Effect of eccentric action velocity on expression of genes related to myostatin signaling pathway in human skeletal muscle

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ABSTRACT: The aim of this study was to investigate the effects of an acute bout of eccentric actions, performed at fast velocity ($210^{o}\cdot s^{-1}$) and at slow velocity ($20^{o}\cdot s^{-1}$), on the gene expression of regulatory components of the myostatin (MSTN) signalling pathway. Participants performed an acute bout of eccentric actions at either a slow or a fast velocity. Muscle biopsy samples were taken before, immediately after, and 2 h after the exercise bout. The gene expression of the components of the MSTN pathway was assessed by real-time PCR. No change was observed in MSTN, ACTRIIB, GASP-1 or FOXO-3a gene expression after either slow or fast eccentric actions ($p > 0.05$). However, the MSTN inhibitors follistatin (FST), FST-like-3 (FSTL3) and SMAD-7 were significantly increased 2 h after both eccentric actions ($p < 0.05$). No significant difference between bouts was found before, immediately after, or 2 h after the eccentric actions (slow and fast velocities, $p > 0.05$). The current findings indicate that a bout of eccentric actions activates the expression of MSTN inhibitors. However, no difference was observed in MSTN inhibitors' gene expression when comparing slow and fast eccentric actions. It is possible that the greater time under tension induced by slow eccentric (SE) actions might compensate the effect of the greater velocity of fast eccentric (FE) actions. Additional studies are required to address the effect of eccentric action (EA) velocities on the pathways related to muscle hypertrophy.

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INTRODUCTION

Several studies have investigated the effects of different exercise modes on muscle fibre hypertrophy response [17, 57, 4]. A particular interest in eccentric actions (EA), performed at slow and fast velocities, has emerged during the last decade or so [57]. Farthing et al. [17] reported greater muscle hypertrophy after fast EA when compared to slow EA at the proximal, mid, and distal muscle sites. Accordingly, Shepstone et al. [57] demonstrated higher type IIa and IIx fibre hypertrophy after fast than slow EA training. However, the intracellular pathways potentiating protein synthesis after fast compared to slow EA training, and, as a consequence, enhancing skeletal muscle hypertrophy, remain to be clarified.

Although there is some evidence that an acute bout of EA could activate intracellular pathways that modulate muscle protein synthesis [16, 58, 56, 19], little information is available regarding pathways that regulate protein degradation. In this regard, growth and differentiation factor 8 (also known as myostatin [MSTN]), a member of the TGF-beta superfamily, seems to play a key role in controlling muscle wasting [41, 42, 30, 32]. For instance, human studies have reported that resistance training (RT) programmes down-regulate MSTN expression and maximize muscle hypertrophy [31, 29, 33, 54]. These findings suggest that the down-regulation of the MSTN signalling pathway may be associated with overload-induced muscle hypertrophy.

As such, it seems reasonable to hypothesize that acute fast EA are able to produce greater muscle mechanical overload than slow EA, maximizing protein synthesis and muscle mass gain. However, the effects of EA velocity on intracellular pathways related to muscle mass degradation remain to be elucidated. Thus, the aim of this study was to investigate the effects of an acute bout of EA, performed at a fast velocity and a slow velocity, on the gene expression of regulatory components of the MSTN signalling pathway.

MATERIALS AND METHODS

Experimental design

This study was a 2 x 3 repeated measures design, employing two groups (slow EA and fast EA) and time (pre, immediately after and

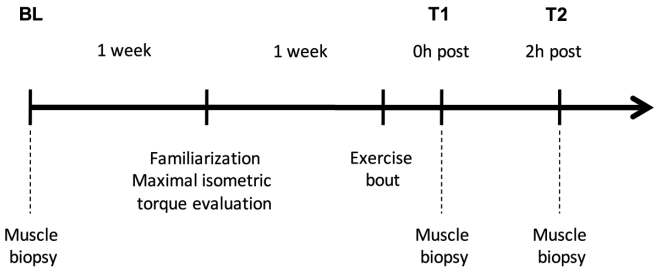

FIG. 1. Experimental design.

2 h after) as factors. Participants performed an acute bout of EA at either a slow or a fast velocity; muscle biopsy samples were taken before, immediately after, and 2 h after the exercise bout (Figure 1).

Subjects

Twenty-four physically active male subjects, without any history of musculoskeletal disorders, participated as volunteers in the study. All subjects were healthy and did not use any drug or nutritional supplement and had not participated in any resistance training programme in the previous 6 months before commencement of the study. Due to personal reasons, 4 subjects did not complete the experimental protocol.

Initially, the maximal voluntary isometric actions (MVIA) were assessed and participants were ranked in quartiles according to their MVIA. Then, participants from each quartile were randomly assigned to either a slow $20°{\cdot}s^{-1}$ (SE – n = 11, 77.2 ± 10.5 kg, 1.76 ± 0.06 m, and 25.36 ± 5.0 years) or fast $210°{\cdot}s^{-1}$ (FE – n = 9, 76.3 ± 9.6 kg, 1.77 ± 0.03 m, and 26.40 ± 4.3 years) eccentric group. A 2-sample t-test assured similar peak torque values between groups ($p > 0.05$). This study was performed according to the Declaration of Helsinki [23] and was approved by the Research Ethics Committee of the Institute of Biomedical Sciences, University of São Paulo, Brazil. All subjects were informed of the inherent risks and benefits before signing an informed consent form.

Maximal voluntary isometric actions (MVIA)

An isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, NY, EUA) was used to assess MVIA. A general warm-up consisting of 5 min in a treadmill at 9 km/h, followed by lower limb stretching exercises, was conducted. Subjects were seated in the dynamometer chair with a 90º hip flexion. Straps were used to prevent unwanted body movements. The subjects were also instructed to keep their arms crossed at chest height. Individual positioning on the dynamometer was recorded for future usage. The knee of the dominant leg was then positioned near the apparatus lever arm and the anatomical axis of rotation of this joint was aligned with the dynamometer's rotation axis. The contact pad on the lever arm was positioned at the nearest proximal site to the lateral malleolus. The knee was positioned at a 60º knee extension from the right horizontal (full extension – 0°). Subjects were allowed 3 to 5 trials (5 s each trial), with 3 min intervals between them. Verbal encouragement was provided at every trial to ensure that maximal effort was performed for each muscle action.

Familiarization session

After the MVIA test, a familiarization session took place. After a general warm-up, subjects were positioned on the dynamometer as described before and performed 2 sets of 10 repetitions at $120°{\cdot}s^{-1}$. A non-specific velocity and the usage of the non-dominant leg were implemented to avoid any velocity-specific effects on the hypertrophy signalling pathways.

Experimental session

Subjects performed a general warm-up before each experimental session. The acute exercise bout consisted of 5 sets of 8 repetitions of eccentric knee extensions (0° to 90°) at either slow ($20°{\cdot}s^{-1}$) or fast ($210°{\cdot}s^{-1}$) velocity on the isokinetic dynamometer, interspersed by 3 min rest intervals. The lever arm was passively returned to the initial position at a fixed velocity of $20°{\cdot}s^{-1}$ after each repetition. Peak torque, work, and impulse were calculated for each repetition throughout the sets of EA. A previous study [4] implemented a similar range of velocities as used in the current study. In the above-mentioned study [4] the velocities used in the present study were tested. The main rationale was to use a very different range of velocities.

Muscle biopsy

Unilateral muscle samples were taken from the mid portion of the vastus lateralis of the subjects' dominant leg using the percutaneous biopsy technique with suction. Muscle specimens were freeze-dried, dissected free from any blood and connective tissue and divided in half. The muscle sample was frozen in liquid nitrogen and stored for RT-PCR analysis. The pre-test biopsy (baseline; BL) was performed 2 weeks before the experimental protocol. This period was chosen to allow full recovery from the biopsy procedure. The post-test biopsies (immediately after (T1) and 2 h after (T2)) were done through an incision adjacent (2 to 3 cm) to the pre-test site at 0 and 120 min after the completion of the EA protocol. All biopsies were carried out in the morning, and the last meal prior to the EA protocol was a standard breakfast (~10-15% of total energy intake – shake; 0.8 g of carbohydrates per kg^{-1} of body mass + 0.4 g of protein per kg^{-1} of body mass); served 2 h before the start of the exercise bout.

Gene expression analysis

Reverse transcription

Total cellular RNA was isolated from muscle samples using the Trizol reagent (Invitrogen, USA). The RNA was quantitatively and qualitatively analysed in the current study. For the quantitative evaluation of the RNA samples, the NanoDrop (ND-2000C, Thermo Scientific,

USA) was used, following the manufacturer's instructions. The 260/280 ratio ranged from 1.74 to 1.91, with an average of 1.83. The qualitative evaluation was conducted using an Agilent 2100 Bioanalyzer (Agilent Technologies, Germany), following the specific protocol provided by the manufacturer. The nanochip used for evaluating the RNA quality generates electrophoresis peaks, from which the RNA integrity number (RIN) is determined. The RIN is considered to be the best predictor for assessing the integrity of the RNA molecules. The RIN is a decimal number ranging from 1 to 10, where 1 is attributed to completely degraded samples and 10 to intact RNA samples with very good quality. The RIN ranged from 6.1 to 7.5, with an average of 6.5. Total RNA (1 μg) was used in a reaction containing oligo dT (500 μg/ml), dNTP (10 mM each), 5× first-strand buffer, 0.1 M dithiothreitol and 200 U of reverse transcriptase (SuperScript II, Invitrogen, USA). Reverse transcription was performed at 70°C for 10 min followed by 42°C for 60 min and 95°C for 10 min.

Primer design

Primer sets were designed using Primer Express version 2.0 software (Applied Biosystems, USA) using sequences accessed through GenBank, and were checked for specificity using the Nucleotide-Nucleotide Blast search (Table 1).

Real-time polymerase chain reaction

All samples were analysed in duplicate and the reaction fluorescence emitted was quantified with an ABI Prism 7300 sequence detector (Applied Biosystems, USA) based on current methodology [3]. The amplification analysis was performed with Applied Biosystems sequence detection software. Results were expressed using the comparative cycle threshold (Ct) method described in the manufacturer's User Bulletin no. 2 (Applied Biosystems, USA).

The Ct represents the polymerase chain reaction (PCR) cycle at which an increase in gene reporter fluorescence above a baseline signal can be detected. For each gene of interest, ΔCt values were calculated in all samples as follows: Ct (gene of interest) - Ct (internal control gene). The RPLP0 (ribosomal protein large P0) gene was used as an internal control and, as expected, no change was observed. The calculation of the relative change in the expression levels of one specific gene was performed by subtracting ΔCt of the control group (used as the calibrator) from the corresponding ΔCts of the two experimental groups. The values and ranges given were determined as follows: $2^{-\Delta\Delta Ct}$ with $\Delta\Delta Ct \pm$ S.E.M (S.E.M. is the standard error of the mean $\Delta\Delta$Ct value; User Bulletin no. 2, Applied Biosystems, USA). The final values for samples were reported as fold differences relative to the expression of the control (calculated as $2^{-\Delta\Delta Ct}$), with the control arbitrarily set to 1.

TABLE 1. Target genes and sequence of primers.

mRNA Target	Sequence of Primers (5´ 3´)
RPLP0	F - CGACCTGGAAGTCCAACTAC R - ATCTGCTGCATCTGCTTG
MSTN	F - GACCAGGAGAAGATGGGCTGAATCCGTT R - CTCATCACAGTCAAGACCAAAATCCCTT
FST	F - CCAGGCTGGGAACTGCTGGC R - TCCTCGGTCCACGAGGTGCT
FSTL3	F - TGGTGCTCCAGACTGATGTCA R - CAGTGGACAAGGCCCAAGA
GASP-1	F - GGATTTCTGGAGGCCTGCTT R - TCCAGAGGTGTGAGCCAGTCT
ACTRIIB	F - TACGAGCCACCCCCGACAGC R - AGCGCCCCCGAGCCTTGAT
SMAD-7	F - CAGATACCCGATGGATTTTCTCA R - CCCTGTTTCAGCGGAGGAA
FOXO-3a	F - GAACGTGGGGAACTTCACTGGTGCTA R - GGTCTGCTTTGCCCACTTCCCCTT

F, Forward; R, Reverse; RPLP0; MSTN, Myostatin; FST, Follistatin; FSTL3, Follistatin-like three; GASP-1, Growth and Differentiation Factor-associated Serum Protein-1; ACTRIIB, Activin receptor IIB; SMAD-7; FOXO-3a, Forkhead box 3a.

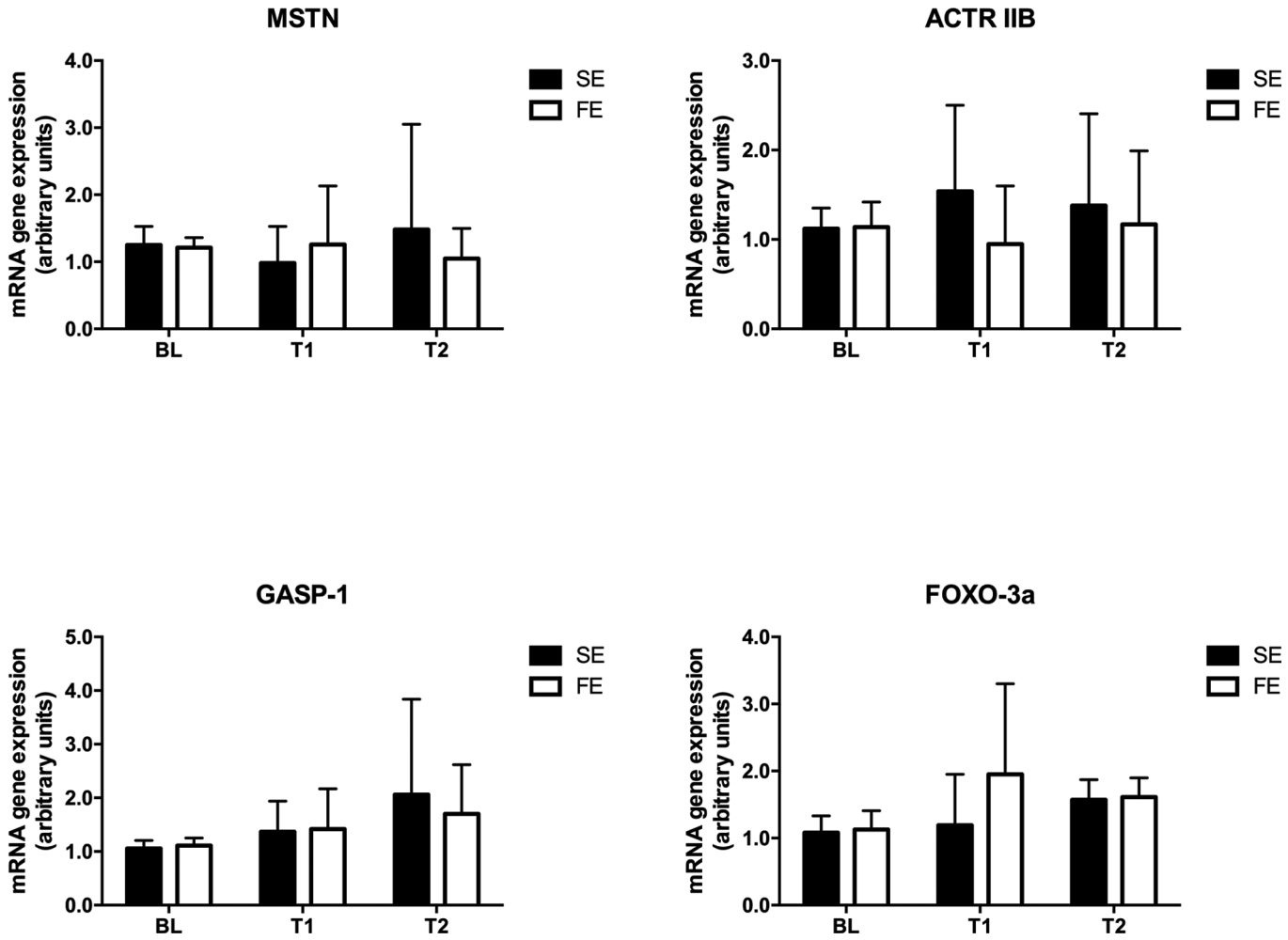

FIG. 2. MSTN, ACTRIIB, GASP-1 and FOXO-3a gene expression is shown for the baseline (BL), slow eccentric (SE) and fast eccentric (FE) conditions in pre and post- exercise (mean ± SD). Data is shown as fold change (ΔΔCT).

Statistical analysis

A mixed model was performed for each gene assessed, having group (slow and fast) and time [baseline (BL), 0 h (T1), and 2 h (T2)] as fixed factors and subjects as a random factor [61]. Additional mixed models using group (slow and fast) and sets (1st, 2nd, 3rd, 4th, and 5th) as fixed factors, and subjects as a random factor, were performed for the variables obtained from the isokinetic dynamometer. Whenever a significant F-value was obtained, a post-hoc test with a Tukey adjustment was performed for multiple comparison purposes. The significance level was set at $p < 0.05$.

RESULTS

Peak torque, work and impulse

There was no difference in peak torque between sets and velocities ($p > 0.05$) (data not shown, see Roschel et al. [51]). Similarly, total work was similar between velocities ($p > 0.05$) (data not shown, see Roschel et al. [51]). On the other hand, the total impulse (453% value, $p < 0.001$) and the impulse per set (454% value, $p < 0.001$) were significantly higher for the SE group (data not shown, see Roschel et al. [51]).

mRNA

No change was observed for MSTN, ACTRIIB, GASP-1 or FOXO-3a mRNA level in either the SE or the FE group ($p > 0.05$) (Figure 2). However, the MSTN inhibitors FST and FSTL3 and SMAD-7 demonstrated a significant increase for both the SE and FE bouts at T2 compared to BL ($p < 0.05$) (Figures 3). No significant difference was found from baseline (BL) value between velocities (SE and FE, $p > 0.05$).

DISCUSSION

In present study, the effect of 2 EA velocities on the gene expressions of components of the MSTN signalling pathway was compared. The

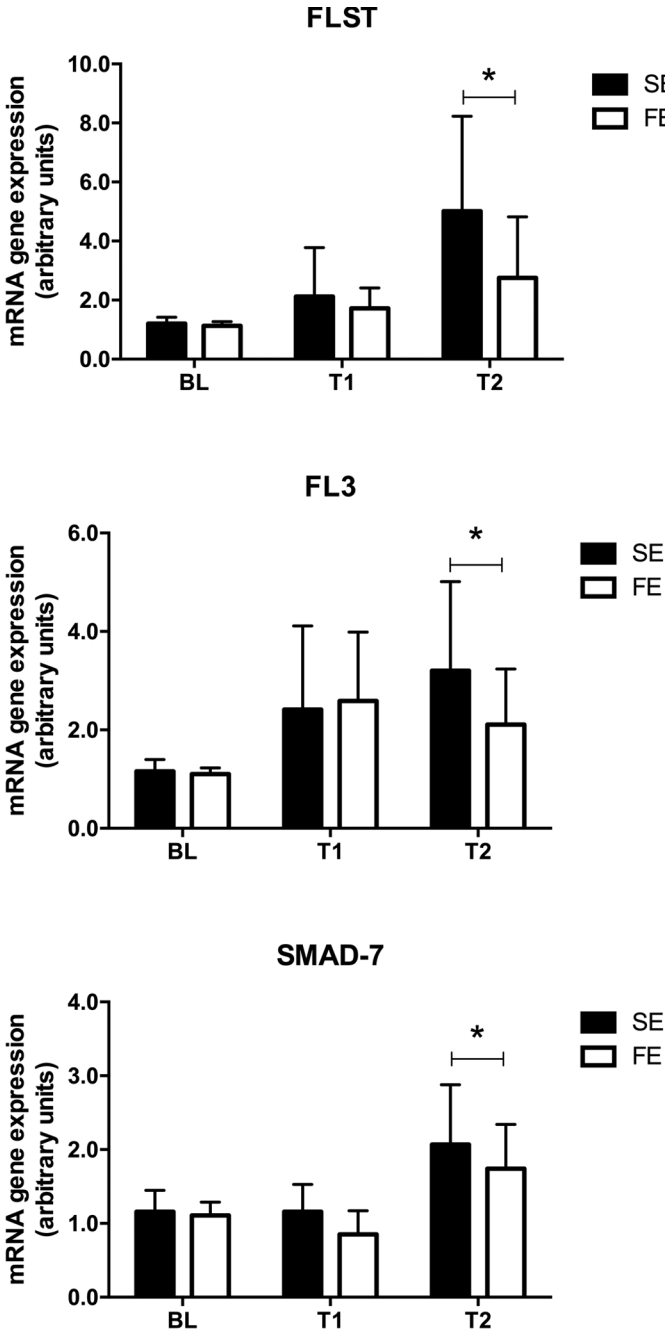

FIG. 3. FST, FSTL3 and SMAD-7 gene expression is shown for the baseline (BL), slow eccentric (SE) and fast eccentric (FE) conditions in pre- and post-exercise (mean ± SD). * Post-test greater than pre-test $p < 0.05$. Data are shown as fold change ($\Delta\Delta$CT).

main finding of this study was the similar increase in gene expression of MSTN inhibitors (e.g. FST, FSTL3 and SMAD-7) produced by the SE and the FE bouts.

Mechanical overload, when associated with resistance exercise (RE), is known to increase protein synthesis by ~ 50% after 4 h and this response peaks after 24 h (~ 115%) [5]. It has also been reported that this increase in protein synthesis remains elevated for up to 48 h after a bout of RE performed by untrained [48] and physically active [14] men and women. This acute increase in the rate of protein synthesis after RE may explain the long-term hypertrophy response; Terzis et al. [59] suggested that the acute activation of the regulatory components of hypertrophy pathways leads to an increase in protein accretion in human skeletal muscle following RT. However, there is little information regarding the effect of mechanical overload induced by EA on pathways that modulate skeletal muscle degradation.

The results of the present study indicate that the EA protocols (SE and FE) successfully induced the expression of FST and follistatin-like 3 (FSTL3). FST and FSTL3 are endogenous inhibitors of activin and other TGF-beta superfamily members [63], including MSTN [35]. This is the first study, to date, to compare the effect of EA (SE vs. FE) on FST and FSTL3 gene expression. Recently, de Souza et al. [10] demonstrated no change in FSTL3 mRNA expression after 8 weeks of interval training, concurrent training or RT. Similarly, Jensky et al. [28] demonstrated no change in the content of FST mRNA at 24 h after a maximal acute EA bout. Later, Jensky et al. [27] compared concentric and EA and found no change in the expression of the FST gene at 8 h after a single exercise bout. In regard to FSTL3, Hulmi et al. [25] found no alteration in gene expression at 48 h after dynamic RE and RT. Recently, no modulation of FST-FSTL3 gene expression was also observed following concurrent strength and endurance training [11, 10]. On the other hand, corroborating the present findings, previous investigations indicate that distinct experimental models able to modulate muscle plasticity successfully increase FST. For example, Willoughby [65] observed a considerable increase (127%) in serum FST-FSTL3 after RT, while Hansen et al. [22] demonstrated that the plasma concentration of FST increased 2-fold at 2 h after RE. In addition, Dalbo et al. [9] observed an increase in FST-FSTL3 mRNA level at 48 h after the first and 24 h after the 3rd sequential bouts of RE. Laurentino et al. [33] noted an increased level of FST and FSTL3 at 48 h after low and high intensity, and low intensity with blood flow restriction RT. A similar outcome was reported by Santos et al. [54] when investigating the effect of RT in patient with inclusion body myositis. Dieli-Conwright et al. [12] observed increased FST and FSTL3 mRNA expression in postmenopausal women 4 h after the acute maximal EA bout with and without hormone replacement. Likewise, the results reported in this study indicate that EA also exerts stimulatory effects on FST and FSTL3 gene expression, which may favour muscle growth. It is possible that the greater FST availability inhibits MSTN signalling, thus maximizing muscle growth.

GASP-1 is a new class of inhibitory binding proteins of TGF-beta; unlike FST and FSTL3, this protein specifically inhibits MSTN [24]. Previous studies have demonstrated increased GASP-1 in serum after RT [21], and after both RT+placebo and RT+creatine supplement intervention [55], besides elevated GASP-1 mRNA content

after RT with blood flow restriction [33]. It seems that exercise is able to affect the expression of GASP-1, regardless of the training mode. Accordingly, Dieli-Conwright et al. [12] demonstrated increased GASP-1 mRNA in the vastus lateralis of postmenopausal women 4 h after an acute maximal EA bout with and without hormone replacement. Interestingly, in the present study, GASP-1 gene expression was not altered by either SE or FE bouts. However, a similar trend toward increased GASP-1 gene expression observed in both experimental groups (polled; $p = 0.06$) may confirm the positive effects of EA on the expression of GASP-1. This increase in GASP-1 could contribute to MSTN latency and skeletal muscle growth. It is noteworthy that the time of muscle biopsy – 4 h [12] vs. 0 and 2 h – might have been a major confounding factor in the absence of a significant change in this study. However, to confirm this supposition, additional studies investigating the time course of GASP-1 expression in response to RE are required.

Another potential inhibitor of MSTN is SMAD-7 [66, 18]. SMAD-7 attenuates the repressive action of MSTN on the growth and development of skeletal muscle [43, 26]. This effect was associated with the growth of the skeletal muscle of rats submitted to chronic stretching, as reported by Aoki et al. [2]. These authors observed high expression of SMAD-7 mRNA compared to MSTN mRNA at 48 and 96 h after the onset of an experimental overload protocol in rats. In humans, Laurentino et al. [33] suggested a positive effect of SMAD-7 on muscle hypertrophy in humans. In this study, a concomitant increase in muscle mass and gene content of SMAD-7 was observed, whereas the expression of MSTN was reduced after RT with and without blood flow restriction [33]. In agreement, de Souza et al. [10] demonstrated an increase in the SMAD-7 mRNA expression after 8 weeks of both training models (RT vs concurrent training) while MSTN mRNA expression was not changed. From this perspective, the increase in SMAD-7 gene expression in the present study might induce MSTN inhibition, leading to skeletal muscle hypertrophy in the long term.

MSTN is a potent negative regulator of muscle mass [41] involved in skeletal muscle atrophy [40, 37]. The effect of EA on MSTN expression has been previously demonstrated in rats. When comparing the molecular response to different EA velocity, Ochi et al. [44] observed a greater MSTN protein content for FE ($180^{o}s^{-1}$) than for SE ($30^{o}s^{-1}$) on days 2 and 7 after the EA bouts. A similar outcome was recently reported by Ochi et al. [46], who observed an increased content of MSTN protein at 24 h after 4 bouts of FE ($180^{o}s^{-1}$), although no change was found for 4 bouts of SE ($30^{o}s^{-1}$). On the other hand, Garma et al. [20] had already demonstrated a decreased MSTN mRNA content at 24 h after 4 EA bouts when evaluating the degree of anabolic response among isometric, concentric and EA bouts. In line with these findings, Ochi et al. [45] demonstrated that content of MSTN protein decreased after 10 sessions (20 days) of EA training. The reduction was lower than that for concentric action training. In humans, the present study showed no change in the expression of MSTN after EA, confirming the findings of Vincent et al. [62], who reported no change in the MSTN mRNA content after acute EA bouts. Contrary to these findings, it has been previously demonstrated that a bout of RE decreases MSTN gene expression [31, 38, 15, 8]. Researching the effects of repeated damage-inducing exercise on expression of myogenic genes, Costa et al. [7] demonstrated a decreased MSTN mRNA level on days 3 and 7 after 6 EA bouts. In turn, Dieli-Conwright et al. [13], investigating the acute response of transcriptions factor, noted a decreased MSTN mRNA content 4 h after the acute EA bouts. Together, these data indicate that the MSTN downregulation may be related to long-term training-induced muscle growth. However, it should be emphasized that acute responses to an exercise bout do not necessarily mimic long-term responses (i.e. the training model). In fact, a previous study reported attenuation of acute exercise responses after several weeks of training [6].

In a long term perspective, Roth et al. [52] and Hulm et al. [25] demonstrated a decrease in the MSTN mRNA content after RT. Taken together, these results indicate that RT may have led to downregulation of MSTN expression, so favouring the observed skeletal muscle hypertrophy [52, 25]. However, Jespersen et al. [29] also observed no effect of chronic RT on MSTN expression. Interestingly, the increase in MSTN gene expression after chronic mechanical loading conditions (e.g. stretching and RT) has also been previously reported. Peviani et al. [47] demonstrated that when skeletal muscle is submitted to short bouts of stretching, an increase in MSTN mRNA level is observed in rats. Furthermore, Willoughby [65] demonstrated an increase in MSTN expression after RT and the occurrence of skeletal muscle hypertrophy, despite the higher MSTN mRNA level observed after training. It seems that MSTN responses to mechanical overload remain to be elucidated. This controversy might be due to differences in the experimental design of the above-mentioned studies (e.g. time of muscle biopsy, overload models implemented, and acute and long-term responses).

ACTRIIB is the membrane receptor that recognizes MSTN [39]. From the interaction between ACTRIIB and MSTN there occurs inhibition of the action of muscle skeletal transcription factors, such as MyoD [60]. As for the ACTRIIB gene, Aoki et al. [2] noted a reduction in the level of ACTRIIB mRNA at 12 and 24 h after the onset of a stretching protocol. However, ACTRIIB mRNA level returned to baseline values at 24 and 96 h, which was associated with substantial longitudinal muscle growth. Previous findings from Dalbo et al. [9], Laurentino et al. [33], de Souza et al. [11], and de Souza et al. [10] showed no change in ACTRIIB mRNA content. These studies analysed distinct RT modes, such as RT with blood flow restriction, RT and concurrent strength and endurance training. Similarly, in the present study, no change in ACTRIIB gene expression was observed. On the other hand, Dieli-Conwright et al. [12] demonstrated that maximal EA induced a significant decrease in ACTRIIB mRNA in groups of postmenopausal women submitted to RE with (or without) hormone replacement. A similar result was observed by Ochi et al. [44], in which ACTIIB protein content was

lower in rats submitted to SE bouts ($30^{o}s^{-1}$) than in the other two groups (rats submitted to FE bouts ($180^{o}s^{-1}$) and the control group). Similarly, in humans, Willoughby [65] observed a reduction in ACTRIIB protein content after RT. Decreased ACTRIIB gene expression has also been demonstrated after RE [25]. However, after 21 weeks of RT this effect was attenuated [25]. Apparently neither an acute nor a chronic mechanical stimulus is able to modulate ACTRIIB gene expression.

FOXO is a transcription factor that is related to protein degradation [36, 53, 58]. In skeletal muscle cells, protein degradation is potentially associated with the 3a isoform. Muscle wasting may be enhanced as FOXO-3a is able to activate the promoter of the gene encoding MSTN [1]. Considering that RT is a potent stimulus for muscle hypertrophy, it is expected that this kind of physical training will negatively modulate FOXO-3a expression. Accordingly, Louis et al. [38] observed a decrease in the expression of FOXO-3a and MSTN after RE. While the gene expression of MSTN decreased over 24 h (1, 4, 8, 12 and 24 h), FOXO-3a expression was reduced only at 8 and 12 h after RE [43]. In rats, previous studies had demonstrated an increased content of FOXO3-a protein after FE bouts ($180^{o}s^{-1}$) but not SE ($30^{o}s^{-1}$) [44, 46, 34]. However, the phosphorylated FOXO-3a content on days 2 and 7 after both FE and SE was decreased [44]. Nevertheless, there was no change in phosphorylated FOXO-3a content either 24 h after the 4^{th} FE and SE bout [46] or on post-EA day 7 [34]. Similarly, Raue et al. [50] and Williamson et al. [64] reported no change in expression of FOXO-3a after RE when performed by young and elderly women. This study also reported no significant change in mRNA content of FOXO-3a after SE and FE actions bouts. In contrast, Dieli-Conwright et al. [13] demonstrated decreased FOXO-3a mRNA expression 4 h after the acute EA bouts. Previously, Roschel et al. [51] reported similar activation of the components of the PI3K/Akt/mTOR pathway after SE and FE acute actions bouts. In line, Rahbek et al. [49], assessing the effect of whey protein hydrolysate (WPH+CHO) vs carbohydrate (CHO) supplementation on molecular response during recovery from muscle-damaging EA, observed an increase in phosphorylated Akt (in the CHO group), mTOR, p70S6K, and rpS6 content (in both WPH+CHO and CHO groups) after 3 h of an acute EA bout and a decrease in the phosphorylated FOXO-3a content (in both WPH+CHO and CHO groups) after 3, 24 and 48 h of an acute EA bout. It is possible that this adaptation (e.g. greater PI3K/Akt/mTOR pathway activity) mitigates the likely transcriptional and proteolytic effects exerted by FOXO-3a.

The current study has some limitations that should be considered when interpreting the outcomes: 1) the small sample size that minimizes statistical power, and 2) the lack of measurement of protein content. Future studies addressing these issues should be conducted to provide additional information regarding the effect of eccentric muscle action velocity on muscle hypertrophy related pathways.

CONCLUSIONS

The current findings indicate that a bout of EA activates the expression of MSTN inhibitors. However, despite the previous reports describing greater muscle hypertrophy responses after a higher velocity EA, no differences were observed for gene expression responses to high and low velocity EA, as employed in the present study. It is possible that the longer time under tension induced by SE might compensate the effect of the greater velocity of FE. Additional studies are required to address the molecular mechanisms (e.g. intracellular pathways) related to muscle hypertrophy in response to different eccentric action velocities.

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