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ORIGINAL CONTRIBUTION

Effects of pre-exercise feeding on serum hormone concentrations and biomarkers of myostatin and ubiquitin proteasome pathway activity

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Abstract
Purpose The aim of the study was to examine the acute effects of pre-exercise ingestion of protein, carbohydrate, and a non-caloric placebo on serum concentrations of insulin and cortisol, and the intramuscular gene expression of myostatin- and ubiquitin proteasome pathway (UPP)-related genes following a bout of resistance exercise.
Methods Ten untrained college-aged men participated in three resistance exercise sessions (3 × 10 at 80 % 1RM for bilateral hack squat, leg press, and leg extension) in a cross-over fashion, which were randomly preceded by protein, carbohydrate, or placebo ingestion 30 min prior to training. Pre-supplement/pre-exercise, 2 h and 6 h post-exercise muscle biopsies were obtained during each session and analyzed for mRNA fold changes in myostatin (MSTN), activin IIB, follistatin-like 3 (FSTL3), SMAD specific E3 ubiquitin protein ligase 1 (SMURF1), forkhead box O3, F-box protein 32 (FBXO32), and Muscle RING-finger protein-1, with beta-actin serving as the housekeeping gene. Gene expression of all genes was analyzed using real-time PCR.
Results Acute feeding appeared to have no significant effect on myostatin or UPP biomarkers. However, resistance exercise resulted in a significant downregulation of MSTN and FBXO32 mRNA expression and a significant upregulation in FSTL3 and SMURF1 mRNA expression ($p < 0.05$).
Conclusions An acute bout of resistance exercise results in acute post-exercise alterations in intramuscular mRNA expression of myostatin and UPP markers suggestive of skeletal muscle growth. However, carbohydrate and protein feeding surrounding resistance exercise appear to have little influence on the acute expression of these markers.

Keywords Hypertrophy · Strength · Insulin · Cortisol · Protein

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Introduction

Myostatin is a known negative regulator of skeletal muscle mass [1–3]. During periods of skeletal muscle catabolism, myostatin expression is increased [4, 5], resulting in increased activity of the ubiquitin proteasome pathway [6]. During periods of skeletal muscle anabolism, myostatin is decreased allowing for satellite cell activation and eventual differentiation resulting in hypertrophy [2]. However, recent work has demonstrated that hypertrophy in the absence of myostatin may occur with little or no input from satellite cells and thus may increase the myonuclear domain by modulating the synthesis and turnover of structural muscle fiber proteins [7]. Primary factors influencing skeletal muscle size are diet and resistance exercise, each of which has been found to influence hormones

associated with skeletal muscle mass [8, 9]. By way of diet, a 2-day food deprivation protocol in mice resulted in a significant increase in myostatin (MSTN), F-box protein 32 (FBXO32), and Muscle RING-finger protein-1 (MURF1) mRNA in fast-twitch tibialis anterior muscle along with a significant decrease in muscle mass and protein content [10]. Likewise, researchers have reported the importance of protein consumption on the rate of muscle protein synthesis [11–13], while other evidence suggests that protein/carbohydrate feeding may attenuate muscle protein catabolism by way of IRS-1/2 → Akt signaling via transcription suppression of atrogenes [14, 15]. Nonetheless, little work has been conducted examining the independent influence of protein and carbohydrate consumption surrounding resistance exercise on biomarkers of myostatin and ubiquitin proteasome pathway activity.

Protein consumption surrounding exercise has garnered much attention in recent years regarding its role at stimulating hypertrophy of skeletal muscle [12, 16–22]. The timing of protein consumption appears particularly important as research suggests that protein consumption before and/or immediately following resistance exercise may be more beneficial in terms of muscle protein anabolism than 5 h before [19] or 2 h following a bout of resistance exercise [23]. In this regard, Hulmi et al. examined the influence of immediate pre- and post-exercise consumption of whey protein isolate after the first bout as well as after 21 weeks of a progressive resistance training program on the mRNA expression of myostatin and other downstream genes in college-aged [21] and older men [20]. Results from these investigations suggest that immediate pre-/post-exercise consumption of whey protein isolate resulted in significantly greater improvements in muscle cross-sectional area than the consumption of a non-caloric placebo following chronic resistance training. Therefore, it is possible these effects may have occurred due to favorable alterations in the mRNA expression of myostatin-related genes [20, 21]. However, this work needs to be expanded as genes that serve a primary regulatory role in myostatin pathway activity were not examined including SMAD specific E3 ubiquitin protein ligase 1 (SMURF1) and follistatin-like 3 (FSTL3) in college-aged men. SMURF1 and FSTL3 function as negative regulators of myostatin pathway activity with SMURF1 tagging SMAD2/3 for degradation [24] while FSTL3 binds to active myostatin preventing binding to its receptor [25].

Resistance training is also known to result in skeletal muscle damage, and myostatin has been found to be a necessary component of catecholamine-induced skeletal muscle atrophy [26, 27]. The ubiquitin proteasome pathway (UPP) is the primary pathway responsible for the selective degradation of skeletal muscle [28], and as a result, prominent biomarkers of the UPP will be examined, including forkhead box O3 (FOXO3), the muscle-specific ubiquitin ligases F-box protein 32 (FBXO32 also known as atrogin-1), and muscle RING-finger protein-1 (MURF1) [29–31].

The purpose of this investigation was to determine the impact of pre-exercise ingestion of whey protein isolate, carbohydrate, and a non-caloric placebo on serum markers of skeletal muscle anabolism/catabolism and intramuscular expression of myostatin- and UPP-related genes. It was hypothesized that resistance exercise would result in favorable alterations in myostatin- and UPP-related gene expression and that these alterations would be further enhanced by protein and carbohydrate consumption.

Methods

Subjects

This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board prior to data collection. Based upon a priori sample-size calculations, 10 untrained, healthy men (21.9 ± 1.3 years) were recruited to participate in this study. Prior to participation, subjects had to sign statements stating that (1) they had no current or past use of anabolic steroids, human growth hormone, or other pharmaceutical drugs that affect muscle mass; (2) they had not partaken in a structured lower-body resistance training program (i.e., one or more workouts per week) for at least 1 year; (3) they had not ingested or were not currently ingesting creatine, β-Hydroxy β-methylbutyric acid (HMB), thermogenic aids, and/or other nutritional supplements (excluding multivitamins) for an 8-week period prior to beginning the study; (4) they were classified as low risk according to ACSM criteria with no medical contraindications to resistance exercise. Subjects were informed of the experimental procedures and signed informed consent statements and medical history forms in adherence with the Institutional Review Board of the University of Oklahoma and the American College of Sports Medicine (ACSM) prior to participation.

Procedures

Research design

This study employed a single-blind, placebo-controlled, cross-over design. The participants completed an initial familiarization session in which their one repetition max (1RM) was assessed on the bilateral leg press, hack squat, and leg extension exercises, following the guidelines of the National Strength and Conditioning Association (NSCA) [32]. Following the assessment of their one repetition

maximum (RM), participants completed two additional familiarization sessions (F1 and F2) whereby they completed three sets of 10 repetitions with 2-min rest between sets and exercises on the bilateral leg press, hack squat, and leg extension exercises at a lifting intensity of 70 % 1RM (F1) and 75 % of one repetition maximum (F2). Following a 1-week latent period, participants returned to their first of three experimental conditions whereby they reported to the lab in a fasted state between 0600 and 0900 hours (Fig. 1). Participants completed three sets of 10 concentric–eccentric repetitions on the bilateral leg press, hack squat, and leg extension exercises at a lifting intensity of 80 % one repetition maximum with 2-min rest periods between sets and exercises. This workout was chosen for each experimental condition as it is representative of a typical lower-body resistance exercise training session. Table 1 presents the characteristics of the participants, along with the training data associated with the experimental conditions. The training volume during each condition was identical, and the macronutrient intake 48 h prior to each experimental condition was not significantly different (Table 2).

During each condition, venous blood samples were collected from an antecubital vein immediately prior to supplement consumption and immediately following exercise. After collection, blood samples were centrifuged for 15 min and stored at −20 °C for later analysis. During each condition, Bergstrom muscle biopsies were obtained from the vastus lateralis through the same incision site at a depth between three and four cm using standard procedures prior to supplement consumption and at 2 and 6 h following resistance exercise. After collection, muscle tissue was placed into a cryogenic tube and flash-frozen in liquid nitrogen. Samples were then transferred for long-term storage into a −80 °C ultra-low freezer until follow-up analyses.

Nutritional supplementation

Upon arrival to each of the experimental conditions, participants were assigned to ingest one of three supplements, at random, under the supervision of the research staff. The supplement groups were as follows: (A) 25 grams of whey protein isolate (NOW® Foods Nutrition, unflavored whey protein isolate, Bloomingdale, IL, USA), (B) 25 grams of maltodextrin (carbohydrate), and (C) water artificially sweetened with Splenda® (placebo) (Table 3). Each supplement was mixed with 350 ml (12 fl oz) of cold water and ingested 30 min prior to the start of the workout under the supervision of researchers.

Serum hormone quantitation

Serum concentrations of insulin and cortisol were determined using enzyme-linked immunosorbent assays (ELISA)

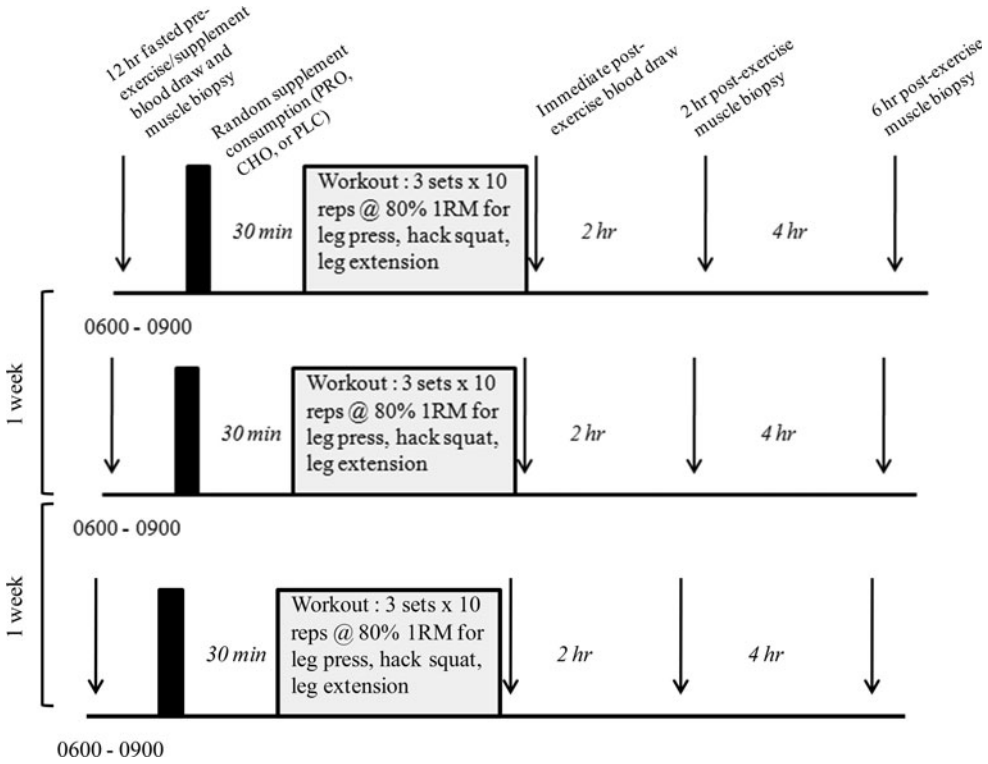

Fig. 1 Design employed in the current investigation

Table 1 Participant characteristics ($N = 10$)

Age (years)	21.9 ± 1.3
Height (cm)	177.5 ± 1.9
Body mass (kg)	77.8 ± 2.6
% body fat	17.8 ± 1.3
Leg press 1RM (kg)	238 ± 16
Hack squat 1RM (kg)	119 ± 5
Leg extension 1RM (kg)	51 ± 3
Training volume[a]	9,788 ± 471

[a] Each training session consisted of three sets of 10 repetitions at a lifting intensity of 80 % 1RM for leg press, hack squat, and leg extension with a 3-min recovery between sets

from Diagnostic System Laboratories (Webster, TX). All assays were performed according to the manufacturer's guidelines, using a wavelength of 450 nm against a known standard curve. Serum concentrations of all samples were determined in duplicate with a Model 680 microplate reader (Bio-Rad Inc, Hercules, CA).

Biochemical analyses

Muscle RNA isolation cDNA synthesis

Approximately 20 mg of muscle was homogenized using 500 µl of Tri reagent with a polypropylene pestle and microcentrifuge tube according to the manufacturer's instructions (Sigma Chemical Co., St. Louis, MO). The resultant RNA pellet was dissolved in 50 µl of RNase-free water and stored at −80 °C until later analyses.

Total RNA concentrations for each sample were determined using a high sensitivity RNA analysis kit with the Experion Automated Electrophoresis platform (Bio-Rad Laboratories, Hercules, CA). This procedure has been previously shown in our laboratory to yield undegraded RNA, free of DNA and proteins, as indicated by prominent 28S and 18S ribosomal RNA bands [33]. The preparation of reagents and the RNA ladder were performed according to the manufacturer's instructions. Furthermore, all RNA samples and the RNA ladder were thawed on ice during the assay to preserve mRNA integrity, and all assays were performed in duplicate.

Real-time PCR to detect post-exercise expression of genes of interest

Forward and reverse oligonucleotide primer pairs were constructed using commercially available software that ensures species- and gene-specific primer sequencing that lack secondary structures (Beacon Designer, Bio-Rad Laboratories). Primers were then commercially synthesized (Integrated DNA Technologies, Coralville, IA) (Table 4). Beta-actin (ACTB) was used as an internal reference for detecting relative change in the quantity of target mRNA due to its consideration as a constitutively expressed housekeeping gene after resistance exercise [34]. Two µl of cDNA was added to each of the eight separate PCR for MSTN, ACVR2B, FSTL3, SMURF1, FOXO3, FBXO32, MURF1, and ACTB. Each PCR contained the following mixtures: 12.5 µl of SYBR Green Supermix (Bio-Rad Laboratories) (100 mM KCl mixture, 40 mM Tris–HCl, 0.4 mM of each deoxynucleoside triphosphate, 50 U/µl of iTaq DNA polymerase, 6.0 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein), 1.5 µl of forward and reverse primers, and 7.5 µl of nuclease-free dH_2O. The PCR were amplified with a thermal cycler (Bio-Rad Laboratories) whereby the amplification sequence involved an initial 3-min cycle at 95 C to activate the *Taq* polymerase followed by a 40-cycle period with a denaturation step at 95 C for 10 s and primer annealing/extension step at 55 C for 30 s. All assays were performed in duplicate, and the critical threshold (C_T) coefficient of variation (C_V) value for each gene was as follows: MSTN = 0.56 %, ACVR2B = 0.73 %, FSTL3 = 0.70 %, SMURF1 = 0.78 %, FOXO3 = 0.76 %, FBXO32 = 1.15 %, MURF1 = 0.76 %, ACTB = 0.96 %. Furthermore, the plate-to-plate C_V value for ACTB critical threshold values using a control cDNA sample was <1.0 %. Gene expression data were calculated using the Pfaffl method [35] (i.e., $2^{-\Delta\Delta CT}$, assuming 100 % primer binding efficiency) where:

$$-\Delta\Delta CT = (C_T \text{ post-exercise gene of interest} - C_T \text{ post-exercise ACTB}) - (C_T \text{ pre-exercise gene of interest} - C_T \text{ pre-exercise ACTB})$$

To be consistent with the previous literature, gene expression values were expressed as percent change from 0,

Table 2 Macronutrient profile for each participant during each condition ($N = 10$)

Condition	Kilocalories (g/kg/day)	Protein (g/kg/day)	Carbohydrate (g/kg/day)	Fat (g/kg/day)
PRO	51.9 ± 2.3	2.4 ± 0.1	6.0 ± 0.3	2.1 ± 0.2
CHO	50.8 ± 2.5	2.3 ± 0.1	5.9 ± 0.4	2.1 ± 0.2
PLC	52.9 ± 3.0	2.4 ± 0.1	5.9 ± 0.2	2.2 ± 0.3

No difference in caloric intake was noted between conditions

PRO protein condition, *CHO* carbohydrate condition, *PLC* placebo condition

Table 3 Nutrient profile for each experimental condition

Nutrient profile	PRO	CHO	PLC
Amount (g)	28	28	28
Energy (kcal)	105	112	3
Protein (g)	25	0	0
Carbohydrate (g)	1	28	1
Fat (g)	0.3	0	0
Sodium (mg)	40	0	0

PRO protein. The protein used was NOW® Foods Nutrition (Bloomingdale, IL USA) unflavored whey protein isolate. *CHO* carbohydrate. The carbohydrate used was maltodextrin. *PLC* Placebo. The placebo used was Splenda® (Tate & Lyle, London, England, UK)

which was the value given to pre-exercise gene expression [36].

Statistical analyses

For normally distributed data (energy consumption, macronutrient consumption, and hormone concentrations), parametric statistics were used to detect within- and between-group differences. Separate one-way ANOVAs were used detect between-condition differences in the macronutrient profile of participants, whereas separate 3 × 2 (supplement condition × time) mixed factorial repeated measures ANOVAs were used to detect interactions and main effects for supplement condition and time. When necessary, separate dependent *t* tests were used to examine within-condition differences over time, while separate one-way ANOVAs with a Bonferroni correction were used to detect between-condition differences at each time point.

Due to the large variance commonly attributed to molecular variables, the Shapiro–Wilk statistic was performed for each dependent variable to ensure a normality in distribution existed. If data exhibited a non-normal distribution (i.e., skewness and/or kurtosis >1.96, or Shapiro–Wilk statistic p value <0.05) and standard transformation approaches were not possible, a nonparametric approach, as has been previously reported [37, 38], was used to analyzed changes in gene expression. The Kruskal–Wallis statistic was employed to detect between-condition differences at each time point. If this statistic yielded a significant p value, Mann–Whitney U statistics were employed as a post hoc measure to determine which condition(s) was significantly different. The Friedman test was used to detect changes in each condition over time. If the Friedman statistic yielded a p value <0.05, then Wilcoxon signed rank tests were employed for each condition as a post hoc measure to determine which time points were significantly different. Significance for all statistical analyses was determined using an alpha level of <0.05.

Results

A significant condition × time interaction ($p = 0.04$) was detected for serum insulin. A significant main effect was

Table 4 Primer sequences used to probe genes of interest expressed in skeletal muscle samples

Gene	Primer sequence (forward and reverse)	GenBank accession no.
MSTN	5′-GAC CAG GAG AAG ATG GGC TGA ATC CGT T-3′	NM_005259
	5′-CTC ATC ACA GTC AAG ACC AAA ATC CCT T-3′	
ACVR2B	5′-GCC TTG CCA TCA GAT TGT G-3′	NM_001106
	5′-GCC ATC AGA ACC AGA TAT ACC-3′	
FSTL3	5′-TGC TCA GAA TCG CCT ACC-3′	NM_005860
	5′-CTC CGT GTT GTC CTC TCC-3′	
SMURF1	5′-TGA AGG AAC GGT GTA TGA AG-3′	NM_020429
	5′-CGG TGC TAT CTG TGT AAG G-3′	
FOXO3	5′-GAA CGT GGG GAA CTT CAC TGG TGC TA-3′	NM_201559
	5′-GGT CTG CTT TGC CCA CTT CCC CTT-3′	
FBXO32	5′-ATG TGC GTG TAT CGG ATG G-3′	NM_058229
	5′-AAG GCA GGT CAG TGA AGC-3′	
MURF1	5′-GCC TTC TTC GCC TTC TCC-3′	NM_002931
	5′-AGC TCA TAC AGA CTC AGT TCC-3′	
ACTB	5′-AGT CAT CAT CTG GCT CTG G-3′	NM_001101
	5′-AAT GGA GGC TTG AGG TAG G-3′	

MSTN myostatin, *ACVR2B* activin IIB, *FSTL3* follistatin-like 3, *SMURF1* SMAD specific E3 ubiquitin protein ligase 1, *FOXO3* forkhead box O3, *FBXO32* F-box protein 32, *MURF1* muscle RING-finger protein-1, *ACTB* beta-actin

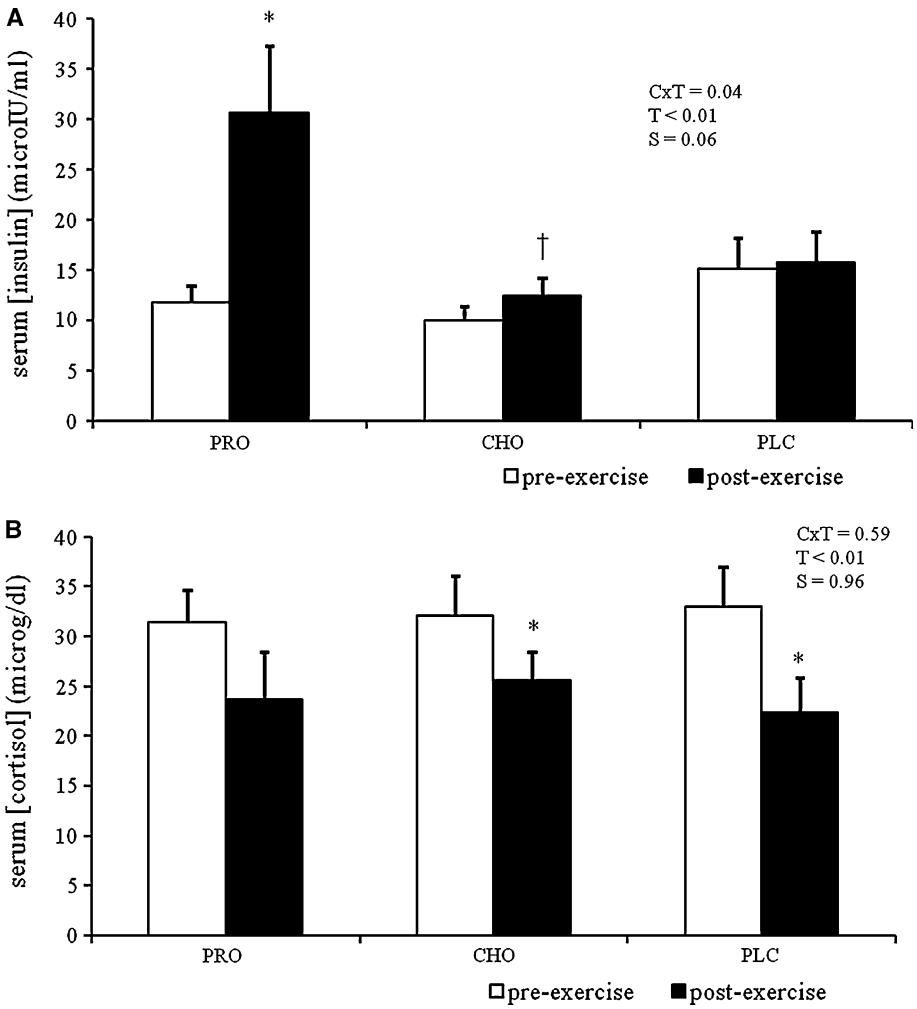

Fig. 2 Serum hormone concentrations. **a** Changes in serum insulin concentrations (μIU/ml) within and across all conditions ($N = 10$). **b** Changes in serum cortisol concentrations (μg/dl) within and across all conditions ($N = 10$). Data expressed as means ± SE. *Symbols*: *Asterisk* significant within-group difference from baseline, $p < 0.05$; *dagger* significant between-group difference compared with 6 h protein condition

detected for time ($p = 0.03$) in which post-exercise insulin concentrations were found to be significantly greater in the protein condition compared with pre-exercise/pre-supplement insulin concentrations. There was a trend detected for condition ($p = 0.06$), and follow-up analyses revealed post-exercise insulin concentrations to be significantly greater during the protein condition compared with the carbohydrate condition ($p = 0.02$) and to be nonsignificantly greater compared with the placebo condition ($p = 0.07$) (Fig. 2a).

No condition × time interaction ($p = 0.59$) was detected for serum cortisol concentrations. No main effect was detected for condition ($p = 0.96$), but there was a significant main effect for time ($p < 0.001$). Follow-up analyses revealed post-exercise cortisol concentrations to be significantly lower during the carbohydrate ($p < 0.01$) and placebo conditions ($p = 0.049$) with a trend decrease present during the protein condition ($p = 0.07$) (Fig. 2b).

Normality distribution statistics revealed that the expression patterns of all analyzed genes were non-normally distributed, and as a result, nonparametric statistics were employed. It should be noted that when examining all within-supplement comparisons over time, no fold change in ACTB CT values from pre-exercise to 2 h or 6 h post-exercise were present within the protein ($p = 0.18$), carbohydrate ($p = 0.40$), or placebo ($p = 0.16$) condition. Percent changes in MSTN mRNA expression following exercise are presented in Fig. 3a. There were no between-group differences 2 h ($p = 0.52$) or 6 h ($p = 0.63$) following exercise. There was a significant time effect on MSTN expression following exercise (protein, $p < 0.01$; carbohydrate, $p < 0.01$; placebo, $p < 0.01$). Within-group tests revealed that MSTN mRNA expression was significantly downregulated 2 h following exercise during placebo ($p < 0.01$) and carbohydrate ($p = 0.04$) conditions and 6 h following exercise in the protein ($p < 0.01$), carbohydrate ($p < 0.01$) and placebo ($p < 0.01$) conditions.

Percent changes in ACVR2B mRNA expression following exercise are presented in Fig. 3b. There were no between-group differences 2 h ($p = 0.36$) or 6 h ($p = 0.82$) following exercise. There were no significant time effects on

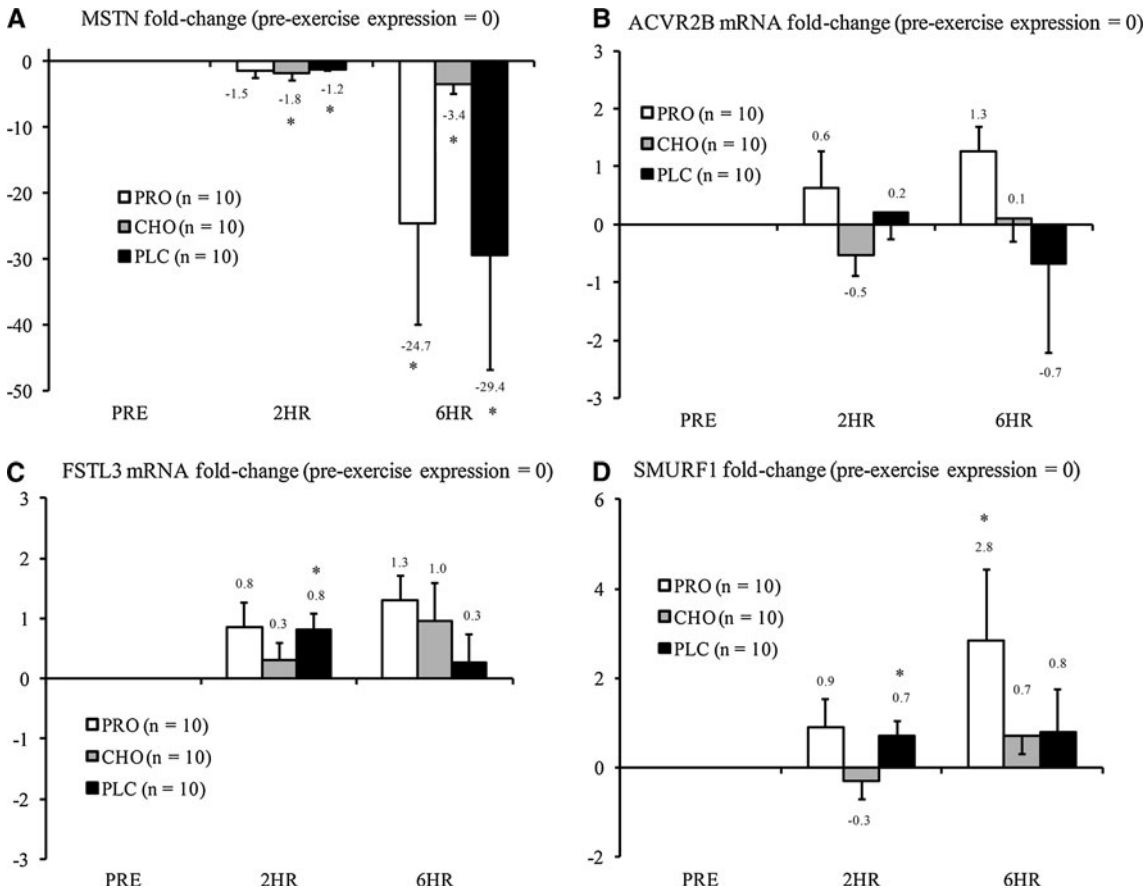

Fig. 3 MSTN-related mRNAs in response to pre-exercise feeding. **a** Changes in muscle MSTN mRNA expression within and across all conditions following resistance exercise ($N = 10$). **b** Changes in muscle ACVR2B mRNA expression within and across all conditions following resistance exercise ($N = 10$). **c** Changes in muscle FSTL3 mRNA expression within and across all conditions following resistance exercise ($N = 10$). **d** Changes in muscle SMURF1 mRNA expression within and across all conditions following resistance exercise ($N = 10$). Data expressed as means ± SE. Results are normalized to ACTB mRNA expression. Mean percent changes are presented above each data point. *Symbols*: *Asterisk* significant within-group difference from baseline, $p < 0.05$

ACVR2B expression in any condition (protein, $p = 0.69$; carbohydrate, $p = 0.25$; placebo, $p = 0.72$).

Percent changes in FSTL3 mRNA expression following exercise are presented in Fig. 3c. There were no between-group differences 2 h ($p = 0.07$) or 6 h ($p = 0.85$) following exercise. There was a significant time effect on FSTL3 mRNA expression following exercise (protein, $p = 0.12$; carbohydrate, $p = 0.30$; placebo, $p < 0.01$).

Percent changes in SMURF1 mRNA expression following exercise are presented in Fig. 3d. There were no between-group differences 2 h ($p = 0.47$) or 6 h ($p = 0.61$) following exercise. There was a significant time effect on SMURF1 mRNA expression following exercise (protein, $p = 0.02$; carbohydrate, $p = 0.03$; placebo, $p = 0.03$). Within-group tests revealed that SMURF1 mRNA expression was significantly upregulated 2 h following exercise during the placebo ($p = 0.04$) condition and 6 h following exercise during the protein ($p = 0.02$) condition.

Percent changes in FOXO3 mRNA expression following exercise are presented in Fig. 4a. There were no between-group differences 2 h ($p = 0.61$) or 6 h ($p = 0.60$) following exercise. There were no significant time effects on FOXO3 expression in any condition (protein, $p = 0.91$; carbohydrate, $p = 0.74$; placebo, $p = 0.30$).

Percent changes in FBXO32 mRNA expression following exercise are presented in Fig. 4b. There were no between-group differences 2 h ($p = 0.22$) or 6 h ($p = 0.76$) following exercise. There was a significant time effect on FBXO32 mRNA expression following exercise (protein, $p = 0.12$; carbohydrate, $p < 0.01$; placebo, $p < 0.01$). Within-group tests revealed that FBXO32 mRNA expression was significantly downregulated 2 h ($p < 0.01$) and 6 h ($p < 0.01$) following exercise during the carbohydrate condition and 6 h ($p < 0.01$) following exercise during the placebo condition.

Percent changes in MURF1 mRNA expression following exercise are presented in Fig. 4c. There were no

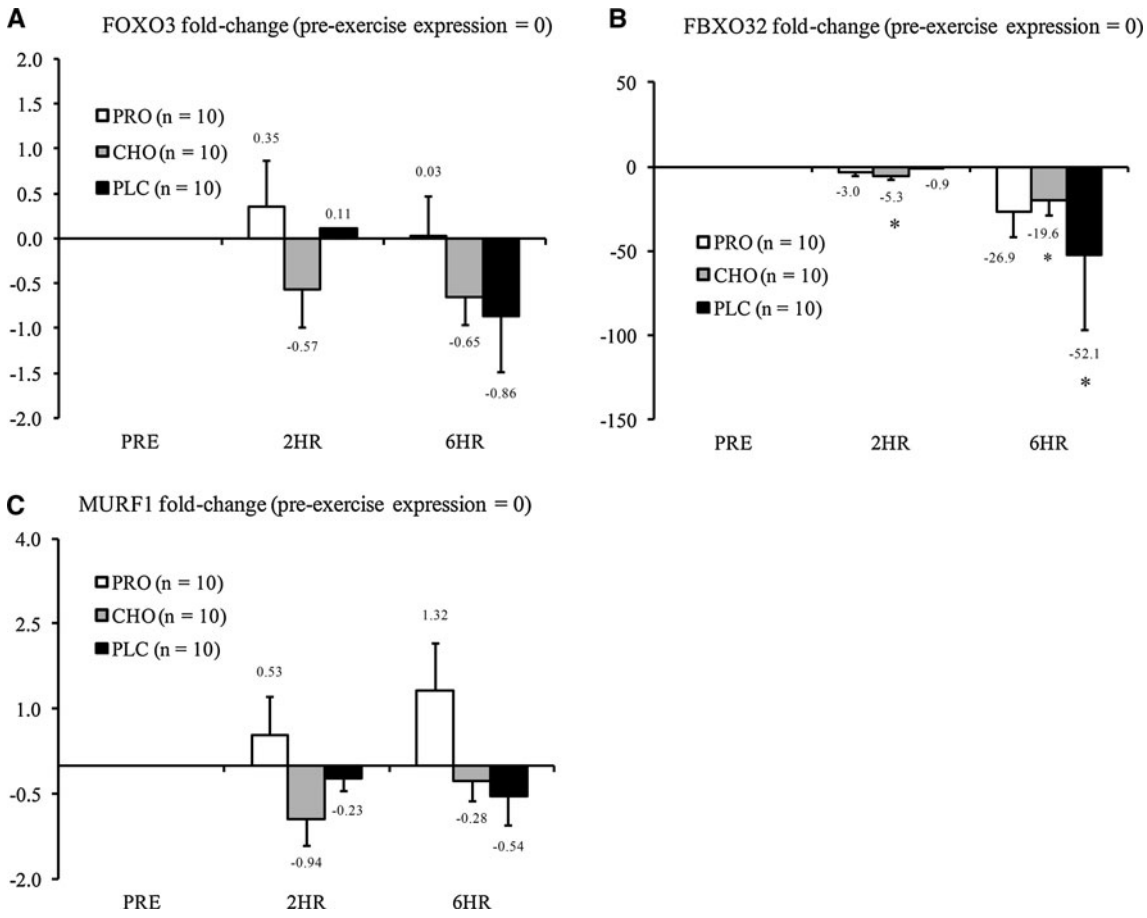

Fig. 4 UPP-related mRNAs in response to pre-exercise feeding. **a** Changes in muscle FOXO3 mRNA expression within and across all conditions following resistance exercise ($N = 10$). **b** Changes in muscle FBXO32 mRNA expression within and across all conditions following resistance exercise ($N = 10$). **c** Changes in muscle MURF1 mRNA expression within and across all conditions following resistance exercise ($N = 10$). Data expressed as means ± SE. Results are normalized to ACTB mRNA expression. Mean percent changes are presented above each data point. *Symbols*: *Asterisk* significant within-group difference from baseline, $p < 0.05$

between-group differences 2 h ($p = 0.39$) or 6 h ($p = 0.06$) following exercise. There was no significant time effect on MURF1 mRNA expression in any condition over time (protein, $p = 0.15$; carbohydrate, $p = 0.12$; placebo, $p = 0.41$).

Discussion

The purpose of the current investigation was to determine the impact of pre-exercise consumption of protein, carbohydrate, and a non-caloric placebo on serum insulin and cortisol concentrations, and the mRNA expression of myostatin- and UPP-related genes. As expected, an acute bout of resistance exercise stimulated a downregulation in the mRNA expression of MSTN- [21, 33, 36, 39] and the UPP-related gene FBXO32 [36, 40]. In this regard, the consumption of protein resulted in the most robust increase in serum insulin concentrations; however, these changes did not appear to influence the mRNA expression of myostatin- or UPP-related genes.

Pre-exercise protein consumption resulted in a significant increase in serum insulin concentrations. In this regard, post-exercise serum insulin concentrations were significantly greater in the protein compared with the carbohydrate condition, with a trend difference existing between the protein and placebo condition ($p = 0.07$). Immediate post-exercise cortisol concentrations were significantly lower in the carbohydrate and placebo conditions compared with baseline with a trend decrease present in the protein condition ($p = 0.07$). Serum cortisol has been reported to be unchanged from baseline following a bout of resistance exercise [41, 42], and cortisol has also been found to be significantly increased during periods of mental stress [43, 44]. As a result, we anticipate that our baseline assessment of serum cortisol was increased due to concern from the blood draw and impending muscle collection protocols.

Myostatin is an important regulator of skeletal muscle mass [1–3], but limited research has examined the influence of nutritional interventions surrounding exercise on myostatin- [20, 21, 39] and UPP-related genes [21, 45]. Results from the current investigation (placebo condition) are consistent with previous work that has reported an acute bout of resistance exercise to result in a significant downregulation of MSTN mRNA [20, 21, 36, 46] for up 24 h post-exercise [33] and to have no effect on ACVR2B mRNA expression [20, 21, 33, 47, 48] up to 48 h post-exercise [33]. Moreover, FSTL3 and SMURF1 mRNAs were significantly upregulated 2 h post-exercise, while FBXO32 mRNA was significantly downregulated 6 h post-exercise, which is in agreement with previous work that reported resistance exercise to have no effect on FBXO32 mRNA expression 3 h post-exercise [49] but to be significantly downregulated 8 h post-exercise [36]. Results from the current investigation provide further evidence that an acute bout of resistance exercise independent of pre-exercise nutritional supplementation stimulates favorable alterations in the mRNA expression of MSTN- and UPP-related genes; a finding that suggests that these plausible transcriptomic changes may precede the long-term benefits of resistance training.

Protein consumption surrounding chronic resistance training has been found to increase skeletal muscle cross-sectional area [16, 50] and lean body mass [51, 52] to a greater extent than chronic resistance training with or without carbohydrate consumption. In the current investigation, pre-exercise protein consumption stimulated a significant increase in serum insulin concentrations immediately post-exercise; however, there were no between-group differences at 2 or 6 h post-exercise for any gene of interest. As a result, nutritional supplementation surrounding an acute bout of resistance exercise appears to have little influence on the mRNA expression of myostatin- and UPP-related genes. Conversely, within-condition differences were not identical during each trial and are in agreement with reports from previous investigations [20, 21]. Of particular interest was the finding that protein supplementation delayed the downregulation of MSTN mRNA expression 2 h post-exercise. This finding is consistent with previous work, which reported the consumption of 15 g of whey protein isolate immediately prior to and immediately following a bout of resistance exercise to prevent the post-exercise decrease in MSTN mRNA expression [20, 21]. The current investigation provides support that the delay in the downregulation of MSTN mRNA was most likely not due to feeding alone as there was a significant decrease in the mRNA expression of MSTN 2 h post-exercise during the carbohydrate and placebo conditions. Furthermore, the delay in the downregulation of MSTN does not appear to be related to mRNA expression patterns of mechano growth factor (MGF), cyclin D1, p21, or MYOD [53]. As a result, the beneficial effects of protein consumption surrounding resistance exercise may be driven by another pathway such as mammalian target of rapamycin (mTOR), which has been reported to be influenced by protein consumption and resistance exercise [54].

ACVR2B expression was unaffected during the placebo, protein, and carbohydrate conditions. These results are consistent with previous work that reported protein consumption to have no effect on ACVR2B mRNA expression 1 and 48 h following resistance exercise. ACVR2B mRNA expression patterns have been reported to be unaffected by age [33], resistance exercise [20, 21, 33, 47, 48], and feeding [20, 21]. As a result, ACVR2B regulation at the mRNA level appears to play a minimal role in influencing MSTN mRNA signaling in response to acute or chronic resistance exercise.

This is the first investigation to examine the effects of protein consumption prior to resistance exercise on FSTL3 mRNA expression in college-aged men. Interestingly, FSTL3 was significantly upregulated 2 h post-exercise during the placebo condition, but was unaffected during the protein condition 2 and 6 h post-exercise. The lack of upregulation of FSTL3 during the protein condition is seemingly unfavorable in terms of promoting skeletal muscle hypertrophy. However, the possibility exists that the upregulation of FSTL3 mRNA during the protein condition was delayed by protein consumption. In this regard, Hulmi et al. [20] reported FSTL3 mRNA expression to be unaffected with or without protein consumption in older men 1 h post-exercise, but to be significantly upregulated 48 h post-exercise in the protein group. Future investigations should examine the protein expression of FSTL3 following chronic resistance exercise with or without protein consumption to determine whether protein consumption surrounding exercise can stimulate an upregulation of FSTL3.

No found work has examined the influence of feeding on the mRNA expression of SMURF1, which functions to mark Smad2/3 for degradation via the UPP, thus acting as a negative regulator of the myostatin signaling cascade [24]. In this regard, only one found study has examined the effects of resistance exercise on SMURF1 mRNA expression [33]. In 2011, Dalbo et al. [33] reported SMURF1 mRNA expression to be unaffected 24 and 48 h following three sequential bouts of resistance exercise in younger and older men. In the current investigation, nutrient consumption appeared to influence SMURF1 mRNA expression. Of particular interest was the finding that during the placebo condition, SMURF1 mRNA was upregulated and MSTN mRNA was downregulated 2 h post-exercise, indicating that these two transcripts are potentially mechanosensitive. In this regard, during the protein condition, SMURF1 mRNA was upregulated and MSTN mRNA was downregulated 6 h post-exercise. Thus, this is preliminary evidence to suggest that this level of myostatin signaling

regulation exists in response to resistance training and, thus, will be of future interest to molecular exercise physiologists.

Myostatin has been found to be a required component for glucocorticoid-induced skeletal muscle atrophy [26, 27]. Resistance exercise with or without supplementation had no effect on FOXO3 or MURF1 mRNA expression at 2 or 6 h post-exercise. There was no significant effect of protein consumption on FBXO32 mRNA expression, which is consistent with previous work [21]. During the carbohydrate condition, there was a significant downregulation of MSTN and FBXO32 mRNA at 2 and 6 h post-exercise, but no significant correlation was present at either time point (2 h: $r = 0.11$, $p = 0.75$; 6 h: $r = 0.29$, $p = 0.41$).

Conclusions

Resistance exercise results in favorable alterations in myostatin- and UPP-related mRNAs at 2 and 6 h post-exercise, which may link these acute changes to skeletal muscle growth following prolonged training. Paradoxically, this study in concert with the other literature suggests that whey protein isolate consumption surrounding resistance exercise may delay the downregulation of MSTN [20, 21] while upregulating FSTL3 [20] and SMURF1 mRNAs. Nonetheless, this study continues to provide mRNA evidence to suggest that resistance training, independent of macronutrient intake, favorably impacts the transcriptomic signature of atrophy-related genes. Furthermore, future studies should continue to elucidate the importance of the SMURF1 in modulating resistance training adaptations in healthy and diseased individuals.

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