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Research article

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Abstract

Resistance exercise (RE) activates cell signaling pathways associated with myostatin. Decorin is located in the extracellular matrix (ECM) and can block the inhibitory effect of myostatin. This study sought to determine the impact of low-load (LL) and high-load (HL) RE on myostatin mRNA and protein expression along with changes in muscle decorin and circulating follistatin. Ten resistance-trained men performed a LL (50% 1RM) and HL (80% 1RM) RE session using the angled leg press and leg extension with load and volume equated. Venous blood samples and muscle biopsies were obtained prior to and at 3h and 24h following each RE session. Muscle myostatin mRNA expression was increased at 24h post-exercise ($p = 0.032$) in LL and at 3h ($p = 0.044$) and 24h ($p = 0.003$) post-exercise in HL. Muscle decorin was increased at 24h post-exercise ($p < 0.001$) in LL and HL; however, muscle myostatin was increased at 24h post-exercise ($p < 0.001$) only in HL. For muscle Smad 2/3, no significant differences were observed ($p > 0.05$). Serum follistatin was increased and myostatin decreased at 24h post-exercise ($p < 0.001$) in LL and HL. Muscle myostatin gene and protein expression increased in response to HL RE. However, serum myostatin was decreased in the presence of increases in decorin in muscle and follistatin in circulation. Therefore, our data suggest a possible mechanism may exist where decorin within the ECM is able to bind to, and decrease, myostatin that might otherwise enter the circulation for activin IIB (ACTIIB) receptor binding and subsequent canonical signaling through Smad 2/3.

Key words: Follistatin, Smad 2/3, extracellular matrix, mechanotransduction

Introduction

Resistance exercise (RE) is well known to orchestrate cell signaling pathways associated with muscle hypertrophy and regeneration. Myostatin, also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily and is a negative regulator of muscle regeneration and growth (Sutrave et al., 1990). In skeletal muscle, myostatin gene expression results in production of an immature pre-promyostatin protein which is subsequently processed to promyostatin (propeptide and myostatin) and released into circulation as an inactive "latent complex" as pro-peptide remains non-covalently bound. In circulation, the myostatin ligand is only capable of binding to its activin IIB (ACTIIB) receptor once the pro-peptide is enzymatically cleaved; otherwise, it can also be inhibited by follistatin. Canonically, myostatin association with the ACTIIB receptor signals intramuscularly through the Smad (homologues of the Drosophila protein, mothers against decapentaplegic) pathway. Receptor-regulated Smads are activated through serine kinase phosphorylation causing Smad 2 and Smad 3 to form hetero-oligomers with Smad4 and translocate the complex to the nucleus to up-regulate myostatin gene expression and repress muscle regeneration and growth (Sutrave et al., 1990) by down-regulating Myo-D and myogenin expression (Langley et al, 2002).

Decorin is a member of the small leucine-rich proteoglycan gene family and consists of a core protein and a dermatan/chondroitin sulfate chain (Miura et al., 2006) that is synthesized and secreted by skeletal muscle (Branden et al., 1991). Decorin binds several types of collagen and participates in regulation of collagen fibril formation and stabilization of collagen fibers in the extracellular matrix (ECM) (Iozzo, 1999). Decorin plays and important role in cell growth, both through direct interactions with cell surface receptors (Patel et al., 1998; Santra et al., 2002) and through modulation of growth factor activities (Schönherr et al., 1998; Yamaguchi et al., 1990) by binding to the core protein (Ständer et al., 1999). Suppression of decorin production significantly decreases the sensitivity to TGF-β dependent inhibition of myogenesis (Riquelme et al., 2001). It has been shown that decorin can block the inhibitory effect of myostatin on myoblast proliferation by immobilizing myostatin in the ECM (Miura et al., 2006).

The ECM is a complex of macromolecules including collagen, elastin, proteoglycans, and glycoproteins forming a matrix and reservoir for growth factors which modulate their activation status (Kresse and Schonherr, 2002). Of these growth factors, TGF-β and its family member, myostatin, have been found to associate with ECM-related proteoglycans, particularly decorin (Taipale and Koski-Oja, 1997). Research has shown that the ECM plays an important role in the development, growth, and repair/regeneration of muscle (Thorsteinsdóttir et al., 2011; Fry et al., 2017). Decorin binds to myostatin by its core

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protein and immobilizes myostatin in the ECM. This activity prevents the diffusion of myostatin across the ECM where it would otherwise be released into circulation, thereby decreasing its ability to bind with ACTIIB receptors and suppressing subsequent myostatin-mediated Smad 2/3 signaling and the inhibitory action towards myocyte cell growth (Muira et al., 2006).

The ECM serves as a conduit in which the mechanotransductive transmission of a muscle's contractile force is relayed intramuscularly to the contractile apparatus (Street, 1983). Decorin levels have been shown to be elevated up to 60 min following a bout of RE (Kanzleiter et al., 2014) and may indicate a role of the ECM for tempering RE-induced increases in muscle myostatin protein release into circulation. However, the responsiveness of myostatin expression and/or activity does not appear to be dependent on exercise intensity and/or volume (Wilborn et al., 2009; Schwarz et al., 2016; He et al, 2018; Shanazari et al., 2019). Even though evidence exists showing myostatin to be transcriptionally down-regulated with RE, studies have also shown RE-induced increases in myostatin gene expression (MacKenzie et al., 2013; Hostrup et al., 2018; Hulmi et al., 2009) and circulating myostatin (He et al., 2018). In the instance of increased myostatin gene expression, it is conceivable that increased myostatin transactivation would likely increase myostatin protein expression and subsequently impact the amount of myostatin available for release into circulation. In response to RE, if myostatin ACTIIB receptor binding capacity becomes reduced due to a reduction in circulating myostatin from decorin-induced inhibition in the ECM then, indeed, a decrease in intramuscular myostatin signaling through Smad 2/3 and a subsequent positive response toward muscle regeneration could feasibly occur. Moreover, a mechanism has been shown to exist in which decorin binding with myostatin within the ECM tempers the amount of transcriptionally-mediated myostatin produced that is released into the circulation and available for ACTIIB receptor binding (Kishioka et al., 2008); however, this mechanism did not involve a RE-induced stimulus.

Changes in mechanical force that are inherent with RE appear to induce mechantransductive mechanisms in the ECM involved with regulating MPS (Rindom and Vissing, 2016). The tensile and/or compressive stresses that are expectedly generated during RE-induced mechanotransductive muscle loading can impact how mechanosensing molecules impact biochemical signaling to directly activate MPS (Aguilar-Agon et al., 2019). Comparatively speaking, since the tensile and compressive stresses should be greater with HL RE, it is conceivable that the mechanosensing molecules contained within the ECM would be more robustly activated. Therefore, the purpose of this study was to determine the impact of a single bout of low- (LL) and high-load (HL) RE on skeletal muscle myostatin mRNA and protein expression and muscle Smad 2/3 and decorin, in addition to concentrations of circulating myostatin and follistatin.

Methods

Eperimental approach

Specific details of the methodological approach of this study can be found published elsewhere (Cardaci et al., 2020), also indicating that all study procedures were approved by the Institutional Review Board at Baylor University (approval #1521229-3) and conformed to the ethical consideration of the Declaration of Helsinki. In brief, 10 apparently healthy, recreationally resistance-trained [regular, consistent resistance training (i.e. thrice weekly) for at least 1 year prior to the onset of the study] men with a mean age, height, and total body mass of 23.2 (±4.68) yr, 176.78 (±04.58) cm, and 87.15 (±5.77) kg served as participants for this study. Resistance training status was confirmed by a leg press one repetition maximum (1RM), which was compared to normal strength-to-body weight ratios. Participants were eligible for inclusion if their strength-to-body weight ratio was ≥2.82 times body weight [Cooper Institute for Aerobics Research (1997) Strength to Weight Ratios Age-Gender Norms 1RM Bench Press and Leg Press the Physical Fitness Specialist Certification Manual, Dallas]. This study involved a cross-over design that involved participants visiting the laboratory on 3 separate occasions.

Visit 1 consisted of an entry/familiarization, medical/physical activity screening, and, per National Strength and Conditioning Association (NSCA) guidelines, RE maximum strength testing using standard 1RM and 10 repetition protocols (10RM) for the leg press and leg extension exercises, respectively. Visit 2 consisted of performing a LL (50% 1RM) RE session. Visit 3 consisted of a HL (80% 1RM) RE session. A duration of 7-10 days was allowed between all visits, and participants were instructed to refrain from exercise for 48 hours prior to all 3 visits. Leg press foot placement was recorded and held constant over all testing conditions in order to maintain consistency. To ensure participants were moving through the full range of motion during each repetition, a goniometer was used to establish 90 degrees of knee flexion on the leg press and leg extension to constitute a completed repetition. Participants warmed up by completing 5 to 10 repetitions at approximately 50% of the estimated 1RM/10RM. Then participants rested for 1 minute and then completed 3 to 5 repetitions at approximately 70% of the estimated 1RM/10RM. The LL and HL RE sessions occurred in a volume-equated manner. As such, participants performed identical RE consisting of the angled leg press and leg extension. During LL RE, participants performed 50% of their 1RM to volitional failure for 4 sets in each exercise. Total exercise load volume (sets x repetitions x load) was calculated and equated in the HL RE to occur during visit 3. During HL, sets were performed until participants reached the necessary volume needed to match the total volume performed during LL. This allowed for volume and intensity to be equated between the two conditions. Due to the greater amount of volume that can be accumulated with a lighter load, additional sets for each exercise were utilized (if necessary) in order to equate volume between LL and HL conditions. In all cases, 2-4-minute rest occurred between all sets and exercises.

Due to the diurnal nature and dietary influence of the biomarkers being investigated, participants reported to the laboratory upon waking and in a fasted state at 08:53

(±0:55) and 08:37 (±1:00) for LL and HL, respectively. Moreover, in order to minimize nutritional mediation of the markers investigated, participants received a standardized nutrition bar 30 minutes prior to RE (Power Bar®, Premier Nutrition Corporation, Kings Mountain, NC, USA, [carbohydrate: 25g, protein: 20g, fat: 6g, fiber: 4g]). Lastly, in an attempt to control for variations in RE performance, skeletal muscle strength, and proper recovery, RE protocols were scheduled within 2 hours of each other and separated by 7-10 days. Each session involved the gathering of venous blood and muscle biopsy samples.

Muscle biopsies

Percutaneous muscle biopsies (~30mg) were obtained from the middle portion of the *vastus lateralis* muscle of the dominant leg (midpoint between the patella and the greater trochanter of the femur) at a depth between 1 and 2 cm using the fine needle aspiration technique. Muscle tissue was extraction using the TRU-CORE® 1 Automatic Biopsy Instrument (Angiotech, Medical Device Technologies, INC., Gainsville, FL, USA) after subcutaneous administration of the local anesthetic (1ml of 1% lidocaine/xylocaine). After the initial biopsy, following biopsy attempts were made to extract tissue from approximately the same location as the initial biopsy by using the pre-biopsy scar, depth markings on the needle, and a successive incision that was made approximately 0.5 cm to the former from medial to lateral. After removal, adipose tissue was trimmed from the muscle specimens and was immediately frozen and stored at -80°C for later analysis. Biopsies were taken pre-exercise and at 3- and 24-hours post-exercise during visits 2 and 3.

Venipuncture

Venous blood samples were obtained from the medial cubital vein using a standard vacutainer apparatus. Blood samples stood at room temperature for 10 minutes and were then centrifuged. The serum was then removed and frozen at -80°C for later analysis. The blood samples were collected pre-exercise and at 3- and 24-hours post-exercise during visits 2 and 3.

Total RNA isolation from skeletal muscle

Total cellular RNA was extracted from the homogenate of biopsy samples with a monophasic solution of phenol and guanidine isothiocyanate contained within the TRI-reagent (Sigma Chemical Co., St. Louis, MO). Total RNA concentrations from each sample were determined spectrophotometrically with an optical density of 260 nm (OD^{260}) and to verify RNA integrity and absence of RNA degradation, indicated by an OD^{260}/OD^{280} ratio of approximately 2.0. Our protocol produced an average (±SD) ratio of 1.92 (± 0.38) for all samples. The RNA samples were stored at -80°C until complementary DNA (cDNA) synthesis.

Reverse transcription and cDNA synthesis

A reverse transcription reaction mixture [200 ng of total cellular RNA, 5× reverse transcription buffer, a dNTP mixture containing dATP, dCTP, dGTP, and dTTP, MgCl2, RNase inhibitor, oligo$(dT)^{15}$ primer, nuclease-free H_2O, and 1 U•µl-1 MMLV reverse transcriptase enzyme (Bio-Rad, Hercules, CA)] were incubated at 42°C for 40 minutes, heated to 85°C for five minutes, and then quick-chilled on ice yielding cDNA, which were then frozen at -80°C until real-time RT-PCR was performed.

Oligonucleotide primers for PCR

The mRNA sequences of human skeletal muscle myostatin (NM_005259) and GAPDH (NM_002046) published in the NCBI Entrez Nucleotide database (www.ncbi.nlm.nih.gov) were used to construct PCR primers (Beacon Designer software, Bio-Rad, Hercules, CA, USA), and then commercially synthesized (Integrated DNA Technologies, Coralville, IA, USA). For myostatin, the sense (5-CAAGAAYAGAAGCCATTAAGATAC-3') and antisense (5'-CGTTGTAGCGTGATAATCG-3') primers amplified a fragment of 156 bp. For GAPDH, the sense(5'-AAAGCCTGCCGGTGACTAAC-3') and antisense (5'-CGCCCAATACGACCAAATCAGA-3') primers amilified a fragment of 172 bp.

Real-time PCR

Aliquots of cDNA were added to each of the PCR reactions for myostatin and GAPDH. Each PCR reaction contained the cDNA template along with 2× SYBR Green Super- mix (Bio-Rad, Hercules, CA) [100 mM KCl mixture, 40 mM Tris-HCl, 0.4 mM of each dNTP, 50 $U•ml^{-1}$ of iTaq DNA polymerase, 6.0 mM MgCl2, SYBR Green I, 20 nM flourescein], sense and anti-sense primers, and nuclease-free dH2O. Each PCR reaction was amplified (Bio Rad, Hercules, CA) and the amplification sequence involved a denaturation step at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 60 seconds. RT-PCR was performed over 40 cycles. Due it being a constitutively expressed housekeeping gene, GAPDH was used as an external reference standard for detecting relative change in the quantity of target mRNA (Thellin et al., 2009). All CT values were assessed in the linear portion of amplification and a DNA melting curve analysis was performed after amplification to assure that the single gene products were amplified in absence of primer-dimers.

The specificity of the PCR was demonstrated with an absolute negative control reaction containing no cDNA template, and single gene products confirmed using DNA melt curve analysis. Based on our previous work (Kerksick et al., 2013), the expression of mRNA was determined from the post-exercise fold-changes in gene expression an alyzed using the Pfaffl method (Pfaffl, 2001) [i.e., E-ΔΔCT = E is 1.00 + (percent primer efficiency/100); ΔΔCT = (CT gene of interest - CT GAPDH) post-exercise - (CT gene of interest - CT GAPDH) pre-exercise]. A 0-fold change is a 100% down-regulation of the gene and a 1-fold change indicated no change in gene expression relative to baseline mRNA levels, whereas a 2-fold and 3-fold change in gene expression indicated a 100% and 200% increase in gene expression, respectively.

Skeletal muscle total protein extraction

A portion of each muscle sample was weighed and homogenized using a commercial tissue extraction reagent (Invitrogen Corporation, Camarillo, CA, USA) and a tissue

homogenizer. Total muscle protein extraction was performed using a cytoplasmic extraction buffer (Aviva Systems Biology Corporation, San Diego, CA, USA). All extracts were supplemented with phenylmethanesulfonyl fluoride and a protease inhibitor cocktail (Sigma Chemical Company, St. Louis, MO, USA) with broad specificity for the inhibition of serine, cysteine, and metallo-proteases. Total protein extracted samples were analyzed in duplicate and determined spectrophotometrically at a wavelength of 750 nm (Bio-Rad Hercules, CA, USA) and using bovine serum albumin as the standard. Total protein content was expressed relative to muscle wet-weight.

Serum analysis of myostatin and follistatin

The concentrations of serum myostatin and follistatin were assessed using commercially-available enzyme-linked immunosorbent assay (ELISA) kits (myostatin: RayBio, Norcross, GA, USA; decorin: Aviva Systems Biology, San Diego, CA, USA; follistatin, MyBiosource, San Diego, CA). The sensitivity of the kits was 0.65 ng.ml, 0.83 ng/ml and 23 pg/ml for myostatin, and follistatin, respectively. Samples were analyzed in duplicate and absorbances were read at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA, USA). Unknown concentrations were determined by linear regression against known standard curves using commercial software (Microplate Manager, Bio-Rad, Hercules, CA, USA). The overall intra-assay percent coefficients of variation were 8.3 (±3.85) and 8.64% (±3.87) respectively, for myostatin and follistatin.

Skeletal muscle analysis of decorin, myostatin, and Smad 2/3

The concentrations of skeletal muscle Smad 2/3 were assessed using a commercially-available enzyme-linked immunosorbent assay (ELISA) kit (Cell Signaling Technology, Boston, MA, USA). The sensitivity of each kits is de termined to be 0.65 ng.ml, 0.83 ng/ml. and 0.057 ng/ml for decorin, myostatin, and Smad 2/3, respectively. Samples were analyzed in duplicate and absorbances were read at a wavelength of 450 nm using a microplate reader (iMark, Bio-Rad, Hercules, CA, USA). Unknown concentrations were determined by linear regression against known standard curves using commercial software (Microplate Manager, Bio-Rad, Hercules, CA, USA). Protein content for decorin and myostatin was expressed relative to total protein content and Smad 2/3 was expressed by absorbance values. The overall intra-assay percent coefficients of variation were 6.77% (±4.36), 8.16% (±3.98), and 7.64 (±3.21), respectively skeletal muscle decorin, myostatin, and Smad 2/3.

Statistical analysis

Statistical analysis was performed by utilizing separate 2 x 3 [Condition (LL, HL) x Time (pre-exercise, 3-hours post-exercise, and 24-hours post-exercise)] factorial analyses of variance (ANOVA) with repeated measures. If a significant interaction was present, analysis of main effects was conducted using the simple main effects, pairwise comparisons with a Bonferroni adjustment to compare dependent variables within each independent variable condition. If no interaction was present, then normal pairwise comparisons with a Bonferroni adjustment were used to test main effects. The magnitude of statistical significance was measured by effect size (Partial Eta-Squared), which estimates the ratio of variance in the dependent variable that is explained by the independent variable. Partial Eta Squared effect sizes (η^2) are characterized 0.1 - 0.3 as small, 0.3 - 0.5 as medium, and ≥0.5 as large [50]. All statistical procedures were performed using SPSS 27.0 software and an alpha level of ≤0.05 was set for all statistical measures.

Results

Skeletal muscle myostatin mRNA expression

For muscle myostatin mRNA expression (Table 1), no significant main effect of condition ($p = 0.091$, $\eta^2 = 0.395$) or time x condition interaction ($p = 0.470$, $\eta^2 = 0.174$) was observed. However, a significant main effect for time ($p = 0.041$, $\eta^2 = 0.624$) was observed. Pairwise comparisons

Table 1. Mean (±SD) of intramuscular and serum biomarkers in response to low-load (LL) and high-load (HL) resistance exercise.

Condition	Pre-Exercise	3h Post	24h Post
Myostatin mRNA Expression (fold-change)			
LL	1.00 (±0.00)	1.41 (±0.51)	2.65 (±0.46) *
HL	1.00 (±0.00)	2.59 (±0.67) *	3.04 (±0.82) *
Muscle Myostatin (ng/mg)			
LL	16.45 (±7.87)	17.74 (±8.47)	18.05 (±12.67)
HL	14.79 (±11.15)	18.71 (±10.93)	20.75 (±10.76) *
Serum Myostatin (ng/ml)			
LL	113.17 (±38.91)	101.42 (±38.63)	93.39 (±46.48)
HL	117.21 (±53.50)	112.05 (±60.37)	75.31 (±51.23) *
Total Smad 2/3 (Absorbance at 450 nm)			
LL	0.32 (±0.02)	0.45 (±0.03)	0.52 (±0.03)
HL	0.35 (±0.04)	0.56 (±0.06)	0.62 (±0.05)
Muscle Decorin (pg/mg)			
LL	511.77 (±186.96)	805.56 (±239.97)	1165.73 (±439.91) *
HL	583.44 (±184.41)	1171.22 (±350.24)	1588.73 (±429.86) *
Serum Follistatin (pg/ml)			
LL	1,468 (±345.92)	1,582.84 (±381.63)	1,978.22 (±531.06) *
HL	1,523 (±367.02)	1,673.68 (±412.84)	1,869,78 (±476.18) *

* Indicates a significant main effect for time ($p < 0.05$); myostatin mRNA ($p = 0.041$), muscle myostatin ($p = 0.029$), serum myostatin ($p = 0.036$), muscle decorin ($p = 0.025$), serum follistatin ($p = 0.034$)

revealed that there was a significant increase in myostatin mRNA expression at 24h post-exercise ($p = 0.032$) in the LL condition and 3h ($p = 0.044$) and 24h ($p = 0.003$) post-exercise in the HL condition.

Skeletal muscle decorin, myostatin, and Smad 2/3

For muscle decorin (Table 1), no significant main effect of condition ($p = 0.218$, $\eta^2 = 0.026$) or time x condition interaction ($p = 0.359$, $\eta^2 = 0.037$) was observed. However, a significant main effect for time ($p = 0.025$, $\eta^2 = 0.826$) was observed. Pairwise comparisons revealed that there was a significant increase in decorin concentrations at 24h post-exercise compared to pre-exercise ($p < 0.001$) in both exercise conditions. For muscle myostatin (Table 1), no significant main effect of condition ($p = 0.299$, $\eta^2 = 0.439$) or time x condition interaction ($p = 0.455$, $\eta^2 = 0.334$) were observed. However, a significant main effect for time ($p = 0.029$, $\eta^2 = 0.765$) was observed. Pairwise comparisons revealed that there was a significant increase in muscle myostatin at 24h post-exercise compared to pre-exercise ($p < 0.001$) in the HL condition only. No significant main effect of time ($p = 0.226$, $\eta^2 = 0.223$), condition ($p = 0.799$, $\eta^2 = 0.367$), or time x condition interaction ($p = 0.872$, $\eta^2 = 0.279$) was observed for Smad 2/3 (Table 1).

Serum myostatin and follistatin

No significant main effect of condition ($p = 0.123$, $\eta^2 = 0.427$) or time x condition interaction ($p = 0.745$, $\eta^2 = 0.095$) were observed for serum myostatin (Table 1). However, a significant main effect for time ($p = 0.036$, $\eta^2 = 0.717$) was observed. Pairwise comparisons revealed that there was a significant decrease in serum myostatin concentrations at 24h post-exercise compared to pre-exercise ($p < 0.001$) in both conditions. For serum follistatin (Table 1), no significant main effect of condition ($p = 0.7428$ $\eta^2 = 0.024$) or time x condition interaction ($p = 0.551$, $\eta^2 = 0.067$) was observed. However, a significant main effect for time ($p = 0.034$, $\eta^2 = 0.741$) was observed. Pairwise comparisons revealed that there was a significant increase in serum follistatin concentrations at 24h post-exercise in both conditions compared to pre-exercise ($p < 0.001$).

Discussion

With volume equated, within skeletal muscle compared to LL RE we showed that HL RE preferentially increased muscle myostatin mRNA at both 3h and 24h post-exercise and muscle myostatin protein at 24h post-exercise. However, HL and LL RE both significantly increased muscle decorin levels, but had no significant impact on the levels of Smad 2/3. In addition, in circulation, we showed significant decreases in myostatin and increases in follistatin at 24h post-exercise in both HL and LL. Collectively, our data have allowed us to hypothesize a possible mechanism (Figure 1) where RE may have created a greater mechanotransductive stimulus in the HL condition to increase muscle myostatin mRNA and protein expression and decorin protein content. This corresponding increase in muscle decorin may be responsible for the increased follistatin and reduced myostatin we observed in circulation. While studies have shown RE decreases myostatin mRNA expression (Wilborn et al., 2009; Schwarz et al., 2016; He et al., 2018), interestingly, we observed significant increases in the HL condition at 3h and 24h post-exercise and at 24h post-exercise for the LL condition. Similarly, it has been shown that a single bout of leg press with 5 sets at a 10RM load increased myostatin mRNA levels by approximately 40% at 48h post-exercise (Hulmi et al., 2009). In addition, another study showed that 12 sets of leg extension exercise at a load corresponding to 12RM resulted in a significant 2-fold increase in myostatin mRNA at 5h post-exercise (Hostrup et al., 2018). In rodents, electrically-induced tetanic contractions of the anterior tibialis induced significant increases of approximately 325% in myostatin mRNA expression at 3h post-exercise that remained elevated by approximately 100% 24h post-exercise (MacKenzie et al., 2013). Our present results suggest that myostatin gene expression does not appear to be preferentially responsive to mechanical load, rather it was responsive to the overall effect of RE, conceivably due to a mechanotransductive effect within the ECM. However, it should be noted that we used participants who were previously resistance-trained. Therefore, our results should be interpreted cautiously as the response in non-resistance-trained individuals may reflect a different mechanism where myostatin expression and decorin activity could possibly be responsive to mechanical load.

We also showed that the levels of myostatin in muscle were increased at 24h post-exercise. While there seems to be limited data regarding muscle myostatin concentrations following RE, a study in humans has shown that a single bout of RE with 12 sets of a 10RM load significantly increased muscle myostatin levels at 48h and 72h post-exercise (Snijders et al., 2014). Since we observed a significant increase in myostatin gene expression, the corresponding increase in myostatin protein expression we also witnessed is not an unexpected response.

A number of studies have shown reductions in serum myostatin levels after periods of resistance training involving such experimental scenarios as increased dietary protein intake (Bagheri et al., 2020), whole-body cryostimulation (Jaworska et al., 2020), and Type 2 diabetes (Rad et al., 2020). There appears to be a paucity of data showing the effects of a single bout of RE on serum myostatin. However, in the present study we showed a significant reduction in serum myostatin at 24h post-exercise. It is conceivable that this reduction in circulating myostatin was due to the corresponding increase in intramuscular decorin which may have resulted in the binding and immobilization of myostatin in the ECM. Tensile stresses are generated during RE. In essence, regarding mechanotransduction, tensile stress likely constitutes a deformation trigger on mechano-sensing muscle proteins during RE and it is conceivable that HL RE, which imposes greater tensile stresses with different modes of RE, likely differentially impacts specific mechano-sensing proteins such as decorin and integrins that tether the ECM to focal adhesion complex proteins (Olson and Nordheim, 2010).

In circulation, myostatin binding to the transmembrane ACTIIB receptor up-regulates the intramuscular Smad signaling cascade leading to the phosphorylation of Smad 2 at specific finger-region residues (Lessard et al.,

2018) prior to its dimerization with Smad 3, thereby subsequently activating Smad 2/3 (Zhu et al., 2004). The activated Smad complexes then translocate to the nucleus where they up-regulate myostatin gene expression in addition to facilitating the expression of the FoxO transcription factors towards muscle proteolysis (Zhou et al, 2020]. In spite of the increase in myostatin mRNA expression in the present study, Smad 2/3 phosphorylation was not affected by RE. This coincides with the study in rodents previously discussed (MacKenzie et al., 2013).

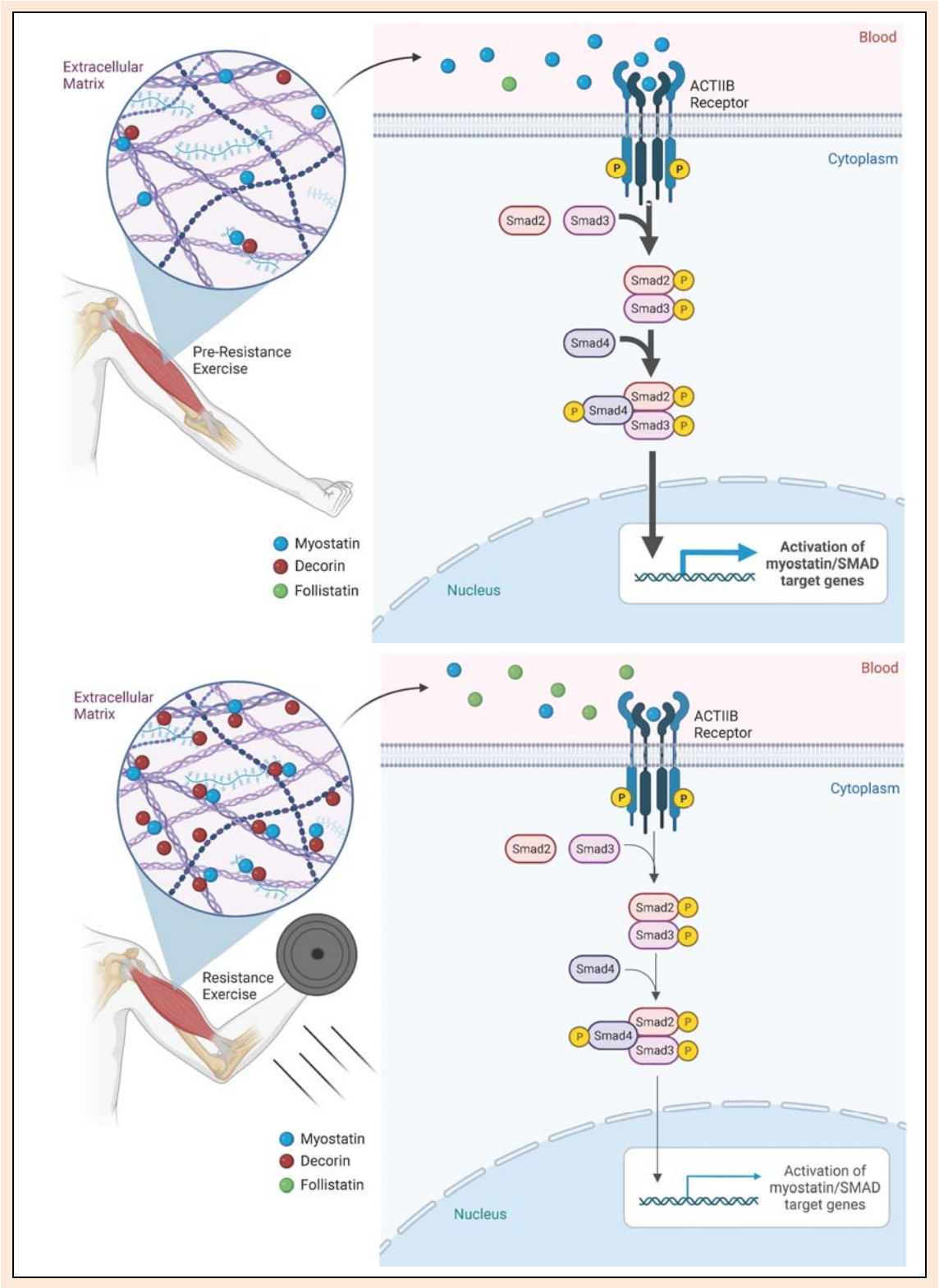

Figure 1. RE-induced up-regulation in myostatin and subsequent increases in decorin bind myostatin in the ECM and reduce Smad 2/3 activity. A hypothetical mechanism of action based on our findings: Based on our results, compared to pre-RE, RE increased myostatin mRNA and protein expression. In addition, muscle decorin was increased which conceivably resulted in more robust binding to myostatin in the ECM which resulted in less myostatin entering the circulation. This decrease, along with a RE-induced increase in circulating follistatin likely resulted in less myostatin binding to the ACTIIB receptor with less subsequent impact on Smad 2/3 signaling.

The ECM of skeletal muscle is essential for the transmission of force during muscle contraction (Kanzleiter et al, 2014). Mechanical loading of the muscle-tendon unit has been shown to up-regulate the autocrine action of stress-responsive growth factors such as TGF-β, connective tissue growth factor (CTGF), and insulin-like growth factor-1 (IGF-1) (Olesen et al., 2006; Heinemeier et al., 2013; Heinemeier et al., 2007A). Mechanical loading and mechanotransduction associated with moderate exercise in humans showed a stimulation of the ECM and increased muscle decorin levels at 6h post-exercise (Heinemeier et al., 2007B). Decorin binds to, and immobilizes, myostatin in the ECM and suppresses myostatin activity and signaling (Kishioka et al., 2008). Decorin has also been shown to increase immediately following a single bout of resistance exercise using an 8RM load (Street, 1983). In the present study, we showed significant increases in muscle and circulating levels of decorin at 24h post-exercise for both exercise conditions.

In vitro data has shown that decorin secreted into the incubation media inhibited exogenous myostatin activity by interfering with myostatin signaling (Kishioka et al., 2008). This could be explained by other *in vitro* data demonstrating that decorin up-regulates follistatin expression (Li et al., 2007; Zhu et al., 2007). Moreover, in *in vivo* settings it has also been shown that high-intensity RE was effective at increasing the levels of circulating follistatin (He et al., 2018). In the present study, we showed both muscle decorin and serum follistatin to be increased at 24h post-exercise. Since the activity of myostatin in circulation is regulated by various proteins, including myostatin propeptide (Theis et al., 2001), follistatin (Amthor et al., 2004), follistatin-related gene, and growth and differentiation factor-associated serum protein-1 (Hill et al., 2002), it is conceivable that decorin may regulate myostatin activity indirectly by stimulating the expression of one or more of these proteins.

Conclusion

The present study shows an increase in both the gene and protein expression of skeletal muscle myostatin in response to RE, particularly that involving HL. As a result, we conclude that RE increases decorin within the ECM which is then able to bind to and subsequently decrease the amount of myostatin that might otherwise enter the circulation for ACTIIB receptor binding and subsequent canonical signaling in muscle through Smad 2/3.

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Key points

- With volume equated, high-load RE increased muscle myostatin mRNA at both 3h and 24h post-exercise and muscle myostatin and decorin protein at 24h post-exercise.
- Significant decreases in serum myostatin and increases in follistatin at 24h post-exercise was observed in both high- and low-load RE conditions.
- RE apparently creates a mechanotransductive mechanism where decorin within the ECM is able to bind to and subsequently decrease the amount of myostatin that might otherwise enter the circulation and negatively impact the response of skeletal muscle to RE.

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