

## Effects of Resistance Training on Muscular Adaptations and Inflammatory Markers in Overweight and Obese Men

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Accepted for Publication: 16 October 2024

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**Conflict of Interest and Funding Source:** The University of Isfahan provided funding sources to perform study procedures. The authors declare no conflict of interest.

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## ABSTRACT

**Purpose:** Obesity may blunt exercise responsiveness to improve muscular adaptations. The effect of resistance training (RT) targeting different body regions on muscle and inflammatory markers is unclear. This study aimed to investigate the impact of upper (upper body exercises), lower (lower body exercises), or combined (upper body + lower body exercises) RT on muscle and inflammatory markers, body composition, and performance in overweight and obese men.

**Methods:** Sixty overweight and obese men (age =  $31 \pm 4$  years) were randomly assigned to one of 4 groups: upper-body RT (UB; n=15), lower-body RT (LB; n=15), combined RT (UB+LB; n=15) or control (C; n=15). The training protocol consisted of 3 exercise sessions per week for 12 weeks. Blood samples for measuring serum markers (follistatin, myostatin, C-reactive protein [CRP], adiponectin, tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ], and irisin) were obtained at baseline and 48 hours after the final training session. Fat mass (FM), body fat percentage (BFP), skeletal muscle mass (SMM), and fat-free mass (FFM) were measured using bioelectrical impedance analysis (Inbody 720). **Results:** SMM, FFM, UB and LB strength and power, follistatin, follistatin:myostatin ratio, adiponectin, and irisin significantly increased while FM, BFP, myostatin, CRP, and TNF- $\alpha$  significantly reduced from pre- to post-training in all training groups ( $p < 0.05$ ). Changes in LB muscle power ( $r = 0.558$ ), both UB ( $r = 0.518$ ) and LB ( $r = 0.419$ ) muscle strength, and follistatin ( $r = 0.545$ ), had moderate positive relationships with  $\Delta$ SMM, while changes in myostatin ( $r = -0.585$ ) had a moderate negative relationship with  $\Delta$ SMM. Also, changes in myostatin ( $r = 0.825$ ) and CRP ( $r = 0.715$ ) had a strong positive relationship with  $\Delta$ FM, while TNF- $\alpha$  ( $r = 0.467$ ) had a moderate positive relationship with  $\Delta$ FM. Follistatin ( $r = -0.789$ ) and adiponectin ( $r = -0.713$ ) had a strong negative relationship with  $\Delta$ FM, while irisin ( $r = -0.426$ ) had a moderate negative relationship with  $\Delta$ FM. **Conclusions:** Combined RT elicits the greatest increases in follistatin, follistatin:myostatin ratio, adiponectin, and decreases in myostatin and CRP compared with other training groups in overweight and obese men. However, systemic improvements may be achieved through performing UB or LB RT alone. **Key Words:** EXERCISE, STRENGTH TRAINING, OBESITY, ADIPOKINES, MYOKINES.

## Introduction

Resistance training (RT) is a popular training modality with numerous health benefits (1-3). Studies have shown that RT prescription factors such as intensity and volume can produce different skeletal muscle adaptations (2, 3). Performing RT targeting different muscle groups, such as upper body (UB) vs. lower body (LB), may also lead to different skeletal muscle adaptations (2, 4). For instance, a 12-week whole-body RT intervention in young and middle-aged men and women reported a greater and earlier increase in UB skeletal muscle mass (SMM) compared to the LB, (5) demonstrating that different skeletal muscles do not have the same responses to an applied stimulus. The reason for this disparity is not well understood, but it may be influenced by differences in body composition, especially skeletal muscle composition of the UB and LB, use of the LB in everyday activities, and the type of exercises performed during RT (5, 6). There is evidence that people with obesity have poorer responsiveness to RT compared to counterparts without obesity (7, 8) in terms of increases in muscular adaptations, particularly increases in SMM (9, 10). This observation may be related to obesity increasing inflammatory markers (i.e., C-reactive protein [CRP] (11), Interleukin-6 [IL-6] (12), Tumor Necrosis Factor-alpha [TNF- $\alpha$ ], etc.) (12). Indeed, studies have demonstrated that chronic inflammation in skeletal muscle hinders muscle protein synthesis in people with obesity (9, 13). RT reduces chronic inflammation by increasing anti-inflammatory markers (i.e., adiponectin, and irisin, etc.) and reducing body fat (14), which subsequently reduces pro-inflammatory markers (i.e., CRP), helping to protect and improve SMM and function (15, 16).

Myostatin and follistatin are two key proteins involved in muscle development and growth (17). Myostatin is produced by skeletal muscle and negatively regulates muscle growth via inhibition of muscle stem cell proliferation and differentiation; it increases ubiquitin-proteasomal activity and down-regulates the IGF-1/PI3K/Akt pathway (2, 4, 18). Follistatin is

produced by several tissues and functions as an antagonist to myostatin by preventing ligand-receptor interactions, leading to increases in muscle cell proliferation and differentiation (2, 18). Studies have shown that RT positively influences follistatin and negatively influences myostatin (2, 4). A recent RCT showed that eight weeks of intense combined (UB + LB) RT using Thera bands in inactive young people led to decreased circulating myostatin and increased follistatin levels (4). Our previous RCT involving eight weeks of UB, LB, or combined RT in middle-aged men showed that the combined group had greater changes in myostatin and follistatin compared with UB or LB RT groups (2). Discrepancies between these studies might be attributed to limited mechanical tension while utilizing Thera bands and lower training volume (three or four exercises performed three days per week) compared with our prior research (six heavy and multi-joint exercises performed three days per week) (2). Nevertheless, these findings suggest that the number of skeletal muscles stimulated during RT may not be predictive of changes in myostatin and follistatin. To the best of our knowledge, no previous studies have explored the effects of UB, LB, or combined RT on myostatin and follistatin in overweight and obese men.

This study aimed to investigate the impact of UB, LB, or combined RT on muscular and inflammatory markers, body composition, and muscular performance in overweight and obese men. Our secondary aim was to explore how changes in body fat and SMM influence changes in RT-related muscular adaptations. We hypothesized that the RT program targeting the greatest quantity of muscle groups (i.e., total-body training; combined group) would elicit the greatest increases in markers of muscle growth and lead to the greatest decrease in markers of inflammation.

## **METHODS**

### **Study Design and Participants**

This 12-week randomized controlled trial (RCT) recruited 60 overweight (body mass index [BMI]: 25–29.9 kg.m<sup>-2</sup>) and obese (BMI:  $\geq$  30 kg.m<sup>-2</sup>) sedentary (performing less than 1 hour of self-reported exercise per week in the previous year) men aged 25-40 years. Exclusion criteria included cardiovascular diseases, diabetes, hypertension, sleep disorders, or other risk factors based on a doctor's examination. Participants were required to self-report, via health and exercise history questionnaires, sleeping at least 7-8 hours during the 24-hour day and not taking any supplements or medications, including non-steroidal anti-inflammatory drugs. Further, participants needed to abstain from the use of steroids or any illegal substances known to enhance muscle size for the past year and not have any musculoskeletal disorders. All participants provided written informed consent before their inclusion in the study. The study protocol was reviewed by the Institutional Human Subject Committee and the ethical committee of the University of Isfahan (IR.UI.REC.1400.036) and carried out in accordance with the Declaration of Helsinki.

### **Randomization**

During baseline assessments, participants familiarized themselves with the study tests and procedures, and after baseline assessments, participants were randomly allocated to one of four groups: UB RT (UB; n=15), LB RT (LB; n=15), combined UB and LB RT (UB+LB; n=15), or control (C; n=15). Randomization was performed using an online resource ([www.randomizer.org](http://www.randomizer.org)). Measurements were collected at baseline and after 12 weeks during the

same time of day (within one hour of the previous collection). 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. Participants' height was measured with a stadiometer (SECA, China) to the nearest 0.1 cm. Body mass, BMI, body fat percentage (BFP), fat mass ([FM]; intraclass correlation coefficient [ICC] = 0.995; coefficient of variation [CV]: <1%), SMM, and fat-free mass (FFM); ICC = 0.996; CV: <1%) were estimated using a multi-frequency bioelectrical impedance (BIA) device (Inbody 720, South Korea). Prior to the measurement, the participant's palms and soles were cleansed using electrolyte tissue. Participants then positioned themselves on the InBody 720 device, aligning the soles of their feet with the electrodes. The equipment obtained the participant's body mass, while a researcher manually entered their age and sex onto the display. Participants then held the handles of the device, making sure that the palm and fingers of each hand were in direct contact with the electrodes. They kept their arms fully extended and abducted at an angle of about 20°. The assessment of body composition was conducted by the unit while individuals maintained a state of minimal movement. Participants were instructed to fast for 12 hours (an overnight fast with at least 8 hours of sleep) and refrain from engaging in physical activity for the previous 36 hours before the test. Participants were also instructed to avoid consuming alcohol for 48 hours before the test.

## Blood Sampling and Analysis

Blood samples were collected from the cubital vein using standard procedures following a 10-hour fast. The initial collection occurred 48 hours before the baseline training session. Blood samples were clotted for 20 minutes at room temperature before being centrifuged at 3000 RPM for 20 minutes. Spun serum was removed from the centrifuge and frozen at  $-70^{\circ}\text{C}$ . Serum myostatin (Intra-Assay:  $\text{CV} < 10\%$ , Inter-Assay:  $\text{CV} < 12\%$ ), follistatin (Intra-Assay:  $\text{CV} < 10\%$ , Inter-Assay:  $\text{CV} < 12\%$ ), CRP (Intra-Assay:  $\text{CV} = 8\%$ , Inter-Assay:  $\text{CV} < 11.5\%$ ), adiponectin (Intra-Assay:  $\text{CV} < 5\%$ , Inter-Assay:  $\text{CV} < 5\%$ ) were measured in duplicate using enzyme-linked immunosorbent assay (ELISA, ZellBio GmbH) kits according to manufacturer instructions. Also, irisin was measured in duplicate using ELISA Kit (A87120) Intra- assay  $\text{CV} < 8\%$ , Inter-assay  $\text{CV} < 10\%$ .

## Muscle Strength Testing

Maximal strength testing took place 24 hours after the body composition measurement. Bench press and leg press exercises were used as measures of UB and LB strength. One-repetition maximum (1RM) testing was performed to individualize RT prescriptions. Before testing commenced, study investigators explained the purpose, risks, and discomforts associated with 1RM testing to participants. Participants were instructed to refrain from drinking 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 completed 10 minutes of general and specific warm-up activities before the 1RM testing. The participants then performed two attempts, recording their highest lifted weight and number of repetitions. The number of repetitions to fatigue did not exceed 10. Participants were allowed 3 to 5 minute rest periods

between attempts, and there was no arousing stimulus during testing. After the testing session, the participant's maximal strength was predicted using the following formula:  $1RM = \text{weight} / (1.0278 - 0.0278 \times \text{reps})$  (19).

## Muscle Power

Twenty-four hours after the determination of the estimated 1RM strength, anaerobic power for the UB and LB was assessed via Monark Wingate cycle ergometer (Monark model 894e, Vansbro, Sweden) as previously described (20, 21). Briefly, participants cycled against a pre-determined resistance (7.5% of body mass for the LB test and 5.5% of body mass for the UB test) as fast as possible for 30 seconds (20). For the LB test, the saddle height was adjusted for each participant to produce 5–10° of knee flexion while the foot was in the lowest position. For the UB test, the position of the chair and ergometer for each participant was standardized so that the center of rotation of the lever arms did not level with the shoulders and the arms were near full extension during arm cranking (22). There was no arousing stimulus during testing. Peak power output was recorded in real-time during the test using Monark Anaerobic test software (3.3.0.0) (20).

## Resistance Training Protocols

**Preparatory Phase.** All participants performed one week of RT, consisting of three exercise sessions, for familiarization before the main training intervention. This phase allowed for supervised instruction of proper lifting technique, familiarization with all exercises, and ensuring that the participants initiated the study with a comparable training base. The preparatory phase included six exercises (barbell squat, lateral raise, leg extension, seated leg

curl, lat pulldown, and rowing). The preparatory phase program was adapted from previous literature (23).

**Training Phase.** Following the preparatory phase, participants in the UB, LB, and UB+LB completed supervised training 3 times per week, separated by at least 48 hours, for 12 weeks. All training sessions were performed between 5 and 6 PM. 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 30 seconds between sets. Following the specific warm-up, training included three sets per exercise in weeks 1-3 (rest interval between sets: 30 seconds, % 1RM: 50, repetitions: 15), 3 sets per exercise in weeks 4-6 (rest interval between sets: 60 seconds, % 1RM: 60, repetitions: 12), 4 sets per exercise in weeks 7-9 (rest interval between sets: 75 seconds, % 1RM: 70, repetitions: 10), and 4 sets per exercise in weeks 10-12 (rest interval between sets: 90 seconds, % 1RM: 80, repetitions: 8). Progressive overload was achieved throughout the study by using the following formula to predict 1RM and determine exercise load every two weeks:  $1RM = \text{weight} / [1.0278 (0.0278 \times r)]$  (19). However, training volume was not standardized across the three training groups. It should be noted that in the event that an individual was unable to attend a session, a makeup session was offered.

The exercises (in order) for each training program, along with detailed RT variables, are outlined in Table 1. All training sessions were completed under the supervision of certified trainers and researchers. The periodized RT programs were adapted from previous literature (24), following recommendations by the National Strength and Conditioning Association (25). Participants in the C group performed no exercise.

## Training Volume

The training volume was calculated using the following formula in each session and was reported weekly (26).

Training volume = [repetitions (n) × sets (n) × load or selected weight (kg)].

## Diet

Participants completed 3-day food records at baseline and weeks 4, 8 and 12 to estimate total energy (kcal) and macronutrient intake over time. Participants were instructed to record all food items for 2 non-consecutive weekdays and 1 weekend day. Diet Analysis Plus version 10 (Cengage, Boston, MA, USA) was used to analyze food records (27).

## Statistical Analysis

A priori sample size calculations were conducted using G-Power 3.1.9.2 software. The rationale for the sample size was based on our previous work, which documented significant decreases in serum myostatin after 8 weeks of combined UB and LB RT in middle-aged males (2). By utilizing the equation for effect size (ES)  $\{(\text{mean before} - \text{mean after RT}) / \text{the pooled standard deviation}\}$ , the previous study reported an ES of 0.62  $\{(3.96 - 3.60) / 0.58\}$  (2). In the present study, based on  $\alpha = 0.05$ , a power  $(1 - \beta)$  of 0.80, and an ES = 0.62 (the highest approximate effect size), a total sample size of at least 52 participants ( $n = 13$  per group) was needed for sufficient power to detect significant between-group changes in serum myostatin. The distribution of all variables was evaluated before performing statistical analyses using the Shapiro–Wilk test and visual inspection of boxplots and histograms; there were no missing values at any time point. Baseline characteristics between groups were reported using mean

(SD). Effects of RT on dependent variables were analyzed using analysis of covariance (ANCOVA) to determine the differences between the groups (using Bonferroni post hoc) over time. Training volume was analyzed using repeated measures of ANOVA. Pearson's correlations were performed, and correlation coefficients ( $r$ ) were reported with 95% confidence intervals (95%CI). Single models (curves) represent that one curve adequately fits all the data sets using extra sum-of-squares F test. Values between 0 and 0.3 indicate a weak linear relationship, values between 0.3 and 0.7 indicate a moderate relationship, and values between 0.7 and 1.0 indicate a strong relationship (28). P-values  $<0.05$  or 95% confidence intervals (CI) not including the null point were considered statistically significant. All analyses were performed using SPSS 26, and figure production was performed using GraphPad Prism (version 8.4.3) and Adobe Illustrator artwork (version 25).

## RESULTS

One hundred participants were assessed for eligibility, and 40 did not meet the inclusion criteria; common reasons for exclusion were not being sedentary and having a lower BMI than required. Sixty participants underwent baseline evaluation and were randomized into four groups as mentioned in randomization section. The distribution of participants with obesity vs overweight in each group was as follows: Control: 20 vs. 80%; Upper: 0 vs. 100%; Lower: 13.3 vs. 86.6%; Combined: 13.3 vs. 86.6%. The BMI range in this study was between 25 and 32.1  $\text{kg}\cdot\text{m}^{-2}$ . In total, 11.66% and 88.34% were obese or overweight, respectively. A one-way ANOVA showed there was no significant difference in BMI between training groups at baseline ( $F = 0.455$ ,  $P = 0.715$ ). All baseline characteristics are shown in Table 2.

## Adverse Events

No adverse events were reported by the participants, or observed by study staff during the testing or training sessions.

## Adherence, Dietary assessments & Training volume

The adherence to training interventions for all three groups was 100%. There were no significant within- or between-group differences for any average daily nutrient or energy intake ( $p > 0.05$ ; Table 3).

Changes in training volume throughout the intervention are shown in Table 4. A significant group-by-time effect was noted ( $p < 0.001$ ). Bonferroni posthoc tests indicated that the increases in training volume in LB and UB+LB were significantly greater than UB ( $p < 0.001$ ). No significant difference was observed between LB and UB+LB groups ( $p = 0.068$ ).

## Body composition

Body composition at pre- and post-intervention are shown in Figure 1. Body mass decreased in UB [-1 kg (95% CI = -1.8 to -0.16)], LB [-1.1 kg (95% CI = -2.1 to -0.01)], and UB+LB [-1.2 kg (95% CI = -2.2 to -0.33)], but not in C ( $p > 0.05$ ). BMI also decreased in UB [-0.34  $\text{kg}\cdot\text{m}^{-2}$  (95% CI = -0.62 to -0.05)], LB [-0.37  $\text{kg}\cdot\text{m}^{-2}$  (95% CI = -0.73 to -0.002)], and UB+LB [-0.41  $\text{kg}\cdot\text{m}^{-2}$  (95% CI = -0.73 to -0.1)], but not in C ( $p > 0.05$ ). FM decreased in UB [-2.3 kg (95% CI = -3.1 to -1.5)], LB [-3 kg (95% CI = -3.5 to -2.4)] and UB+LB [-5 kg (95% CI = -5.6 to -4.3)], but not in C ( $p > 0.05$ ). Also, BFP decreased in UB [-2.4 % (95% CI = -3.4 to -1.3)], LB [-3.1 % (95% CI = -3.7 to -2.4)] and UB+LB [-5.6 % (95% CI = -6.3 to -4.8)], but not in C ( $p > 0.05$ ). Body mass, BMI, and FM decreased in all training groups compared with the C group

( $p < 0.001$ ). SMM in UB [1.2 kg (95% CI = 0.6 to 1.7)], LB [1.3 kg (95% CI = 0.7 to 1.9)], and UB+LB [2.1 kg (95% CI = 1.1 to 3.1)] and FFM in UB [1.3 kg (95% CI = 0.07 to 2.5)], LB [1.9 kg (95% CI = 0.7 to 3)], and UB+LB [3.7 kg (95% CI = 2.7 to 4.7)] increased but not in C ( $p > 0.05$ ). However, no between-group differences were observed ( $p > 0.05$ ). Moreover, after adjusting training volume, a significant difference was observed between training groups for SMM (UB+LB vs UB  $p = 0.293$ , (UB+LB vs LB  $p = 0.003$ , LB vs UB  $p = 0.003$ ) and body mass (UB+LB vs UB  $p = 0.020$ , UB+LB vs LB  $p = 0.024$ , LB vs UB  $p = 0.989$ ).

## Performance

Measures of muscular performance at pre- and post-intervention are shown in Figure 1. Bench press strength increased in UB [3.8 kg (95% CI = 3.1 to 4.6)], LB [1.9 kg (95% CI = 1.3 to 2.5)], and UB+LB [4.9 kg (95% CI = 4.1 to 5.7)], but not in C ( $p > 0.05$ ). The increase in UB+LB was significantly greater than the LB group ( $p < 0.001$ ). Bench press strength also increased in all training groups compared with C ( $p < 0.001$ ). Leg press strength significantly increased in UB [2 kg (95% CI = 1.5 to 2.4)], LB [6.2 kg (95% CI = 5.4 to 7)], and UB+LB [6 kg (95% CI = 5.4 to 6.5)], but not in C ( $p > 0.05$ ). The increase in UB+LB was significantly greater than in the UB group ( $p < 0.001$ ). Leg press strength also increased in all training groups compared with C ( $p < 0.001$ ). UB power significantly increased in UB [16.8 W (95% CI = 8.7 to 24.9)], LB [10.6 W (95% CI = 3.3 to 17.9)], and UB+LB [20.4 W (95% CI = 11.1 to 29.8)], but not in C ( $p > 0.05$ ). The increase in all training groups was significantly greater than C ( $p < 0.001$ ). LB power significantly increased in UB [29.4 W (95% CI = 19.9 to 38.9)], LB [65.6 W (95% CI = 52.9 to 78.2)], and UB+LB [71.8 W (95% CI = 65.4 to 78.3 to)], but not in C ( $p > 0.05$ ). The increase in UB+LB was significantly greater than C and UB ( $p < 0.001$ ). Also, the LB group had

greater improvements in LB power than UB, and both LB and UB training groups had greater improvements than the C group ( $p < 0.001$ ).

### **Muscle-related factors**

Muscle markers at pre- and post-intervention are shown in Figure 2. Myostatin significantly decreased in UB [ $-7.8 \text{ pg.mL}^{-1}$  (95% CI =  $-7.2$  to  $-8.4$ )], LB [ $-8 \text{ pg.mL}^{-1}$  (95% CI =  $-7.1$  to  $-8.8$ )], and UB+LB [ $-13 \text{ pg.mL}^{-1}$  (95% CI =  $-11.8$  to  $-14.1$ )], but not in C ( $p > 0.05$ ), and the reduction in UB+LB was significantly greater than all other groups ( $p < 0.001$ ). Follistatin significantly increased in UB [ $54.1 \text{ pg.mL}^{-1}$  (95% CI =  $59.8$  to  $48.3$ )], LB [ $61.8 \text{ pg.mL}^{-1}$  (95% CI =  $72.1$  to  $51.5$ )], and UB+LB [ $128.3 \text{ pg.mL}^{-1}$  (95% CI =  $144.7$  to  $111.9$ )], but not in C group ( $p > 0.05$ ) and the increase in UB+LB was significantly greater than all other groups ( $p < 0.001$ ). Follistatin:Myostatin ratio significantly increased in UB [ $2.5 \text{ pg.mL}^{-1}$  (95% CI =  $2.27$  to  $2.89$ )], LB [ $2.61 \text{ pg.mL}^{-1}$  (95% CI =  $2.26$  to  $2.97$ )], and UB+LB [ $5.12 \text{ pg.mL}^{-1}$  (95% CI =  $4.64$  to  $5.61$ )], but not in C group ( $p > 0.05$ ). The increase in UB+LB was significantly greater than all other groups; changes were significantly greater in training groups compared with C ( $p < 0.001$ ). Moreover, after adjusting training volume, no significant differences were observed between training groups ( $p > 0.05$ ).

### **Inflammatory markers**

Inflammatory markers at pre- and post-intervention are shown in Figure 2. CRP decreased in UB [ $-0.2 \text{ ng.mL}^{-1}$  (95% CI =  $-0.2$  to  $-0.3$ )], LB [ $-0.5 \text{ ng.mL}^{-1}$  (95% CI =  $-0.2$  to  $-0.7$ )], and UB+LB [ $-1 \text{ ng.mL}^{-1}$  (95% CI =  $-0.8$  to  $-1.1$ )], but not in C ( $p > 0.05$ ); the reduction in UB+LB was significantly greater than all other groups ( $p < 0.001$ ). CRP also decreased more in

LB than UB, and both groups had greater decreases in CRP compared with C ( $p < 0.001$ ). TNF- $\alpha$  decreased in UB [ $-0.2 \text{ ng.mL}^{-1}$  (95% CI =  $-0.2$  to  $-0.3$ )], LB [ $-0.2 \text{ ng.mL}^{-1}$  (95% CI =  $-0.06$  to  $-0.4$ )], and UB+LB [ $-0.4 \text{ ng.mL}^{-1}$  (95% CI =  $-0.1$  to  $-0.6$ )], but not in C ( $p > 0.05$ ), and the reduction in UB+LB was greater than in C ( $p < 0.001$ ). There were no between-group differences in TNF- $\alpha$  between the UB and LB and C groups ( $p > 0.05$ ). Adiponectin increased in UB [ $3.1 \text{ ng.mL}^{-1}$  (95% CI =  $3.6$  to  $2.6$ )], LB [ $1 \text{ ng.mL}^{-1}$  (95% CI =  $1.5$  to  $0.6$ )], and UB+LB [ $5.6 \text{ ng.mL}^{-1}$  (95% CI =  $6$  to  $5.1$ )], but not in C ( $p > 0.05$ ), and the increase in UB+LB was significantly greater than all other groups ( $p < 0.001$ ). Increases in adiponectin were also greater in UB and LB compared with C ( $p < 0.001$ ). Irisin increased in UB [ $0.3 \text{ ng.mL}^{-1}$  (95% CI =  $0.5$  to  $0.04$ )], LB [ $0.8 \text{ ng.mL}^{-1}$  (95% CI =  $1.1$  to  $0.5$ )], and UB+LB [ $1.1 \text{ ng.mL}^{-1}$  (95% CI =  $1.6$  to  $0.6$ )], but not in C ( $p > 0.05$ ), and the increase in UB+LB was significantly greater than C and UB groups ( $p < 0.001$ ). Irisin increases were also greater in LB compared with C ( $p = 0.003$ ). Moreover, after adjusting training volume, no significant differences were observed between training groups ( $p > 0.05$ ).

## Correlations

Associations between changes in SMM ( $\Delta$ SMM) and FM ( $\Delta$ FM), with changes in muscle and inflammatory markers as well as muscular performance ( $\Delta$  variable; whole cohort), are presented in Figures 3 and 4. Changes in LB muscle power, both UB and LB muscle strength, and follistatin had moderate positive relationships with  $\Delta$ SMM, while changes in myostatin had a moderate negative relationship with  $\Delta$ SMM. Changes in myostatin and CRP had a strong positive relationship with  $\Delta$ FM, while TNF- $\alpha$  had a moderate positive relationship with  $\Delta$ FM. Follistatin and adiponectin had a strong negative relationship with  $\Delta$ FM, while irisin had a moderate negative relationship with  $\Delta$ FM. For linear regression of individual  $\Delta$  (variable) as a

function of  $\Delta$ SMM and  $\Delta$ FM, data were examined by the extra sum-of-squares F test to first consider if pooled data could be considered as a single model. Only TNF- $\alpha$  was considered a single group. All the muscular performance variables except for UB power along with follistatin and myostatin showed a significant relationship with changes in SMM (Figure 3B-G). In addition, all inflammatory and muscular markers showed a significant relationship with changes in FM (Figure 4B-G).

## DISCUSSION

This 12-week RCT demonstrated that combined RT increased follistatin, follistatin:myostatin ratio, and adiponectin while reducing myostatin and CRP compared with UB or LB RT in young, overweight and obese men. Muscle strength and body composition improved in all training groups relative to control. After adjusting for training volume, most between-group differences became non-significant. These findings suggest that UB or LB RT can have beneficial effects (both site-specific and systemic) on muscle-related growth factors, body composition, and inflammation; however, combined training leads to the greatest improvements, largely attributed to higher training volume.

Decreases in myostatin and increases in follistatin were observed in all RT groups compared with control. These findings have been well-documented (2, 29-31); concurrent reductions in myostatin and increases in follistatin suggest a shift towards a more anabolic environment (32), reflected by the increases in SMM and function we observed. SMM, bench press and leg press strength and UB and LB power increased in all training groups, and in the combined group, UB measures measured generally improved relative to the LB group, while LB measures improved relative to the UB group. Furthermore, myostatin decreased while follistatin

increased in the combined group relative to the UB and LB groups. Regarding irisin, the increase in combined was significantly greater than C and UB groups. Given that most prescription factors were consistent between groups (exercise mode, frequency, intensity, and duration), this finding seemed to be driven by higher training volume in combined RT compared with UB.

Our findings are also consistent with a previous study in middle-aged men showing that changes in myostatin and follistatin were greater in those who performed 8 weeks of combined RT compared with counterparts who completed UB or LB RT alone (2). Interestingly, our study showed that changes in muscle strength had stronger associations with changes in myostatin and follistatin than changes in SMM, and changes in FM were a stronger predictor of changes in myostatin and follistatin compared with SMM. These findings might be related to the use of BIA to estimate SMM in the current study, which has some limitations in populations with obesity (33, 34).

With respect to the stronger associations between changes in muscle strength and changes in myostatin and follistatin compared to changes in SMM, the underlying mechanism remains unclear. However, this may be attributable to the fact that neural adaptations, which are predominant during the early stages of RT in untrained individuals, play a crucial role in strength gains prior to significant increases in SMM (35).

These adaptations, which enhance the maximal volitional force-generating capacity of skeletal muscles, are typically observed within the first 2–4 weeks of training and are believed to be the primary drivers of early strength improvements (36). This phenomenon is supported by various behavioural observations, such as the task-specificity of strength improvements without substantial morphological changes (37), the disproportionate increase in muscle force relative to muscle size (38), and the rise in voluntary activation during the initial training weeks (39).

Consequently, in short-term studies, changes in muscle strength may better predict fluctuations in myostatin and follistatin levels than changes in SMM. Future studies comparing associations between myostatin and follistatin and muscle strength and mass may benefit from using more accurate estimates of SMM, such as dual-energy x-ray absorptiometry (DXA), magnetic resonance imaging (MRI), computed tomography (CT), or the D3-creatine dilution method (40).

Inflammation decreased within all RT groups, and both the LB and combined groups had greater decreases in CRP relative to the UB group. Decreases in CRP following RT have been reported in several meta-analyses (41-43) and occur via RT-induced anti-inflammatory cytokine release and reductions in FM (44). In our study, both the LB and combined groups had higher 12-week training volumes relative to the UB group, and the combined group had greater decreases in both FM and CRP relative to the other training groups. Furthermore, after adjusting for training volume, between-group differences in inflammatory markers became non-significant. Interestingly, only the combined group had a significant decrease in TNF- $\alpha$  relative to control, which might also be explained by this group having the most substantial decreases in FM relative to all other groups (45). Decreases in FM are associated with decreases in TNF- $\alpha$ , irrespective of whether decreases are achieved via exercise (45-48). The anti-inflammatory effects of RT in this study are also highlighted by significant within-group increases in adiponectin in all training groups relative to control. Adiponectin also increased in the combined group relative to the UB and LB groups. Similar to TNF- $\alpha$ , decreases in FM are associated with increases in adiponectin when achieved with and without exercise in overweight and obese populations (45). These RT-related changes in inflammatory markers may be clinically important, as studies have shown that increased CRP levels are associated with a higher risk for

cardiovascular disease (49, 50), while higher adiponectin and lower TNF- $\alpha$  levels are associated with a lower risk for incident type 2 diabetes (51).

We observed transfer effects whereby performing UB or LB RT led to minor improvements in the untrained region; this has been reported in several previous studies. In a previous study, 20 resistance-trained men were randomly assigned to either a high-intensity or a mixed high-volume and high-intensity RT program for six weeks (52). The high-intensity group followed a high-intensity training protocol for both UB and LB (4-5 reps at 88%-90% of 1RM), whereas the mixed group performed high-volume training sessions focused on muscle hypertrophy for LB (10-12 reps at 65%-70% of 1-RM) and a high-intensity protocol for the UB (52). It was shown that targeting LB muscle hypertrophy and maximal strength for UB can stimulate greater strength and power gains in the UB compared with high-intensity RT programs for both the UB and LB. Also, the greater gains in arm muscle size (using skinfold measurements) that occurred in the mixed high volume and high intensity RT group suggest that the anabolic effects of high-volume sessions of squat may stimulate gains in UB muscles (52). However, the high-intensity squat workouts, comprising 5 sets of 3-4 reps, may not have been sufficient to activate a transfer effect between the LB and the UB. In a blood flow restriction study by Madarame et al., 15 untrained men were randomly assigned into the occlusive training group (OCC, n = 8) or a normal training group (NOR, n = 7) (53). Both groups performed the same unilateral arm exercise (arm curl) at 50% of 1RM without occlusion (three sets, 10 reps) (53). Either the dominant or nondominant arm was randomly chosen to be trained (OCC-T, NOR-T) or to serve as a control (OCC-C, NOR-C). After the arm exercise, OCC performed leg exercise with blood flow restriction (30% of 1RM, three sets, 15-30 reps), whereas NOR performed the same leg exercise without occlusion (53). The authors reported that increased arm

muscle size and strength were only observed when LB RT with blood flow restriction was added to an UB RT program (53). Häkkinen et al. also noted a greater relative effect on UB isometric strength gains when LB training was combined with UB training compared with UB training alone (54). As we previously reported, endocrine markers seem to be related to the amount of SMM activated during exercise in the training protocol used (UB and LB exercises) (2). Although speculative, our UB and LB training protocols in the present study involved several compound exercises (e.g., squats, shoulder press, etc.) targeting several muscle groups (including some in untrained regions) that likely increased follistatin while reducing myostatin. Systemic changes in these myokines may have also influenced whole-body protein synthesis, leading to minor muscular performance improvements in the untrained regions. Transfer effects between the LB and UB may also be related to neural mechanisms. It has been suggested that intense LB training could influence arm strength by reducing inhibitory feedback from the Ib afferent nerves in the Golgi tendon organs (55). Inhibitory interneurons activated by Golgi tendon organs can be down-regulated by corticospinal pathways stimulated by RT (55). Both central and peripheral neural mechanisms, enhanced by LB or UB training, may have stimulated neural adaptations in motor units not directly involved in LB or UB exercises. Finally, the increases in UB and LB muscle power we observed, irrespective of whether UB or LB RT was performed, might be partly related to reductions in body mass that occurred in training groups, resulting in reduced loads during the Wingate test.

The results of this study suggest that men with overweight and obesity should consider performing combined UB and LB RT, instead of UB or LB RT alone, where possible. Combined UB and LB RT targets multiple muscle groups, reducing the overall time commitment required to achieve fitness goals. Combined training targeting more muscle groups may lead to higher

caloric expenditure, which can be advantageous for individuals aiming to reduce FM. This is especially important in body mass management and obesity prevention, where increasing energy expenditure is crucial. Overall, the findings support the use of combined RT as a practical, effective, and versatile approach to RT that can be easily applied across various populations and settings, offering a balance of efficiency and effectiveness that is well-suited to real-world scenarios. Future studies should compare the effectiveness of UB vs LB vs combined RT in other populations, particularly trained athletes.

A key strength of this RCT was the successful delivery of an effective RT program with a standardized protocol in a supervised and controlled setting. Limitations of this study include the study population consisting of only young men; therefore, our results cannot be generalized to other populations, including older adult or female populations. We also used BIA to estimate body composition, which can be influenced by factors such as hydration. However, we attempted to control for this confounder by asking participants to perform an overnight fast, void urine, and refrain from exercising before testing. Future studies may benefit from using more accurate measures of body composition, such as DXA, MRI, CT, or the D3-creatine dilution method (40). Also, we recommend future studies using BIA to perform phase angle analysis, if possible, which is considered an indicator of cellular health, with higher values indicating higher cellularity, cell membrane integrity, and better cell function (56). We did not use validated questionnaires to classify the level of physical activity, such as the International Physical Activity Questionnaire (IPAQ) (57); future studies should consider using this validated questionnaire. The assessment of dietary saturated and unsaturated fat intake could help to understand the responses of inflammatory markers to RT.

The lack of training volume standardization between groups explains most of the RT-related muscular and inflammatory marker changes we observed. Protein intake was approximately 0.8-0.9 g/kg/day in most groups, which is sufficient for maintaining nitrogen balance and minimizing muscle loss, but we may have observed greater improvements in SMM and strength following RT if protein intake increased to 1.2-1.6 g/kg/day (58-60). Future studies should recruit diverse populations, including older adults and females, include various training protocols of UB, LB, or combined RT (e.g., concurrent or endurance training, etc.) training protocols, and explore whether sex differences in training responses exist.

## CONCLUSIONS

In conclusion, this study demonstrated that a combined RT program led to significant improvements in muscle-related growth factors (decreased myostatin and increased follistatin) that are reflective of a more anabolic environment, compared with completing UB or LB RT alone. Combined RT also led to improvements in body composition, muscular performance, and inflammatory profile relative to UB or LB RT alone. Although combined RT was superior to UB or LB RT, all types of RT had beneficial effects on the abovementioned outcomes, and training at one body site improved muscular performance at the opposite site, supporting the systemic effects of RT. These findings suggest that targeting either the UB or LB during RT has beneficial effects on the abovementioned outcomes, and targeting more muscle groups generally leads to greater improvements in overweight and obese men.

## **Acknowledgments**

The University of Isfahan provided funding sources to perform study procedures. The authors declare no conflict of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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## FIGURE LEGENDS

**Figure 1.** Effects of resistance training on body composition and muscular performance. **A)** skeletal muscle mass (SMM), **B)** fat-free mass (FFM), **C)** fat mass (FM), **D)** body fat percentage (%); **E)** bench press strength, **F)** leg press strength, **G)** upper body power (UB Power), **H)** lower body power (LB power). N=15 per group, error bars represent 95% confidence interval (CI), and p-values above time points indicate paired sample t-test results.

**Figure 2.** Effects of resistance training on muscle-related and inflammatory makers. **A)** Myostatin, **B)** Follistatin, **C)** Follistatin:Myostatin ratio, **D)** C-reactive protein [CRP], **E)** Tumor Necrosis Factor- $\alpha$  [TNF- $\alpha$ ], **F)** Adiponectin, and **G)** Irisin. N=15 per group, error bars represent 95% confidence interval (CI), and p-values above time points indicate paired sample t-test results.

**Figure 3.** Correlation matrix of  $\Delta$  SMM and muscular and inflammatory markers as well as muscular performance, r values as shown. The key indicates the magnitude of r (grey = 1 or -1, red = 0). (B–G) linear regression (Pearson's) of  $\Delta$  (muscular performance) as a function of  $\Delta$  SMM (kg).

**Figure 4.** Correlation matrix of  $\Delta$  FM muscular and inflammatory markers as well as muscular performance, r values as shown. The key indicates the magnitude of r (grey = 1 or -1, red = 0). (B–G) linear regression (Pearson's) of  $\Delta$  (performance) as a function of  $\Delta$  SMM (kg).

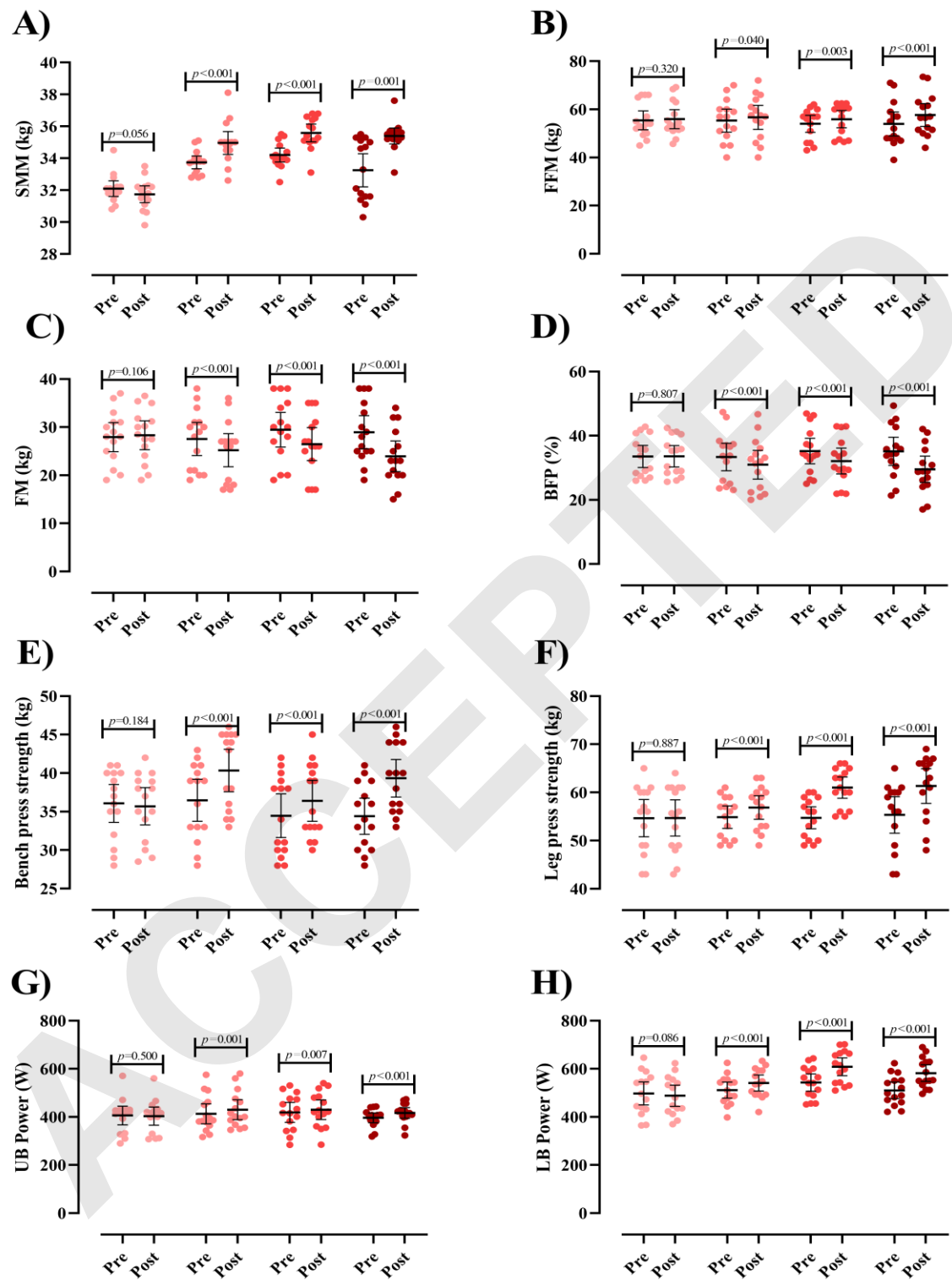


Figure 1

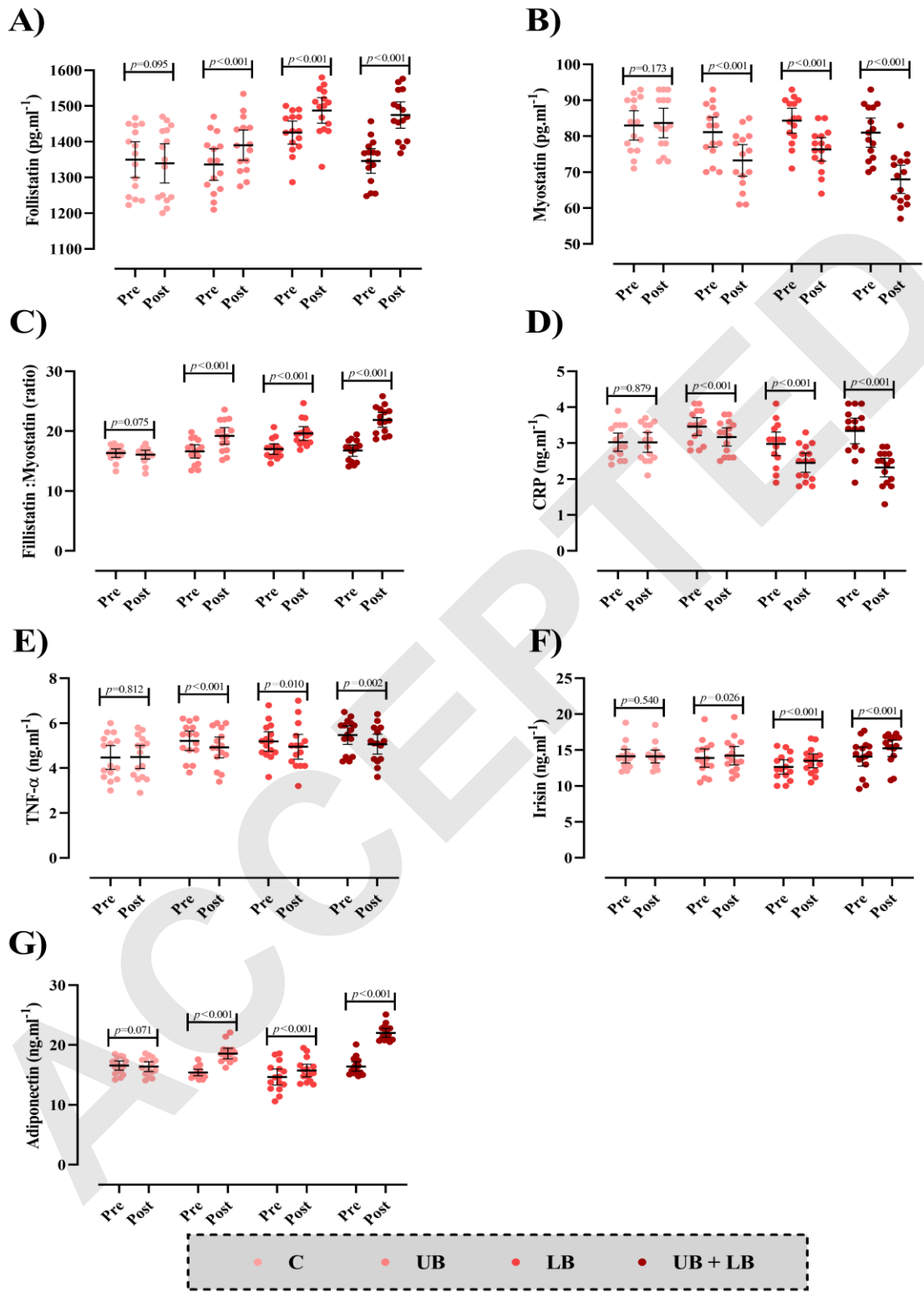
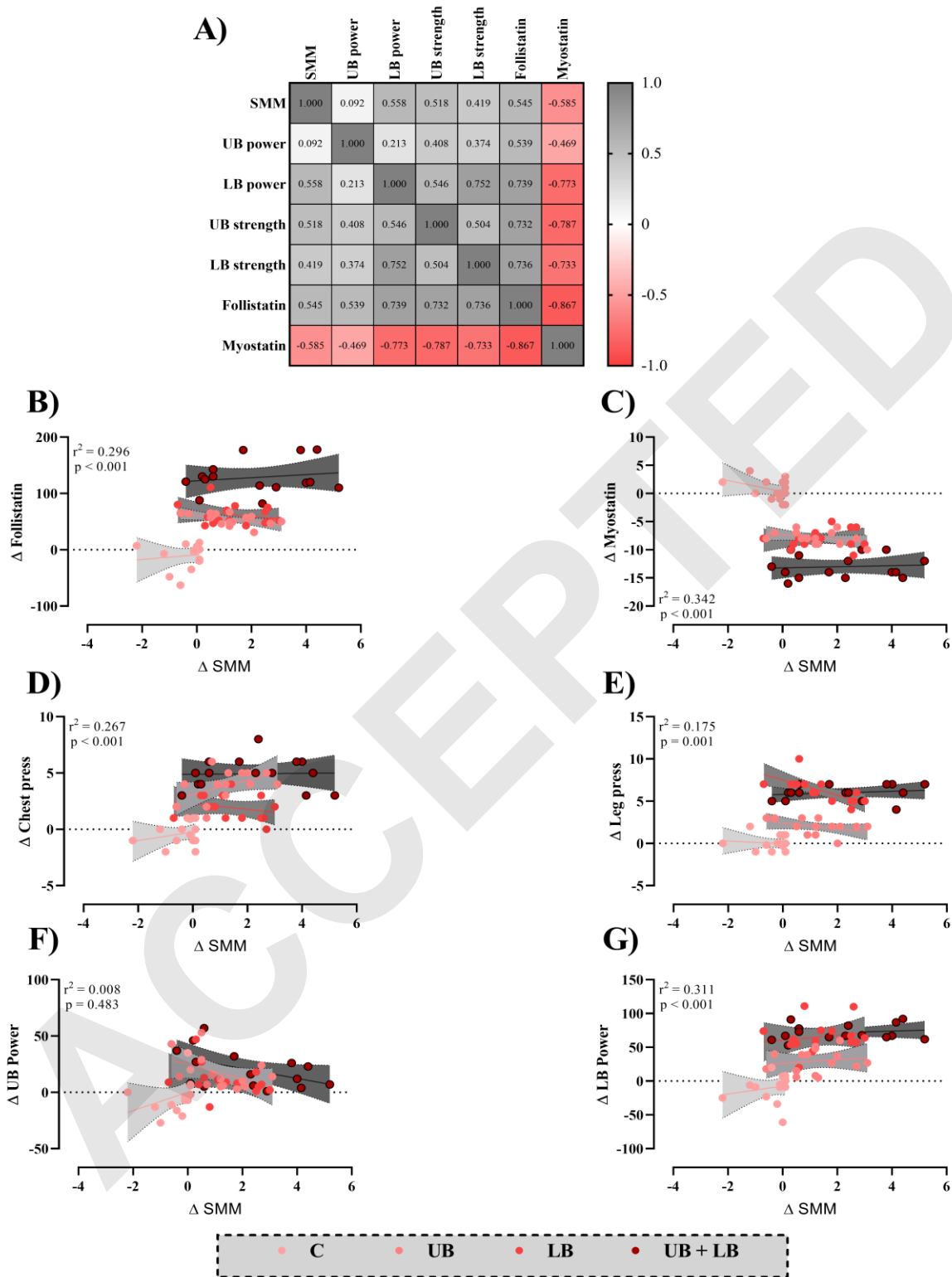
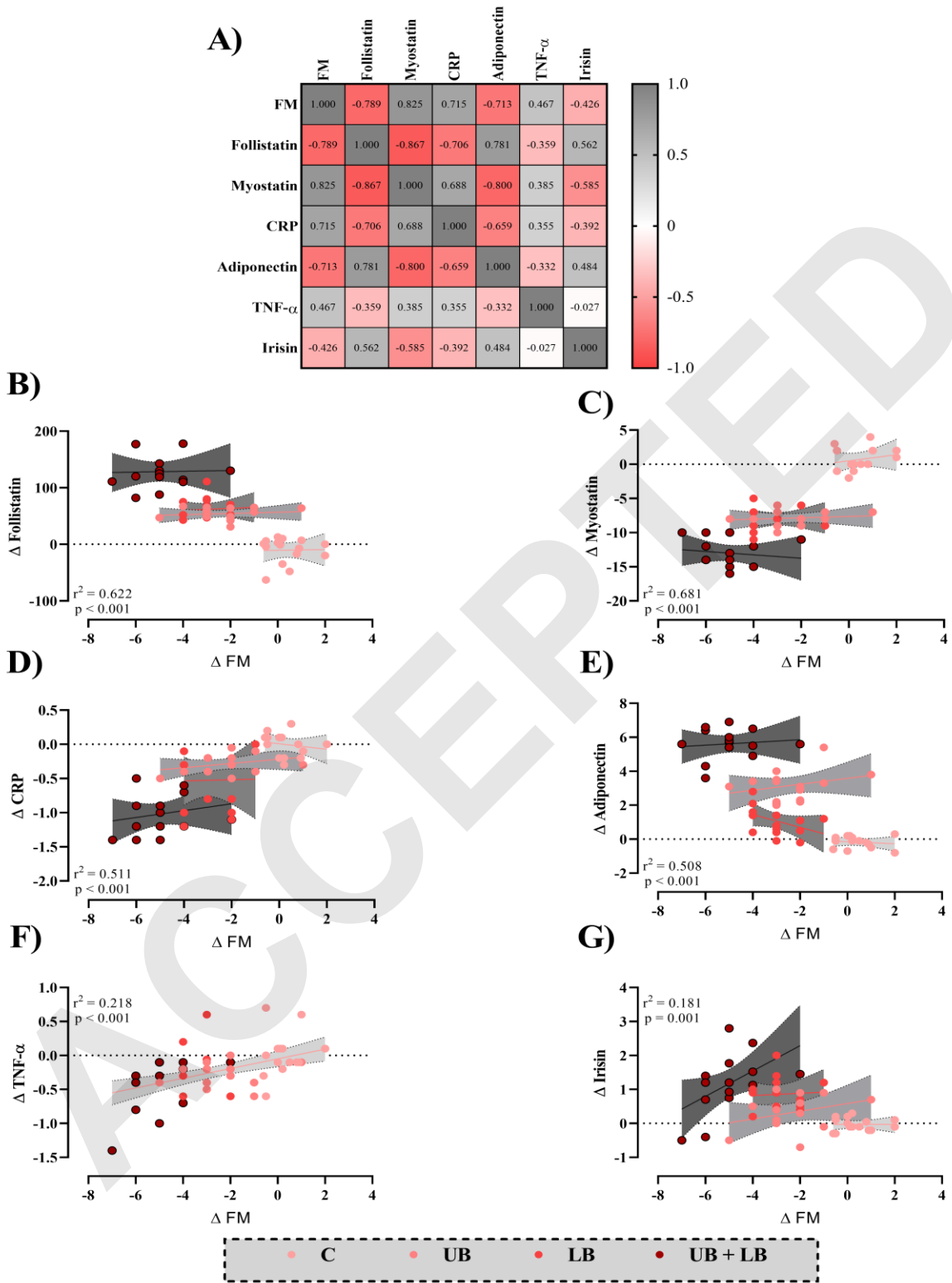


Figure 2



**Figure 3**



**Figure 4**

**Table 1.** Resistance training procedures for all training groups.

Week	Set	Load	Rest intervals	Repetitions
1	3	50%	30	15
2	3	50%	30	15
3	3	50%	30	15
4	3	60%	60	12
5	3	60%	60	12
6	3	60%	60	12
7	4	70%	75	10
8	4	70%	75	10
9	4	70%	75	10
10	4	80%	90	8
11	4	80%	90	8
12	4	80%	90	8
Exercises for each group				
UB	lateral pulldown, chest press, barbell shoulder press, lateral raise, standing barbell biceps curl, and cable triceps pushdown.			
LB	barbell squat, hack squat, lunges, leg extension, lying leg curls and standing calf raises.			
UB+LB	lateral pulldown, barbell squat, chest press, lunges, lateral raise and standing calf raise.			

**Table 2.** Baseline characteristics of the participants.

	C	UB	LB	UB + LB
<b>Measure</b>				
<b>Anthropometry and body composition</b>				
Age (y)	31 ± 4	30 ± 4	30 ± 3	31 ± 5
Height (m)	170 ± 4	170.9 ± 6.8	171.2 ± 4	171.3 ± 3.7
Body mass (kg)	83.3 ± 6.6	82.8 ± 6	83.4 ± 5.9	82.8 ± 6
BMI (kg.m <sup>-2</sup> )	28.7 ± 1.7	28.3 ± 0.7	28.4 ± 1.4	28.2 ± 1.6
SMM (kg)	32 ± 0.8	33.7 ± 0.7	34.2 ± 0.7	33.2 ± 1.8
FFM (kg)	55.4 ± 7.1	55.3 ± 8.6	54 ± 6.3	53.9 ± 9
FM (kg)	27.9 ± 5.4	27.5 ± 6.2	29.4 ± 6.4	28.9 ± 6.2
BFP (%)	33.5 ± 6.2	33.3 ± 7.7	35.2 ± 7.1	35.1 ± 7.9
<b>Muscular performance and markers</b>				
Bench press strength (kg)	36 ± 4.4	36.4 ± 4.9	34.5 ± 5.1	34.4 ± 4.2
Leg press strength (kg)	54.6 ± 6.9	54.8 ± 4.2	54.7 ± 4.1	55.3 ± 6.8
Upper body power (w)	406.1 ± 70.1	413 ± 74.9	419.1 ± 75	396.4 ± 36.7
Lower body power (w)	497.6 ± 86.6	511.5 ± 59.9	543 ± 64.3	510.3 ± 62.3
Follistatin (pg.ml <sup>-1</sup> )	1349.8 ± 90.3	1336.1 ± 79.6	1425.5 ± 58.3	1346 ± 6
Myostatin (pg.ml <sup>-1</sup> )	83 ± 7.3	81.1 ± 7.5	84.3 ± 6.2	81 ± 7.3
Follistatin:Myostatin ratio	16.3 ± 1.2	16.6 ± 2	17 ± 1.5	16.7 ± 1.7
<b>Inflammatory markers</b>				
TNF-α (ng.ml <sup>-1</sup> )	4.4 ± 0.9	5.2 ± 0.7	5.1 ± 0.7	5.4 ± 0.7
CRP (ng.ml <sup>-1</sup> )	3 ± 0.4	3.4 ± 0.4	2.9 ± 0.5	3.3 ± 0.6
Irisin (ng.ml <sup>-1</sup> )	14.1 ± 1.7	13.8 ± 2.3	12.6 ± 1.8	14 ± 2.3
Adiponectin (ng.ml <sup>-1</sup> )	16.5 ± 1.4	15.4 ± 0.9	14.6 ± 2.4	16.4 ± 1.4

Values are presented as mean ± standard deviation. **Abbreviations:** BMI, body mass index; SMM, skeletal muscle mass; FM, Fat mass; FFM, fat-free mass; BFP, body fat percentage; CRP, C-reactive protein; TNF-α, Tumor Necrosis Factor-alpha.

<b>Table 3. Average dietary intake at baseline and post-intervention.</b>		
	<b>Time</b>	
	<b>Baseline</b>	<b>Post-intervention</b>
<b>Energy (kcal. kg<sup>-1</sup>.d<sup>-1</sup>)</b>		
C	26.38 ± 4.15	26.20 ± 3.07
UB	27.52 ± 6.33	27.47 ± 3.86
LB	26.67 ± 6.69	26.38 ± 4.38
UB + LB	27.21 ± 3.42	27.38 ± 3.25
<b>Protein (g.kg<sup>-1</sup>.d<sup>-1</sup>)</b>		
C	0.78 ± 0.16	0.76 ± 0.13
UB	0.91 ± 0.17	0.92 ± 0.18
LB	0.82 ± 0.28	0.83 ± 0.20
UB + LB	0.81 ± 0.21	0.80 ± 0.16
<b>Carbohydrate (g.kg<sup>-1</sup>.d<sup>-1</sup>)</b>		
C	4.04 ± 0.86	4.06 ± 0.67
UB	4.14 ± 1.06	4.21 ± 0.71
LB	3.97 ± 0.98	4.06 ± 0.73
UB + LB	4.49 ± 0.84	4.46 ± 0.69
<b>Fat (g.kg<sup>-1</sup>.d<sup>-1</sup>)</b>		
C	0.78 ± 0.11	0.76 ± 0.12
UB	0.80 ± 0.29	0.77 ± 0.19
LB	0.83 ± 0.33	0.75 ± 0.24
UB + LB	0.66 ± 0.17	0.70 ± 0.10

**Abbreviations.** C, control; UB, upper body, LB, lower body; UB + LB, upper body + lower body

**Table 4.** Training volume over 12 weeks of resistance training.

Week	Group			Repeated measures of ANOVA		
	UB	LB	UB + LB	Main effect	Groups effect	Interaction effect
1 <sup>st</sup> – 3 <sup>rd</sup>	479.88 ± 58.26	845.18 ± 114.19	769.18 ± 54.53	F=3365.87 P=0.001*	F=84.78 P=0.001**	F=84.47 P=0.001***
4 <sup>th</sup> – 6 <sup>th</sup>	460.68 ± 55.93 <sup>a</sup>	811.38 ± 109.62 <sup>a</sup>	738.41 ± 52.35 <sup>a</sup>			
7 <sup>th</sup> – 9 <sup>th</sup>	597.18 ± 72.50 <sup>be</sup>	1019.83 ± 137.77 <sup>be</sup>	957.20 ± 67.86 <sup>be</sup>			
10 <sup>th</sup> – 12 <sup>th</sup>	682.50 ± 82.86 <sup>cfig</sup>	1202.04 ± 162.41 <sup>cfig</sup>	1093.94 ± 77.56 <sup>cfig</sup>			
1 <sup>st</sup> – 12 <sup>th</sup>	2220.25 ± 269.57 <sup>§©</sup>	3878.44 ± 523.85	3558.74 ± 252.32			

Main effect \*, groups effect \*\*, interaction effect \*\*\*  
<sup>a</sup>. difference between 4<sup>th</sup> – 6<sup>th</sup> weeks to 1<sup>st</sup> – 3<sup>rd</sup> weeks <sup>b</sup>. difference between 7<sup>th</sup> – 9<sup>th</sup> weeks to 1<sup>st</sup> – 3<sup>rd</sup> weeks <sup>c</sup>. difference between 10<sup>th</sup> – 12<sup>th</sup> weeks to 1<sup>st</sup> – 3<sup>rd</sup> weeks <sup>d</sup>. difference between 7<sup>th</sup> – 9<sup>th</sup> weeks to 4<sup>th</sup> – 6<sup>th</sup> weeks <sup>e</sup>. difference between 10<sup>th</sup> – 12<sup>th</sup> weeks 4<sup>th</sup> – 6<sup>th</sup> weeks <sup>f</sup>. difference between 10<sup>th</sup> – 12<sup>th</sup> weeks 7<sup>th</sup> – 9<sup>th</sup> weeks <sup>g</sup>  
<sup>§</sup>. Difference between upper body vs. lower body  
<sup>©</sup>. Difference between upper body vs. combined  
<sup>¥</sup>. Different between lower body vs. combined

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