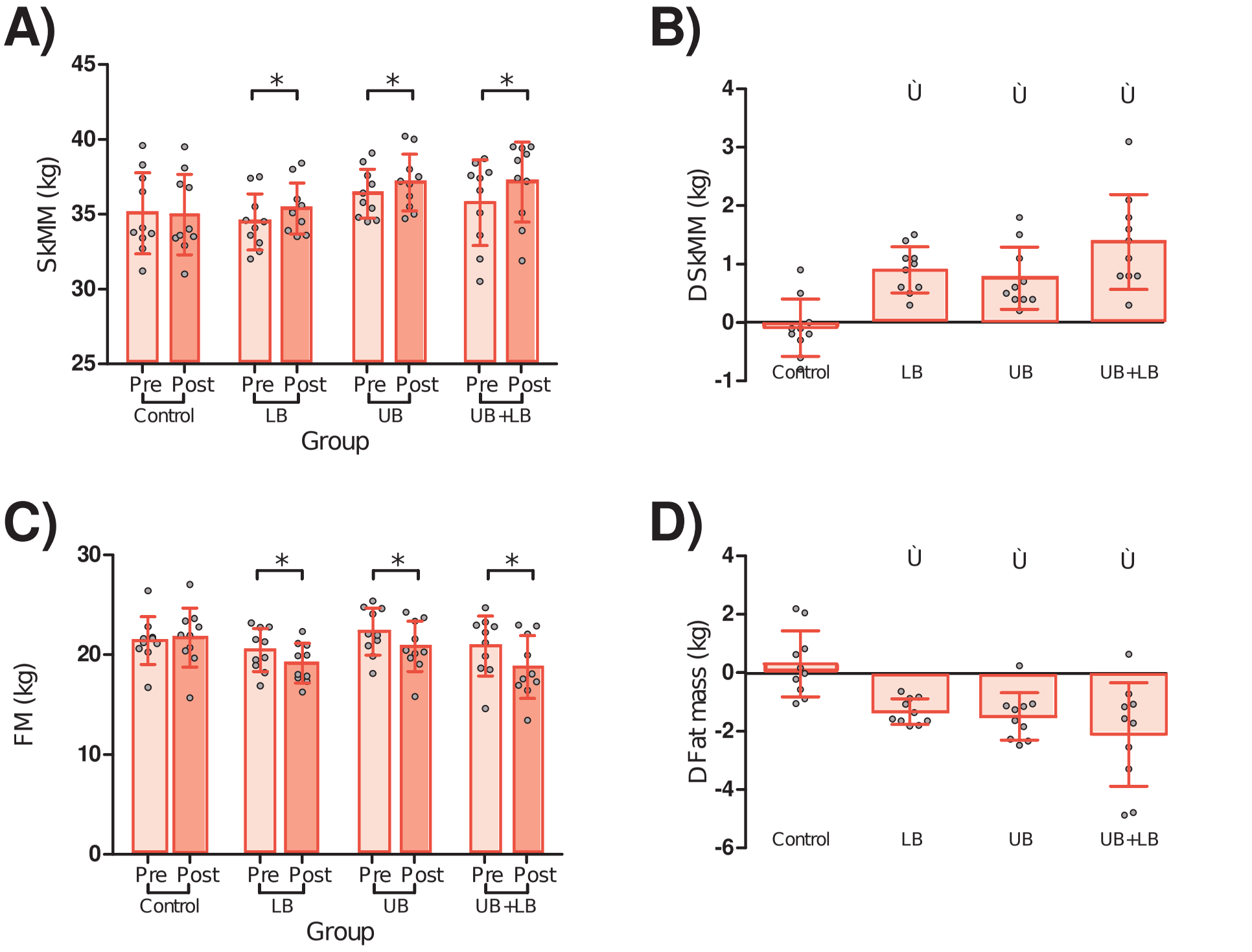

Figure

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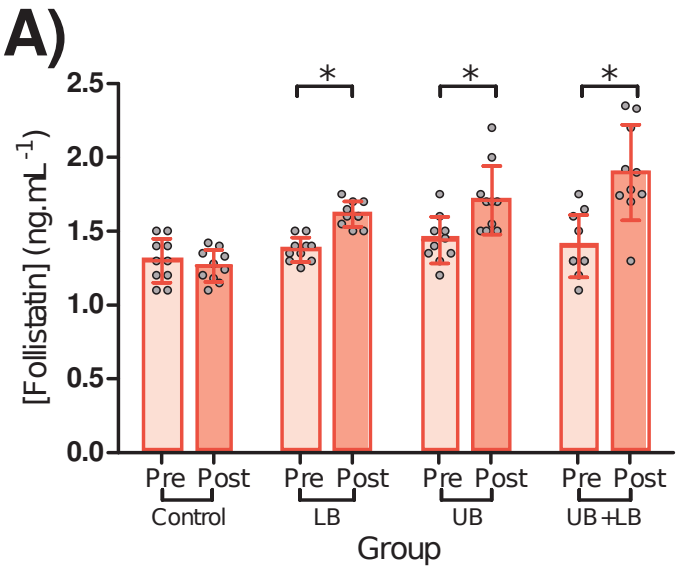

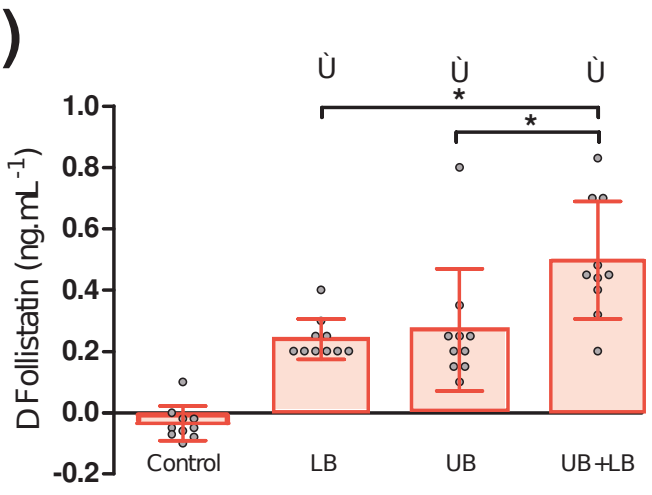

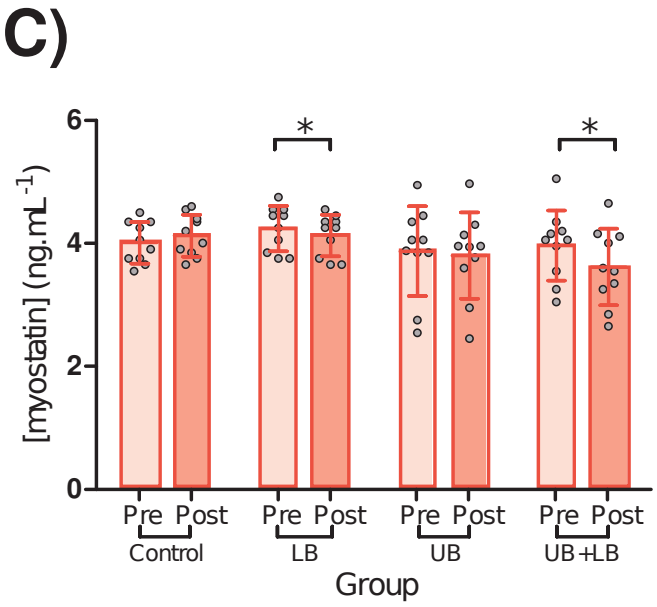

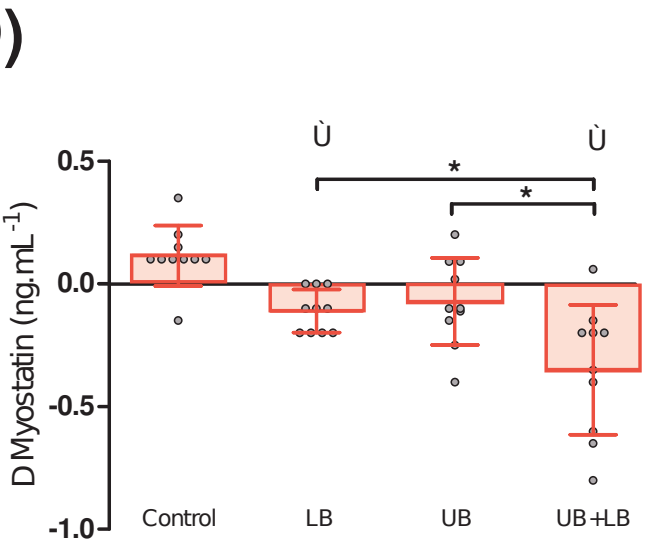

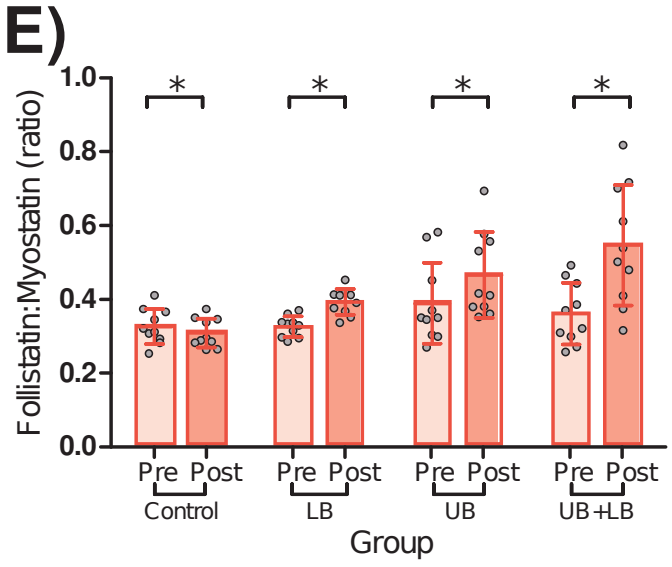

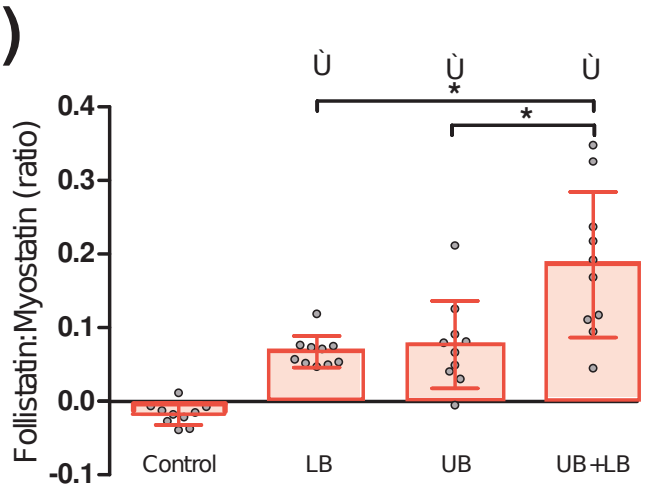

Table

Week	Intensity (1RM)	Repetitions	Sets	Rest (second)
1	50%	15	3	30
2	50%	15	3	30
3	60%	12	3	60
4	60%	12	3	60
5	70%	10	4	75
6	70%	10	4	75
7	80%	8	4	90
8	80%	8	4	90

Table 4. Variables in the resistance training protocols

Table

Variable	Group	Pre-training	Post-training	P-value
		M±SD	M±SD	
Age (year)	UB+LB	45.6 ± 3.2	---	---
	UB	47.9 ± 1.9	---	---
	LB	45.3 ± 2.8	---	---
	C	47.5 ± 3.9	---	---
Height (cm)	UB+LB	175.9 ± 4.2	---	---
	UB	177.8 ± 2.5	---	---
	LB	174.3 ± 3.9	---	---
	C	176.7 ± 2.7	---	---
Weight (Kg)	UB+LB	83.7 ± 6.1	82.2 ± 5.9	0.00
	UB	86.8 ± 5.0	85.9 ± 4.8	0.004
	LB	81.1 ± 5.3	80.1 ± 5.3	0.001
	C	85.4 ± 5.5	85.8 ± 5.6	0.23
BMI (kg/m^2)	UB+LB	27.1 ± 1.1	26.6 ± 1.1	0.00
	UB	27.5 ±1.6	27.2 ± 1.6	0.004
	LB	26.7 ± 1.4	26.4 ± 1.5	0.001
	C	27.4 ± 1.5	27.5 ± 1.5	0.25
LBM (kg)	UB+LB	63.2 ± 3.9	64.1 ± 3.9	0.01
	UB	64.1 ± 3.7	64.4 ± 3.9	0.18
	LB	60.7 ± 4.0	61 ± 4.1	0.07
	C	64.1 ± 4.0	64.0 ± 3.9	0.92
PBF (%)	UB+LB	24.3 ± 2.5	21.8 ± 2.4	0.001
	UB	26.1 ± 1.2	25 ± 1.6	0.007
	LB	25.2 ± 1.8	23.8 ± 1.6	0.00
	C	25.2 ± 1.8	25.2 ± 2.5	0.54
SkMM (kg)	UB+LB	35.9 ± 2.7	37.4 ± 2.4	0.00
	UB	36.1 ± 1.8	36.8 ± 2.1	0.001
	LB	34.4 ± 1.8	35.3 ± 1.7	0.00
	C	35.0 ± 2.7	34.9 ± 2.6	0.57
FM (kg)	UB+LB	20.4 ± 3.0	18.1 ± 2.8	0.001
	UB	22.7 ± 1.8	21.5 ± 1.8	0.004
	LB	20.4 ± 2.1	19.1 ± 1.9	0.00
	C	21.4 ± 2.3	21.7 ± 2.9	0.41

Table 1. Physiological characteristics of the participants. Abbreviations: BMI, Body Mass Index; LBM, Lean Body Mass; PBF, Percent Body Fat; SkMM, Skeletal Muscle Mass; FM, Fat Mass.

Table

	Group	Pre-training	Post-training	P-value
Quadriceps strength test (kg)	UB+LB	66.1 + 5.1	69.8 + 4.2	0.00
	UB	67.4 + 6.4	69.8 + 6.9	0.00
	LB	67.3 + 5.9	71.9 + 5.8	0.00
	C	69.3 + 4.8	68.5 + 5.4	0.19
Handgrip strength test (left hand; kg)	UB+LB	42.9 + 2.8	45.2 + 2.8	0.00
	UB	42.3 + 2.3	45.4 + 3.1	0.001
	LB	41.8 + 4.2	43.3 + 4.8	0.01
	C	42.3 + 3.5	42 + 4.3	0.49
Handgrip strength test (right hand; kg)	UB+LB	44.5 + 2.3	47.2 + 2.2	0.00
	UB	46.6 + 2.8	51.3 + 3.5	0.00
	LB	44.2 + 2.1	46.6 + 1.8	0.00
	C	45.8 + 2.4	45.4 + 3.4	0.47

Table 2. Values for the Quadriceps and Handgrip strenght tests

Table

Week	Exercises	Intensity (1RM)	Repetitions	Rest intervals	Sets
1	BS, LP, LE, R, SLC, LR	40	12	30 sec	3

Table 3. Adaptation phase training protocol. Abbreviations: Barbell squat, BS; Lateral raise, LR; Leg Extension, LE; Seated Leg Curl, SLC; Lat pulldown, LP; Rowing, R.

Table

	Group	Pre-training	Post-training	p-value
Energy (kcal/kg/d)	UB+LB	26.1 + 3.8	25 + 3.3	0.41
	UB	26.7 + 4.6	26 + 5.1	0.56
	LB	26.6 + 5.4	25.5 + 3.2	0.43
	C	26.2 + 5.6	26.3 + 5.7	0.97
Protein (g/kg/d)	UB+LB	0.83 + 0.13	0.86 + 0.09	0.51
	UB	0.84 + 0.18	0.85 + 0.23	0.73
	LB	0.85 + 0.18	0.85 + 0.16	0.96
	C	0.83 + 0.16	0.83 + 0.13	0.91
Fat (g/kg/d)	UB+LB	0.84 + 0.21	0.81 + 0.19	0.78
	UB	0.8 + 0.18	0.79 + 0.22	0.84
	LB	0.78 + 0.23	0.76 + 0.17	0.79
	C	0.8 + 0.29	0.8 + 0.24	0.81
CHO (g/kg/d)	UB+LB	3.8 + 0.54	3.5 + 0.3	0.25
	UB	4.0 + 0.7	3.8 + 0.8	0.42
	LB	4.0 + 0.7	3.8 + 0.6	0.34
	C	3.9 + 0.8	3.9 + 0.9	0.87

Table 5. Energy and macronutrients (Mean ± SD). Abbreviations: kcal, kilocalories; kg, kilogram of body weight; d, day; g, grams.

Author Contribution Statement

Dr. Rashidlamir conceived and designed research.

Reza Bagheri conducted the experiments and wrote the paper.

Dr. Wong and Mehrabani fixed grammatical mistakes. In addition, Dr. Wong analyzed data, contributed in writing the paper and preparing revisions.

M Motevalli analyzed the nutrition data.

Dr. Elliott analyzed the other data, wrote the results sections and fixed all the section of the paper academically.

Finally all authors read and approved the manuscript.