



## OPEN ACCESS

## EDITED BY

Tania Gamberi,  
University of Florence, Italy

## REVIEWED BY

Susan Tsivitse Arthur,  
University of North Carolina at Charlotte,  
United States  
Vanessa Azevedo Voltarelli,  
Hospital Sirio Libanes, Brazil  
Teppeji Fujikawa,  
University of Texas Southwestern Medical  
Center, United States

## \*CORRESPONDENCE

Wilian A. Silveira,  
✉ [wilian.silveira@uftm.edu.br](mailto:wilian.silveira@uftm.edu.br)

<sup>†</sup>These authors have contributed equally to  
this work

RECEIVED 27 July 2023

ACCEPTED 08 January 2024

PUBLISHED 22 January 2024

## CITATION

Abdalla-Silva RL, Zanetti GO, Lautherbach N,  
Schavinski AZ, Heck LC, Gonçalves DAP,  
Kettelhut IC, Navegantes LCC and Silveira WA  
(2024),  $\beta_2$ -Adrenoceptors activation regulates  
muscle trophic-related genes following acute  
resistance exercise in mice.  
*Front. Physiol.* 15:1268380.  
doi: 10.3389/fphys.2024.1268380

## COPYRIGHT

© 2024 Abdalla-Silva, Zanetti, Lautherbach,  
Schavinski, Heck, Gonçalves, Kettelhut,  
Navegantes and Silveira. This is an open-access  
article distributed under the terms of the  
[Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/).  
The use, distribution or reproduction in other  
forums is permitted, provided the original  
author(s) and the copyright owner(s) are  
credited and that the original publication in this  
journal is cited, in accordance with accepted  
academic practice. No use, distribution or  
reproduction is permitted which does not  
comply with these terms.

# $\beta_2$ -Adrenoceptors activation regulates muscle trophic-related genes following acute resistance exercise in mice

Ronaldo L. Abdalla-Silva<sup>1†</sup>, Gustavo O. Zanetti<sup>2</sup>,  
Natalia Lautherbach<sup>3,4</sup>, Aline Zanatta Schavinski<sup>3</sup>, Lilian C. Heck<sup>3</sup>,  
Dawit A. P. Gonçalves<sup>2,5</sup>, Isis C. Kettelhut<sup>4</sup>, Luiz C. C. Navegantes<sup>3</sup>  
and Wilian A. Silveira<sup>1\*†</sup>

<sup>1</sup>Department of Biochemistry, Pharmacology and Physiology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil, <sup>2</sup>Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, <sup>3</sup>Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil, <sup>4</sup>Department of Biochemistry/Immunology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil, <sup>5</sup>Sports Training Center, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Resistance exercise (RE) training and pharmacological stimulation of  $\beta_2$ -Adrenoceptors ( $\beta_2$ -ARs) alone can promote muscle hypertrophy and prevent muscle atrophy. Although the activation of the sympathetic nervous system (SNS) is a well-established response during RE, the physiological contribution of the endogenous catecholamines and  $\beta_2$ -ARs to the RE-induced changes on skeletal muscle protein metabolism remains unclear. This study investigated the effects of the  $\beta_2$ -ARs blockade on the acute molecular responses induced by a single bout of RE in rodent skeletal muscles. Male C57BL6/J mice were subjected to a single bout of progressive RE (until exhaustion) on a vertical ladder under  $\beta_2$ -AR blockade with ICI 118,551 (ICI; 10 mg kg<sup>-1</sup>, i. p.), or vehicle (sterile saline; 0.9%, i. p.), and the gene expression was analyzed in *gastrocnemius* (GAS) muscles by qPCR. We demonstrated that a single bout of RE acutely increased the circulating levels of stress-associated hormones norepinephrine (NE) and corticosterone (CORT), as well as the muscle phosphorylation levels of AMPK, p38 MAPK and CREB, immediately after the session. The acute increase in the phosphorylation levels of CREB was followed by the upregulation of CREB-target genes *Sik1*, *Ppargc1a* and *Nr4a3* (a central regulator of the acute RE response), 3 h after the RE session. Conversely,  $\beta_2$ -AR blockade reduced significantly the *Sik1* and *Nr4a3* mRNA levels in muscles of exercised mice. Furthermore, a single bout of RE stimulated the mRNA levels of the atrophic genes *Map1lc3b* and *Gabarap1* (autophagy-related genes) and *Mstn* (a well-known negative regulator of muscle growth). Unexpectedly, the gene expression of *Igf-1* or *Il-6* were not affected by RE, while the atrophic genes *Murf1/Trim63* and *Atrogin-1/Mafbx32* (ubiquitin-ligases) were increased only in muscles of exercised mice under  $\beta_2$ -AR blockade. Interestingly, performing a single bout of RE under  $\beta_2$ -AR blockade increased the mRNA levels of *Mstn* in muscles of exercised mice. These data suggest that  $\beta_2$ -ARs stimulation during acute RE stimulates the hypertrophic gene *Nr4a3* and prevents the overexpression of atrophic genes such as *Mstn*, *Murf1/Trim63*,

and *Atrogin-1/Mafbx32* in the first hours of postexercise recovery, indicating that the SNS may be physiologically important to muscle adaptations in response to resistance training.

#### KEYWORDS

resistance exercise,  $\beta$ 2-adrenoceptor, skeletal muscle, myostatin, NR4A3

## 1 Introduction

Skeletal muscle is the most abundant and plastic tissue in the human body, comprising approximately 40%–50% of total body mass (Sartori et al., 2021). This tissue is fundamental for locomotion, breathing, thermogenesis, energy expenditure, and glycemic control (Pedersen, 2013; Schnyder and Handschin, 2015). Due to these important functions, muscle wasting and weakness have been associated with reduced quality of life and higher mortality risks of all causes, cancer, chronic obstructive pulmonary disease (COPD) and aging (Mathur et al., 2014; Santana et al., 2019; Zhou et al., 2023). Despite these alarming findings, there is no effective pharmacological treatment for preventing muscle atrophy in such conditions.

Resistance exercise (RE) training, on the other hand, may increase muscle mass (hypertrophy) and strength (Hornberger and Farrar, 2004; Ruas et al., 2012) and prevent muscle wasting in catabolic situations, such as cancer, glucocorticoids treatment, and sarcopenia (Kim et al., 2016; Padilha et al., 2021; Testa et al., 2022; Macedo et al., 2023). These long-term adaptations to RE training involve acute and transient changes in mRNA expression of various genes in response to each single bout of exercise, including the muscle-derived myokines insulin-like growth factor 1 (IGF-1), myostatin (MSTN) and interleukin 6 (IL-6), among others (Piccirillo, 2019). Such changes in mRNA expression occur between 3h and 12h after an exercise bout and result in a gradual modification in protein content and activity (Egan and Zierath, 2013). Ultimately, the transcriptional and post-translational regulation induced by RE leads to muscle growth, when the rate of protein synthesis exceeds protein degradation over time (Phillips et al., 1997), while reduced rates of protein degradation seem to be necessary for attenuating muscle atrophy (Graham et al., 2021). Although the health benefits of regular RE training on skeletal muscle physiology are well established, the acute molecular mechanism and signaling pathways controlling protein metabolism after a single bout of RE remains elusive.

Most physiological systems of the body (i.e., nervous, cardiovascular, respiratory, endocrine and musculoskeletal) are stimulated during RE (Athanasίου et al., 2023). For example, the RE acutely increases the secretion of catabolic hormones such as glucocorticoids (e.g., cortisol in humans; corticosterone in rodents) and reduces the anabolic hormone insulin (INS) (Kraemer and Ratamess, 2005; Athanasίου et al., 2023), extracellular alterations associated with decreased protein kinase B (PKB)/Akt and mitogen-activated protein kinases (MAPKs) ERK1/2 phosphorylation/activation in skeletal muscle (Osman et al., 2000; Yang et al., 2008; Hu et al., 2009). On one hand, the dephosphorylation of these intracellular mediators of the INS can activate the Forkhead box class O (i.e., FoxO), leading to transcription of several components of the ubiquitin-proteasome (UPS; *Atrogin-1/*

*Mafbx32* and *MuRF1/Trim63*) and autophagic-lysosomal (ALS; *Map1lc3b* and *Gabarapl1*) systems, which degrade most cellular proteins and organelles in skeletal muscle (Yang et al., 2008; Milan et al., 2015). Accordingly, the rate of protein degradation increases immediately after the cessation of an acute RE session, probably to prevent the accumulation of damaged proteins and organelles (Phillips et al., 1997; Grumati et al., 2011; Aweida and Cohen, 2021). On the other hand, decreased Akt and ERK1/2 phosphorylation and their downstream targets GSK-3 and mTOR activities are associated with reduced protein synthesis (Rommel et al., 2001; Figueiredo and Markworth, 2015; Miyazaki and Takemasa, 2017). Despite that, enhanced myofibrillar and mitochondrial protein synthesis appear to be a critical step in the recovery of muscle homeostasis in the hours after acute RE (Wilkinson et al., 2008; Egan and Zierath, 2013). Even though the molecular mechanisms involved in the increase of the protein synthesis are well characterized, the role of the proteolytic systems after a single bout of RE and during adaptive muscle hypertrophy is not completely understood.

As a physiological stress, RE also stimulates the activity of the sympathetic nervous system (SNS), a branch of the autonomic nervous system (ANS), to meet the metabolic demand of active skeletal muscles during exercise (Athanasίου et al., 2023). Indeed, RE stimulates the sympathoadrenal axis to increase the plasma catecholamines levels (epinephrine [EPI] and norepinephrine [NE]) as a function of exercise intensity (Kraemer and Ratamess, 2005; Athanasίου et al., 2023), which return to the basal levels between 5 and 15 min after the end of exercise (Goto et al., 2008; Fatouros et al., 2010). Moreover, it has been shown that muscle sympathetic nerve activity (MSNA) also increases in proportion to the rise in intensity (Katayama and Saito, 2019). Based on that, the adrenergic actions of SNS can be mediated by a hormonal (catecholamines released from adrenal medulla) and/or a neural mechanism (NE secreted from sympathetic nerve ends) in many tissues, including skeletal muscle (Graça et al., 2013; Khan et al., 2016; Rodrigues et al., 2019).

In skeletal muscle, there is a significant proportion of GTP-binding protein stimulatory (Gas)-coupled  $\beta$ -adrenoceptors ( $\beta$ -ARs), predominantly of  $\beta_2$ -subtype ( $\beta_2$ -AR; ~90%), that can be activated by endogenous catecholamines via circulation and/or direct muscle noradrenergic innervation (Elfellah et al., 1989; Lynch and Ryall, 2008; Khan et al., 2016). Upon binding of catecholamines, Gas-coupled  $\beta_2$ -ARs activate adenylyl cyclase (AC), leading to the increase in intracellular second messenger cyclic adenosine monophosphate (cAMP), and subsequent activation of cAMP-dependent protein kinase (PKA) (Lynch and Ryall, 2008). Once activated, the free catalytic subunits of PKA phosphorylate several substrates, including regulatory enzymes (i.e., glycogen synthetase and hormone-sensitive lipase), the ryanodine receptor (RyR), and the cAMP response element-binding protein

(CREB) transcription factor, resulting in enhanced catabolism of energy substrates, muscle contractility, and gene expression, respectively (Altarejos and Montminy, 2011; Berdeaux and Stewart, 2012; Cairns and Borrani, 2015). In contrast to these acute effects of catecholamines, chronic treatment with  $\beta_2$ -AR agonists promotes muscle growth and can be used for attenuating muscle wasting (Kim and Sainz, 1992; Mersmann, 1998; Yimlamai et al., 2005; Harcourt et al., 2007; Ryall et al., 2007). The molecular mechanisms by which  $\beta_2$ -AR agonists induce these anabolic and anti-catabolic effects on skeletal muscle are not completely understood but may involve canonical (i.e., cAMP/PKA) and/or non-canonical signaling pathways (Lynch and Ryall, 2008; Gonçalves et al., 2012; Silveira et al., 2020). Indeed, it has been postulated that  $\beta_2$ -AR agonists stimulate muscle hypertrophy and prevent muscle atrophy by activating Akt/mTOR and Akt/FoxO pathways, respectively, via the subunits  $\beta\gamma$  of GTP-binding inhibitory protein-coupled  $\beta_2$ -AR (G $\alpha$ -G $\beta\gamma$ ) (Xiao, 2001; Kline et al., 2007; Lynch and Ryall, 2008; Koopman et al., 2010). Moreover,  $\beta_2$ AR-induced skeletal muscle growth and strength might also be mediated by  $\beta$ -arrestin 1, a multifunctional adaptor protein that function as a signal transducer required for the activation of other signaling molecules in muscle cells, such as p38 MAPK, ERK1/2, CREB and AMPK (Kim et al., 2018). On the other hand, the canonical signaling pathway cAMP/PKA seems to be important to maintain muscle mass under basal conditions by restraining FoxO transcriptional activity and the expression of multiple components of the UPS and ALS (Silveira et al., 2020). Moreover, these  $\beta_2$ -AR agonists also induce a shift in muscle composition from slow to fast fiber type (Kim et al., 2018; Gonçalves et al., 2019). Thus, although RE training and pharmacological stimulation of  $\beta_2$ -ARs alone promote muscle remodeling and adaptation, the physiological role of endogenous catecholamines and  $\beta_2$ -ARs in mediating muscle anabolism to RE remains unknown. Therefore, the current study was undertaken to investigate the effects of the  $\beta_2$ -ARs blockade on the acute molecular changes induced by a single bout of RE in rodent skeletal muscles. By using a pharmacological approach, we demonstrate that the  $\beta_2$ -AR activation during RE is required to induce *Nr4a3* and to prevent the overexpression of atrophic genes such as *Mstn*, *Murfl1/Trim63*, and *Atrogin-1/Mafbx32*.

## 2 Material and methods

### 2.1 Animals and treatments

C57Bl6/J mice (8-week-old male mice, ~20–23 g) were housed in a room with a 12–12 h light–dark cycle (starting at 6:00 a.m.) and given free access to water and a normal lab chow diet (Nuvilab-CR1; Nuvital, Curitiba, PR, Brazil) until the start of the experiments. The sample size used for each experiment is indicated in the figure legends. The animals were randomly divided into two or three groups as follows: control (CON) and resistance exercise (RE) (experiment one); or CON, RE and RE under  $\beta_2$ -AR blockade (ICI + RE) (experiment two). Mice from ICI + RE group were treated intraperitoneally (i.p.) with 10 mg kg<sup>-1</sup> of the selective  $\beta_2$ -AR antagonist (ICI 118,551; Sigma-Aldrich) 30 min before the RE bout

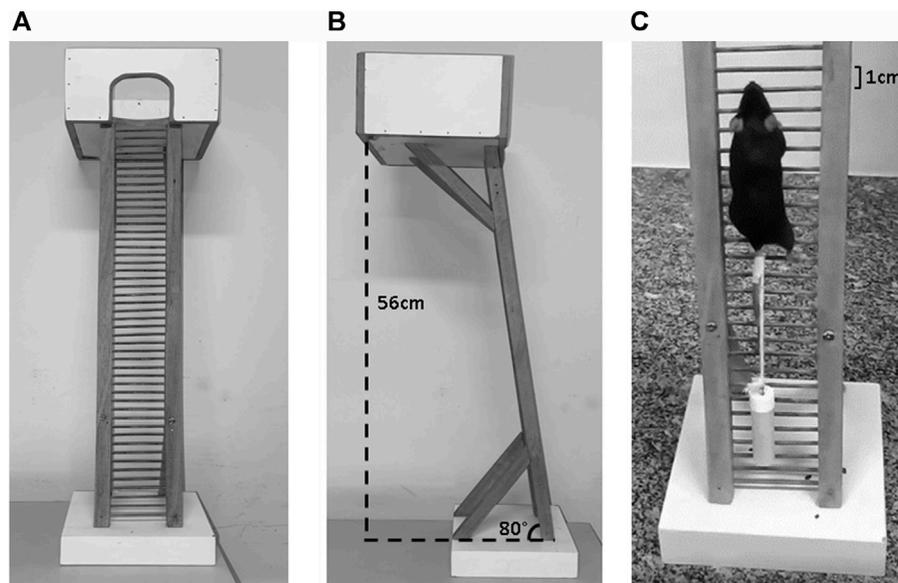
(Azevedo Voltarelli et al., 2021), whereas mice from CON and RE groups were injected with vehicle (0.9% saline). Immediately after (0 h) and 3 hours (3 h) after exercise cessation, the animals were anesthetized with isoflurane by using the open-drop technique (Risling et al., 2012) and euthanized by decapitation with the aid of a rodent guillotine for collecting blood and muscle samples (experiment one) or by cervical dislocation when collecting muscle samples only (experiment two). For this, when the animal was fully anesthetized, the thumb and index finger were positioned on either side of the neck at the base of the skull. With the other hand, the base of the tail was quickly pulled, causing separation of the cervical vertebrae from the skull. After euthanasia, muscles were rapidly harvested, weighed, frozen in liquid nitrogen, and stored at –80°C until further analyses. All experiments were performed accordingly to the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation and were approved by the Ribeirão Preto Medical School from the University of São Paulo - Commission of Ethics in Animal Research (no. 207/2014).

### 2.2 Experimental design

As above mentioned, the present study was divided into two experiments. Experiment one was performed to test whether the acute RE protocol would be able to increase sympathetic activity. For that, the animals were randomly divided into two groups: 1) CON and 2) RE groups. After the familiarization period with the ladder apparatus for RE and the maximum voluntary carrying capacity (MVCC) test, mice from the RE group were subjected to a single bout of RE (see *Resistance Exercise Protocol* section). Immediately after (0 h) the exercise session, the animals were anesthetized with isoflurane and euthanized by decapitation for collecting blood and muscle samples in order to analyze plasma catecholamines by HPLC and muscle proteins content, and phosphorylation by immunoblotting. Experiment two was performed to determine the contribution of  $\beta_2$ -ARs to skeletal muscular transcriptional response following a single bout of RE. For that, the animals were randomly divided into three groups: 1) CON, 2) RE, and 3) ICI + RE. After the familiarization period with the ladder apparatus for RE and MVCC test, mice from RE and ICI + RE groups were submitted to the RE protocol. Three hours (3 h) after the exercise session, the animals were anesthetized with isoflurane and euthanized by cervical dislocation, and muscles were rapidly harvested and stored at –80°C until qPCR and Western blotting analysis. The 3 h period was chosen because rodents subjected to a single bout of exercise showed transient changes in mRNA levels in the first hours of recovery, typically between 3h and 12 h after exercise cessation (Egan and Zierath, 2013; Egan and Sharples, 2023). Mice from the CON group remained confined in their cages during both experimental protocols, except when all animals were submitted to the familiarization period with the ladder apparatus for RE and MVCC test.

### 2.3 Resistance exercise protocol

The resistance exercise (RE) protocol was based on previous reports (Frajacomo et al., 2015; Padilha et al., 2019) and consisted of



**FIGURE 1** (A) The apparatus for resistance exercise (RE) in mice. (B) At the top of the ladder is a housing chamber where the mice are allowed to rest between climbs (60 s). (C) A mouse is shown climbing a 56 cm, 80° incline ladder with the weight attached to the tail.

ladder climbing (80° incline; 1.0 cm space between steps, and 56 cm height) using progressive overload (see **Figures 1A–C**). Initially, all mice were familiarized to voluntary climbing the ladder over five consecutive days. For this, mice were positioned on the base of the ladder and encouraged to climb by pushing them to initiate movements. These stimuli were applied until each animal was able to complete an entire climb (**Padilha et al., 2017**). Forty-8 h after the last familiarization session, the animals were submitted to MVCC test, which consisted of carrying loads equivalent to 50%, 75%, 90%, and 100% of body mass attached to the tail of each mouse. When the animal reached the covered chamber (**Figure 1A**), an interval of 60 s was given between each climbing bout. After climbing the ladder with 100% of its body mass, a 3 g load was added for the next climbs until a load that incapacitated the animal to fully climb the ladder length. The maximum load (MAX) achieved in the MVCC test was used as a parameter to determine the carrying load during the acute RE session. Finally, 48 h after MVCC test, only the animals from RE and ICI + RE groups were submitted to MAX-based protocol of acute RE (50% of MAX, 75%, 90%, 100%, 100% plus 3 g until failure). Failure was defined as the incapacity to carry the weight even after two successive attempts. All animals remained food deprived from the start of the experiments until euthanasia (about 3.5 h).

## 2.4 Catecholamines and corticosterone levels

Both plasma catecholamines, epinephrine (EPI) and norepinephrine (NE), and the tissue NE from *gastrocnemius* (GAS) and *tibialis anterior* (TA) muscles were assayed as previously described (**Garofalo et al., 1996**) using HPLC (LC-7A, Shimadzu Instruments) with a 5- $\mu$ m Spherisorb ODS-2

**TABLE 1** Antibodies for Western blot.

Protein	Dilution for WB	Manufacturer
phospho-Ser <sup>133</sup> CREB	1:2000	Cell Signaling
phospho-Ser/Thr PKA	1:1000	Cell Signaling
phospho-Thr <sup>172</sup> AMPK	1:1000	Cell Signaling
phospho-Ser <sup>256</sup> FoxO1	1:1000	Cell Signaling
phospho-Thr <sup>24</sup> FoxO1	1:1000	Cell Signaling
phospho-Thr <sup>32</sup> FoxO3	1:1000	Cell Signaling
phospho-Thr <sup>202</sup> ERK1	1:2000	Cell Signaling
phospho-Tyr <sup>204</sup> ERK2	1:2000	Cell Signaling
phospho-Thr <sup>180</sup> /Tyr <sup>182</sup> p38-MAPK	1:1000	Cell Signaling
phospho-Ser <sup>473</sup> AKT	1:1000	Cell Signaling
phospho-Ser <sup>235/236</sup> S6	1:1000	Cell Signaling
phospho-Ser <sup>240/244</sup> S6	1:1000	Cell Signaling
phospho-Ser <sup>21</sup> GSK3 $\alpha$	1:1000	Cell Signaling
phospho-Ser <sup>9</sup> GSK3 $\beta$	1:1000	Cell Signaling
phospho-Thr <sup>286</sup> CaMKII	1:1000	Cell Signaling
Atrogin-1	1:1000	Santa Cruz
$\alpha$ - Tubulin	1:2000	Santa Cruz
$\beta$ -actin	1:2000	Santa Cruz

reversed-phase column (Sigma-Aldrich). Serum corticosterone levels were measured by specific radioimmunoassay as previously described (**Durlo et al., 2004**).

TABLE 2 Oligonucleotide primers used for qPCR analysis.

Gene	Forward primer (5'-3')	Reverse primer (3'-5')
<i>Ppargc1a</i>	AATCCAGCGGTCTTAGCACT	TTTCTGTGGGTTTGGTGTGA
<i>Sik1</i>	TCCACCACCAAATCTCACCG	GTTTCGGCGCTGCCTCTTC
<i>Map1lc3b</i>	CGTCCTGGACAAGACCAAGT	TCCGTCTTCGCTTCATAGG
<i>Gabarapl1</i>	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCCATGGTAG
<i>Ctsl</i>	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCCATGGTAG
<i>Bnip3</i>	TTCCACTAGCACCTTCTGATGA	GAACACCGCATTACAGAACAA
<i>Nr4a1</i>	AGCTTGGGTGTGATGTTCC	AATGCGATTCTGCAGCTCTT
<i>Nr4a3</i>	TGCAGAGCCTGAACCTTGAT	TTAACCATGTCGCTCTGTG
<i>Mstn</i>	TTGCAAAATTGGCTCAAACAGC	AAGGGATTACAGCCATCTTCTC
<i>Igf-1</i>	CTCAGACAGGCATTGTGGATGAGT	GGTCTGTTCCTGCACCTTCCTCT
<i>Il-6</i>	TAGTCCTTCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Murf1</i>	TGTGCAAGGAACACGAAG	TGAGAGATGATCGTCTGC
<i>Atrogin-1</i>	GCAGAGAGTCGGCAAGTC	CAGGTCGGTGATCGTGAG
<i>Adr2</i>	GAGCACAAAGCCCTCAAGAC	TGGAAGGCAATCCTGAAATC
<i>Myh1</i>	ACCTTGTGGACAAACTGCAA	AGCTTGTGACCTGGGACTC
<i>Myh2</i>	TTGGTGGATAAACTCCAGGC	CAGCTTGTGACCTGGGACT
<i>Myh4</i>	ACAGACTAAAGTGAAAGCCTACAA	CACATTTGTGATTTCTCCTGTAC
<i>Myh7</i>	AGCAGGAGCTGATTGAGACC	TGTGATAGCCTTCTTGGCCT
<i>Rpl39</i>	TCCTGGCAAAGAAACAAAAGC	TAGACCCAGCTTCGTTCTCCT

## 2.5 Western blotting analysis

GAS and TA muscles were homogenized in RIPA buffer containing 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM sodium orthovanadate, 5  $\mu\text{g mL}^{-1}$  of aprotinin, 1  $\text{mg mL}^{-1}$  of leupeptin, and 1 mM phenylmethyl-sulfonyl fluoride (PMSF). Lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using antibodies listed in Table 1. Primary antibodies were detected using peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence (ECL) reagents on ChemiDoc XRS + System (Bio-Rad). Band intensities were quantified using the software ImageJ/Fiji (version 1.52 d, National Institutes of Health, United States).

## 2.6 Real-time qPCR

The analysis of qPCR was performed at the Laboratory of Metabolism Control from Ribeirão Preto Medical School (University of São Paulo). After the exercise protocol described above, GAS muscles were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Total RNA was extracted from muscle using TRIzol (50 mg of muscle was added to 0.5 mL of TRIzol, Invitrogen<sup>®</sup>). Samples were homogenized in tubes using a TissueLyser II (Qiagen<sup>®</sup>) with 5 mm stainless steel beads for  $2 \times 1$  min cycles at 30 Hz, resting on ice in between. Homogenates were cleared by centrifugation at

10,000  $\times$  g for 5 min at  $4^{\circ}\text{C}$ . RNA extraction was performed according to TRIzol manufacturer's instructions (Invitrogen<sup>®</sup>). RNA was eluted in 50  $\mu\text{L}$  of RNase-free water and stored at  $-80^{\circ}\text{C}$ . RNA samples were treated with DNase I, RNase-free (Thermo Fisher Scientific<sup>®</sup>), to remove genomic DNA contamination. RNA samples were quantitated using NanoDrop One spectrophotometer (Thermo Fisher Scientific<sup>®</sup>), following the manufacturer's instructions. The same device was used to assess the purity of RNA by measuring 260/280 and 260/230 ratios of absorbance values. Samples presenting a 260/280 ratio of  $\sim 2$  and 260/230 ratio of 2–2.2 were accepted as "pure" for RNA. According to the manufacturers' protocols, 1  $\mu\text{g}$  of RNA was reverse transcribed into cDNA using 0.5  $\mu\text{L}$  of SuperScript IV First-Strand Synthesis System (Invitrogen<sup>®</sup>). cDNA was diluted 25-fold with nuclease-free water. For qPCR, the total volume per reaction was 10  $\mu\text{L}$  containing 5  $\mu\text{L}$  of cDNA (2  $\text{ng}/\mu\text{L}$ ), 4.8  $\mu\text{L}$  of PowerUp SYBR Green Master Mix (Thermo Fisher<sup>®</sup>), and 0.2  $\mu\text{L}$  of primers (forward and reverse mixture; 50  $\mu\text{mol/L}$  stock; Table 2). qPCR run on Applied Biosystems<sup>™</sup> 7,500 Real-Time PCR System, using the recommended cycling conditions as follows: a pre-incubation of 2 min at  $50^{\circ}\text{C}$  and 10 min at  $95^{\circ}\text{C}$ , followed by a two-step amplification program of 40 cycles set at  $95^{\circ}\text{C}$  for 15 s (denaturation) and  $60^{\circ}\text{C}$  for 1 min (annealing + extension) and, finally, a dissociation stage set at  $95^{\circ}\text{C}$  for 15 s,  $60^{\circ}\text{C}$  for 1 min and  $95^{\circ}\text{C}$  for 15 s. The last stage was performed to evaluate the quality of qPCR reactions regarding of nonspecific amplification and primer-dimer formation in a dissociation curve for each gene. The amplification specificity for each primer was confirmed by

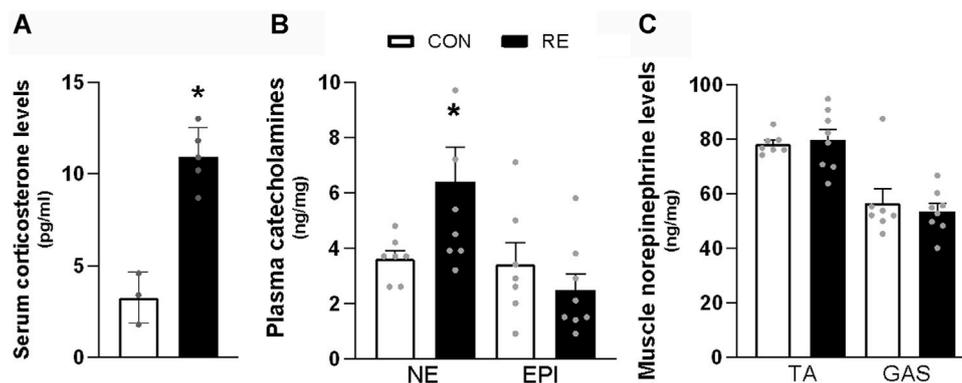


FIGURE 2

Acute effect of a single bout of resistance exercise (RE) on the activation of sympathetic-adrenomedullary and the hypothalamus-pituitary-adrenal (HPA) axis of mice. (A) Serum corticosterone, (B) plasma catecholamines, and (C) content of norepinephrine in *tibialis anterior* (TA) and *gastrocnemius* (GAS) muscles of mice, immediately after (0 h) the RE session. Data are presented as mean  $\pm$  SEM of 7–8 mice. (\* $p \leq 0.05$  vs CON group, Student's t-test). NE, norepinephrine; EPI, epinephrine.

observing the single melt curve peak after the completion of qPCR. Primer sequences were designed utilizing Primer3Plus (<https://www.primer3plus.com/>) in conjunction with OligoAnalyzer 3.1 (<https://eu.idtdna.com/site>) and cross-referenced using the Basic Local Alignment Search Tool program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). A six-point relative standard curve was prepared for each gene by using five-fold serial dilutions of pooled cDNA samples in duplicate. No threshold cycle quantification value for the no template control was detected. The relative expression levels of target genes were calculated using the  $2^{-\Delta\Delta CT}$  method (Schmittgen and Livak, 2008). Data from the target genes were normalized by the expression of *Rpl39* as a reference gene.

## 2.7 Statistics

The distribution and variance homogeneity were tested using Shapiro-Wilk test. The data are expressed as means  $\pm$  standard error mean (SEM). According to each experimental design, non-paired Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* were used in normally distributed variables or those that showed a normal distribution after log transformation. Kruskal–Wallis test followed by Dunn's *post hoc* test was used for non-parametric data. Data were analyzed using JASP 0.16.2 software (GNU Affero GPL v3 license, Department of Psychological Methods, University of Amsterdam) and GraphPad Prism version 7.0 (Graph Pad Softwares, San Diego, CA, United States). The significance level adopted was 5% ( $p \leq 0.05$ ).

## 3 Results

### 3.1 A single bout of RE acutely increases the circulating levels of norepinephrine and corticosterone

Since it has been demonstrated that RE may stimulate a stress response by activating both the sympathetic-adrenomedullary and

the hypothalamus-pituitary-adrenal (HPA) axis (Kraemer and Ratamess, 2005; Athanasiou et al., 2023), we first investigated the acute effect of the ladder climbing-based RE protocol on circulating catecholamines and corticosterone levels, immediately after (0 h) RE cessation. As expected, a single bout of RE acutely increased the serum corticosterone (~3-fold) and plasma NE (~2-fold) levels (Figures 2A, B). On the other hand, plasma EPI levels were not altered (Figure 2A). Moreover, because muscle sympathetic nerve activity (MSNA) has also been reported to increase during exercise (Katayama and Saito, 2019), we evaluated the NE levels in GAS and TA muscles of mice immediately after (0 h) the RE cessation. As shown in Figure 2C, the NE levels in both muscles were unchanged following RE.

### 3.2 A single bout of RE acutely stimulates intracellular pathways involved in energy metabolism and adrenergic signaling in skeletal muscle

Because RE stimulated systemic SNS activity, we examined whether the canonical  $\beta$ -AR signaling pathway (*i.e.*, PKA/CREB) was also activated in the muscles of exercised mice immediately after (0 h) the RE cessation. Although TA muscles were unaffected, a single bout of RE acutely increased the phosphorylation levels of CREB (~30%) in GAS muscles, without altering the phosphorylation levels of other PKA substrates at Ser/Thr (Figures 3A–C). These findings suggest that the activity of transcription factor CREB was increased, but other upstream kinases than PKA may have also been responsible for such an effect. In addition to PKA, the protein kinases AMPK, CAMKII, and p38 MAPK (via mitogen- and stress-activated kinase 1; MSK1), may induce CREB phosphorylation at Ser<sup>133</sup> (Shaywitz and Greenberg, 1999; Bengal et al., 2020). Indeed, we observed a substantial increase in the phosphorylation levels of p38 MAPK (2-fold) and AMPK (2-fold), but not in CaMKII, in GAS muscles (Figures 4A–D). On the other hand, we did not observe any significant difference in phosphorylation status of main signaling pathways controlling muscle protein synthesis (*i.e.*, Akt, ERK1/2,

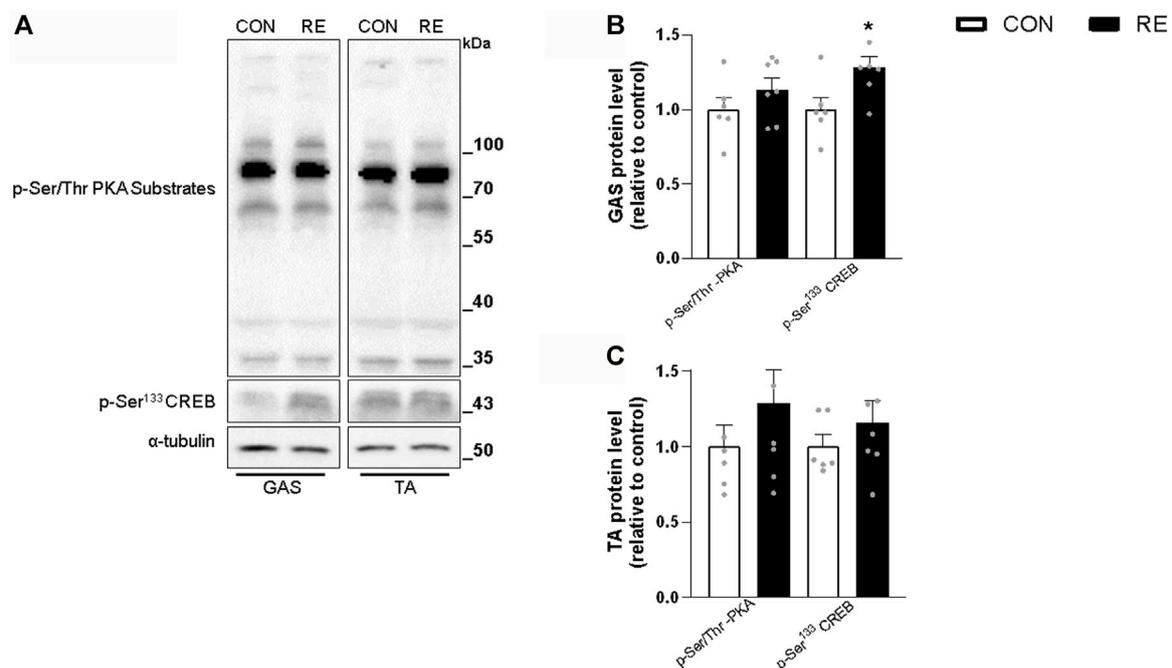


FIGURE 3

Acute effect of a single bout of resistance exercise (RE) on the PKA/CREB signaling pathway in skeletal muscle from mice. (A) Representative western blots of phosphorylation levels of PKA substrates and CREB in muscles of exercised mice, immediately (0 h) after the RE session. (B, C) Densitometric and statistical analysis of the p-Ser/Thr PKA substrates and p-Ser<sup>133</sup> CREB protein content in (B) *gastrocnemius* (GAS) and (C) *tibialis anterior* (TA) muscles. Phosphorylated proteins were normalized to  $\alpha$ -tubulin. Data are presented as mean  $\pm$  SE of 6–7 muscles. (\* $p \leq 0.05$  vs CON group, Student's t-test).

and their downstream targets GSK-3 and S6), and protein degradation (i.e., FoxO1 and FoxO3a), when assessed immediately after exercise cessation (Figures 5A–D). Together, these data indicate that a single bout of RE acutely stimulates plasma NE and CREB phosphorylation in muscles, probably via activation of the p38 MAPK and/or AMPK pathways (Thomson et al., 2008).

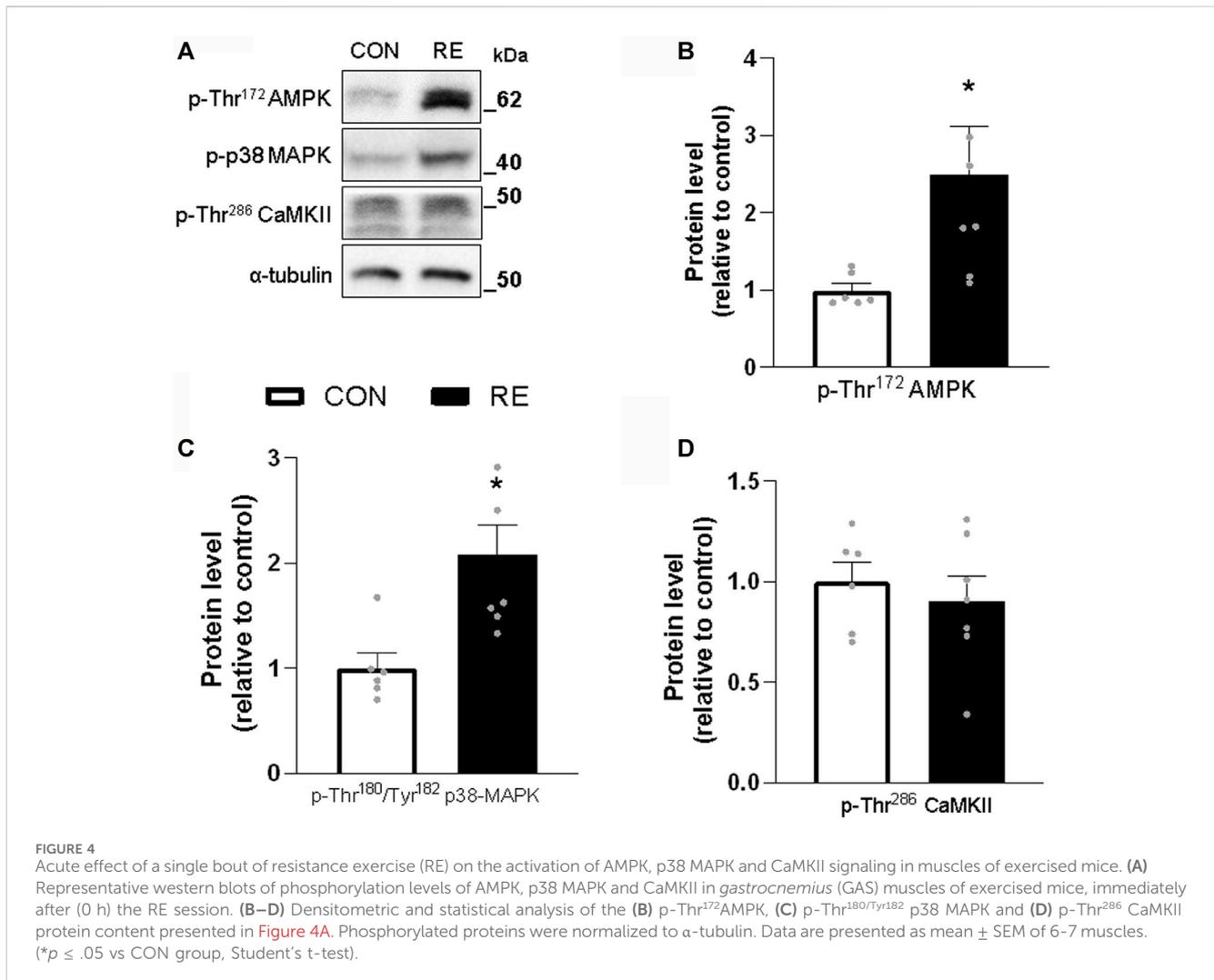
### 3.3 $\beta_2$ -adrenoceptor blockade reduces the CREB-target genes expression in muscles from exercised mice

Because most of the metabolic actions of catecholamines in skeletal muscle are exerted through PKA/CREB signaling pathway (Berdeaux and Stewart, 2012), we hypothesize that the acute increase in plasma NE levels induced by RE would lead to enhanced muscle transcriptional activity of CREB through a  $\beta_2$ -AR-dependent mechanism. To test this hypothesis, we first subjected mice to the maximum voluntary carrying capacity (MVCC) test under  $\beta_2$ -AR blockade with the selective  $\beta_2$ -AR antagonist ICI 118,551 (ICI; 10 mg kg<sup>-1</sup>, i. p., 30 min prior RE session), in order to evaluate whether ICI pre-treatment would affect mice performance. As shown in Table 3, the mean values of the ICI + RE group were very similar to saline treated-group (41.7  $\pm$  1.1 and 42.8  $\pm$  0.7g, for maximal carrying load; 9.0  $\pm$  1.2 and 10.0  $\pm$  0.7 for number of climbs until exhaustion; 290.69  $\pm$  23.7, and 326.8  $\pm$  24.3g for training volume; and 16.0  $\pm$  0.9 and 14.5  $\pm$  1.2min for duration of RE session). Thereafter, we measured the phosphorylation levels of CREB and the

mRNA expression of its specific target genes (i.e., *Sik1*, *Nr4a3*, *Nr4a1*, *Ppargc1a* and *Adrb2*), 3 h after the exercise cessation. As shown in Figure 6, *Sik1* (~3-fold), *Nr4a3* (~6-fold) and *Ppargc1a* (~3.5-fold) mRNA levels were upregulated in GAS muscles 3 h after acute RE, even though CREB phosphorylation returned to the basal values (Figures 7E, F). Performing RE under  $\beta_2$ -AR blockade (ICI + RE) resulted in reduced levels of *Sik1* (Figure 6A) and *Nr4a3* mRNA expression (~50%; Figure 6B), when compared with RE alone. On the other hand, ICI + RE did not alter the mRNA expression of *Ppargc1a* (Figure 6D). Interestingly, the *Adrb2* mRNA was not affected by acute RE or ICI + RE (Figure 6E). Thus, our data suggest that acute RE requires the activation of  $\beta_2$ -AR to regulate the muscle expression of specific CREB-target genes, raising the possibility that at least part of this effect might be directly mediated by plasma NOR.

### 3.4 $\beta_2$ -AR blockade stimulates the expression of atrophic genes in muscles from exercised mice

Because we have shown that catecholamines and  $\beta_2$ -AR agonists induce anti-catabolic effects on skeletal muscle protein metabolism (Graça et al., 2013; Gonçalves et al., 2019) we next investigated the role of  $\beta_2$ -AR activation in the expression of myokines and UPS- and ALS-related genes 3 h after a single bout of RE. As shown in Figure 7A, the mRNA levels of the negative regulator of muscle mass myostatin (*Mstn*; ~70%), but not the growth factor *igf-1* or the cytokine *Il-6*, was increased by a single bout of RE in GAS muscles. Moreover, acute RE only slightly increased the mRNA expression of

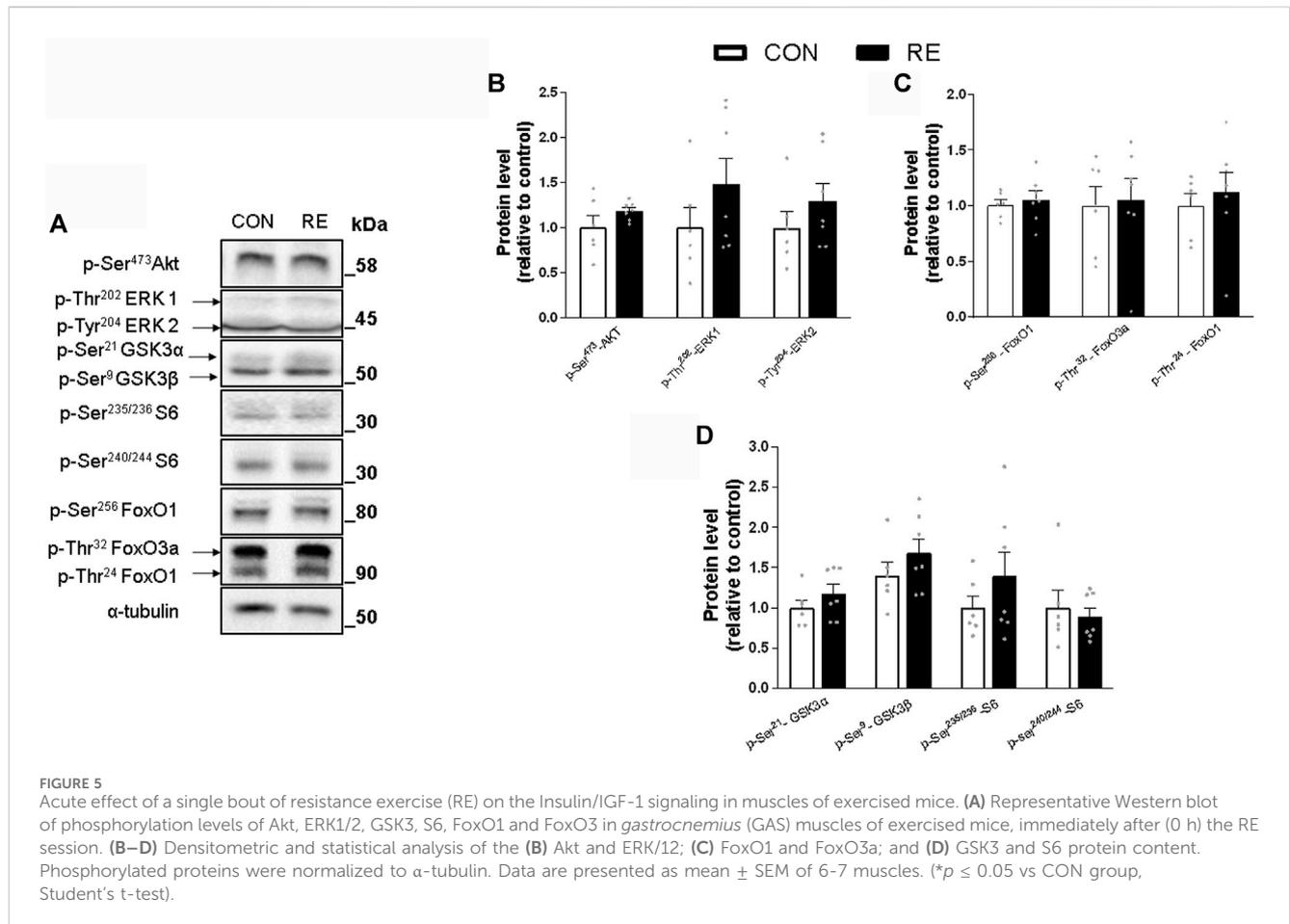


*Map1lc3b* (~30%) and *Gabarapl1* (~50%), an effect that was not observed in *Ctst* and *Bnip3* (Figures 7B, C). In contrast, the  $\beta_2$ -AR blockade increased the gene expression of *MurF1/Trim63*, (~3.5-fold), *Atrogin-1/Mafbx32* (~50%), and *Bnip3* (~40%) (Figures 7B, C), as well as the protein content of Atrogin-1 (~80%) (Figure 7E). Importantly, the increase in *Mstn* mRNA expression was higher (~40%) in GAS muscles from ICI + RE, when compared to RE group (Figure 7A). Because both chronic treatment with  $\beta_2$ -AR agonists and RE training induce a shift in muscle composition from slow to fast phenotype (Mounier et al., 2007), we also measured the mRNA levels of the genes that encodes for myosin heavy chain (MHC) isoforms MHC-I, MHCIIA, MHCIIX and MHCIIB (*Myh7*, *Myh2*, *Myh1* and *Myh4*, respectively). As shown in Figure 7D, neither RE nor ICI + RE affected the mRNA levels of these genes. Altogether, these data indicate that the  $\beta_2$ -AR activation decreases the expression of atrophic genes after a single bout of RE.

## 4 Discussion

The present study provides molecular evidence that  $\beta_2$ -AR activation during a single bout of RE upregulates the *Nr4a3* gene,

a central regulator of the acute RE response, and downregulates the expression of atrophic genes (i.e., *Mstn*, *MurF1/Trim63*, *Atrogin-1/Mafbx32*, and *Bnip3*) in mouse skeletal muscle after the exercise session. These data provide further evidence that the SNS may be physiologically important to muscle adaptations in response to resistance training. Here, we demonstrated that mice performing a single bout of a ladder climbing-based RE protocol showed a marked increase in plasma NE levels, without significant change in plasma EPI. The increase in plasma catecholamines was expected, because exercise is considered a stressful stimulus that stimulates the sympathoadrenal system to meet the physiological demand of active skeletal muscles (Athanasidou et al., 2023). Although both plasma NE and EPI levels continuously increase as a function of RE intensity (Kraemer and Ratamess, 2005), the plasma NE response is greater when compared to EPI (Greiwie et al., 1999). Similar to plasma catecholamines, muscle sympathetic nerve activity (MSNA) gradually increases in proportion to the rise in exercise intensity (Katayama and Saito, 2019). More important, we and others have recently demonstrated that the SNS directly innervates neuromuscular junctions and regulates skeletal muscle metabolism, fiber type composition, and cross-sectional area in normal conditions (Khan et al., 2016; Rodrigues et al., 2019). In the present study,



**TABLE 3** Body mass and maximum voluntary carrying capacity (MVCC) data.

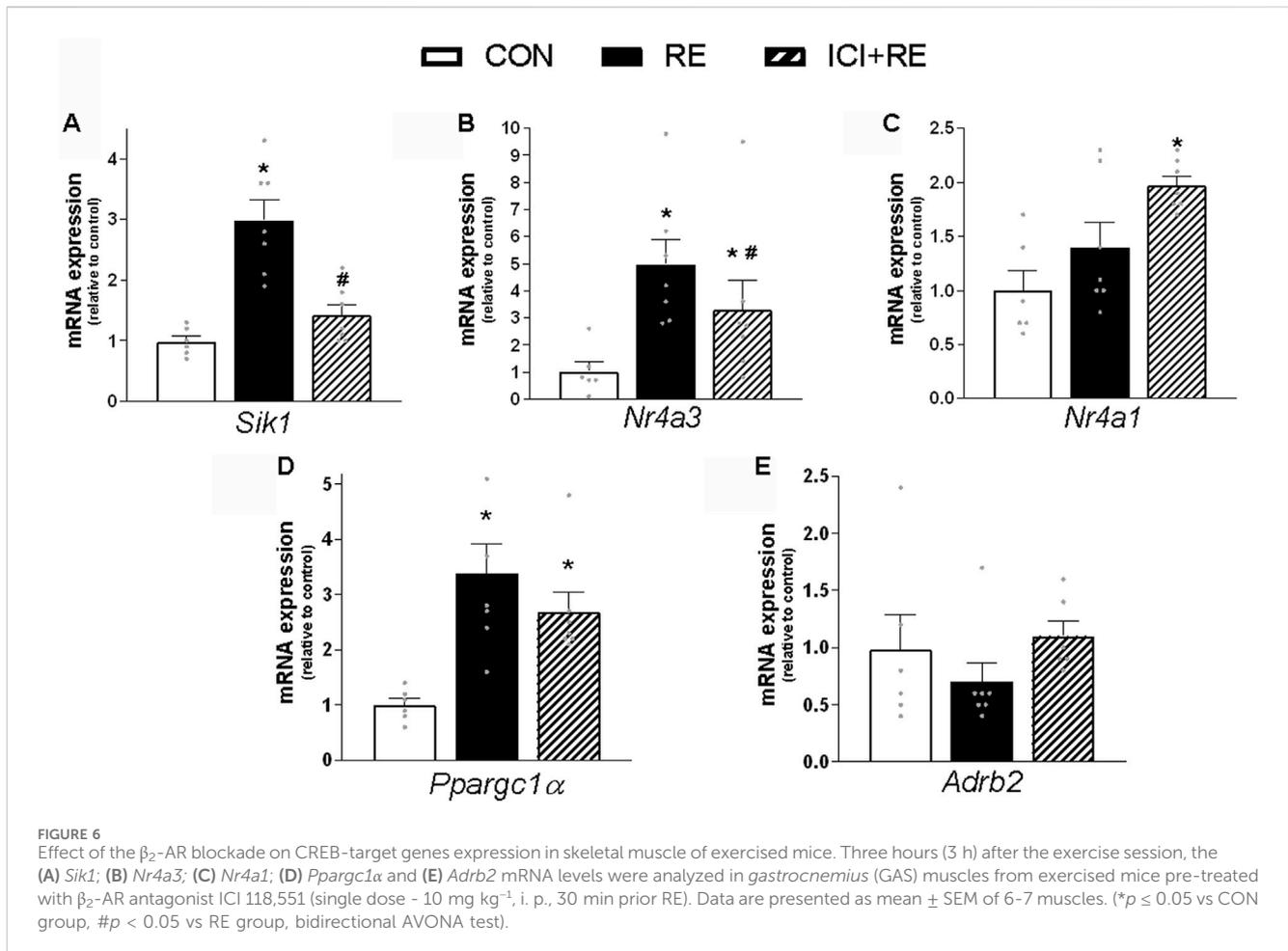
Parameter	CON	RE	RE + ICI
Body mass (g)	21.6 $\pm$ 0.7	21.8 $\pm$ 1.49	21.1 $\pm$ 0.6
Maximal load (g)	43.7 $\pm$ 1.3	41.7 $\pm$ 1.1	42.8 $\pm$ 0.7
Number of climbs	-	9.0 $\pm$ 1.2	10.0 $\pm$ 0.7
Training volume (g)	-	290.69 $\pm$ 23.7	326.8 $\pm$ 24.3
Protocol time (min)	-	16.0 $\pm$ 0.9	14.5 $\pm$ 1.2

All values are presented as means  $\pm$  standard error of the mean; CON: control group; RE: resistance exercise group; ICI + RE: resistance exercise under  $\beta_2$ -AR, blockade group.

however, the increase in muscle content of NE was not observed after the RE session, most likely due to NE diffusion from synapses (mainly from active muscles) into the plasma (Rowell and O'Leary, 1990). Indeed, plasma NE concentration is greatly influenced by the rate of NE diffusion. Therefore, it cannot be excluded that an increase of NE release directly into muscle may have occurred during the RE. The measurement of muscle NE turnover (Graça et al., 2013) and muscle sympathetic denervation (Silveira et al., 2014) would be necessary to determine the direct contribution of muscle sympathetic activity to the molecular changes induced by acute RE.

Independently of the systemic or local origin of NE, we have pieces of evidence that SNS stimulates muscle cells in response to RE. The finding in GAS muscle that a single bout of RE increased the

phosphorylation levels of CREB, a well-established target of PKA, and its specific target genes (i.e., *Sik1*, *Nr4a3*, and *Ppargc1a*), suggests that the canonic  $\beta_2$ -AR/PKA/CREB signaling pathway were stimulated. Consistently, the  $\beta_2$ -AR blockade downregulated the *Sik1* and *Nr4a3* gene expression in GAS muscles from exercised mice. Similar findings were reported by Bruno et al. (Bruno et al., 2014) showing that the expression of *Sik1* mRNA was markedly induced by a high-intensity exercise, and this response was prevented by the pre-treatment with propranolol, a pan  $\beta$ -AR antagonist (Bruno et al., 2014; Goode et al., 2016). However, a few studies have investigated the role of the protein kinase SIK-1 on skeletal muscle physiology. Berdeaux et al. (Berdeaux et al., 2007) proposed that, under adrenergic stimulation with the  $\beta$ -adrenergic agonist isoproterenol, CREB activates the myogenic

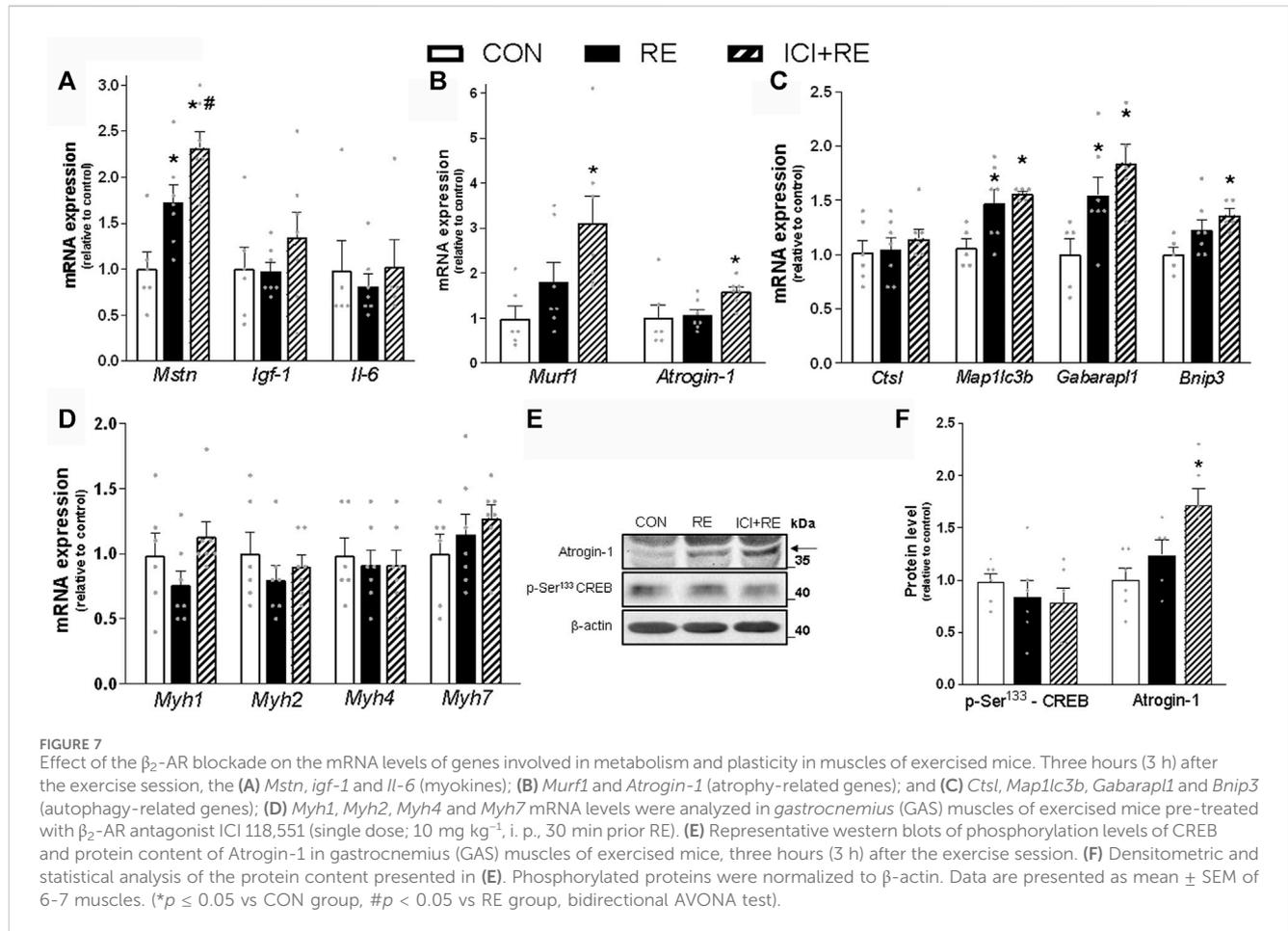


program by increasing the amount of SIK-1 in C2C12 cells and adult skeletal muscles. When activated (dephosphorylated form), SIK-1 phosphorylates class II histone deacetylases (HDACs) and indirectly promotes the expression of myocyte enhancer factor 2 (MEF2) target genes (Berdeaux et al., 2007). Accordingly, the overexpression of SIK1 in muscle cells induces nuclear export of HDAC5 and increases MEF-2C transcriptional activity *in vitro* (Takemori et al., 2009). Despite these data, there is no evidence that SIK-1 regulates muscle protein metabolism in response to exercise.

In contrast to our data, Goode et al. (2016) suggested that the increase in *Nr4a3* mRNA after endurance exercise is independent on  $\beta$ -AR signaling, since its expression was not attenuated by the treatment with the  $\beta$ -AR antagonist propranolol. These conflicting results may be due to differences in the experimental design, including pharmacological treatment (selective versus non-selective  $\beta_2$ -AR antagonist) and type of exercise bout (endurance exercise versus RE). Despite that, recent evidences have shown that the *Nr4a3* gene was robustly induced by a novel RE model in mice (Cui et al., 2020) and by acute RE in human skeletal muscle (Nader et al., 2014). By using gene ontology to highlight pathways activated by inactivity, aerobic versus resistance, and acute versus chronic exercise, Pillon et al. (2020) identified *Nr4a3* as the most exercise- and inactivity-responsive gene, and determined its role in the regulation of mitochondrial function and glucose uptake in response to electrical pulse stimulation in human myotubes *in vitro*. Additionally, it has been

shown that transgenic overexpression of NR4A3 promotes skeletal muscle hypertrophy, oxidative phenotype, and vascularization in mice (Goode et al., 2016). Although it has already been shown that acute exercise (Goode et al., 2016; Cui et al., 2020) and  $\beta_2$ -AR stimulation (Berdeaux et al., 2007; Pearen et al., 2009) alone may induce the expression of *Sik1* and *Nr4a3* mRNA, to our knowledge, this is the first study to demonstrate that the pre-treatment with the selective  $\beta_2$ -AR antagonist downregulates the expression of both genes in muscles from exercised mice. Further studies are needed to reveal the specific role of *Nr4a3* and *Sik-1* expression in mediating the effects of  $\beta_2$ -AR stimulation on muscle protein metabolism in response to chronic RE training.

The reason for differences between TA and GAS muscles in response to acute RE cannot be accounted for in the present study, but exercised muscles are either under the influence of extrinsic (e.g., neural and hormonal) and intrinsic (e.g., mechanical and metabolic) factors that activate/repress several intracellular signaling pathways (Egan and Zierath, 2013). For example, the finding that CREB phosphorylation was induced only in GAS muscles reinforces the hypothesis that acute RE increased the sympathetic activity probably by a metaboreflex-induced mechanism (Fisher et al., 2015), since GAS muscle, but not TA, is highly recruited during climbing movements (Lourenço et al., 2020). On the other hand, the fact that the expression of *Pparg1\alpha* mRNA induced by RE was not affected by  $\beta_2$ -AR blockade raise the possibility that the expression of this transcription



co-activator is under control of intrinsic signals, such as increased intracellular calcium concentration [Ca<sup>2+</sup>]<sub>i</sub>, AMP:ATP ratio and mechanical tension, among others (Egan and Zierath, 2013). Accordingly, these signals may trigger the activation of protein kinases involved in several metabolic processes including AMPK, CaMKII, and p38 MAPK. In agreement with this notion, we demonstrated that the phosphorylation levels of AMPK and p38 MAPK, but not CaMKII, increased in GAS muscle immediately after acute RE. It is important to mention that these kinases seem to be activated by an intensity-dependent manner and may stimulate *Pparg1a* gene transcription by different regulators, such as activating transcription factor2 (ATF2), myocyte enhancer factor 2 (MEF2), CREB, and HDACs (Wojtaszewski et al., 2000; Rose et al., 2006; Akimoto et al., 2008; Egan and Zierath, 2013). Indeed, it has been shown that p38 MAPK can phosphorylate and activate the ATF2 transcription factor, whereas AMPK can directly phosphorylate CREB during exercise, upregulating *Pparg1a* mRNA (Akimoto et al., 2008; Thomson et al., 2008). Taken together, these data suggest that acute RE may upregulate *Pparg1a* expression by multiple muscle intrinsic signals in a  $\beta_2$ -AR-independent manner.

As previously reported (Silvestre et al., 2017), our ladder climbing-based progressive RE increased serum corticosterone (CORT) levels immediately after exercise, and probably reduced insulin (INS) secretion in exercised mice (Raastad et al., 2000; Kraemer and Ratamess, 2005). During catabolic conditions, such as fasting and type 1 diabetes mellitus, the high levels of glucocorticoids associated

with low levels of circulating insulin drive the activation of muscle proteolysis by UPS and ALS in order to support liver gluconeogenesis and the energy requirements of the organism (Hu et al., 2009; Graça et al., 2013). In fact, Hu et al. (2009) have demonstrated that both impaired INS signaling and increased endogenous glucocorticoids are required to stimulate muscle proteolysis by UPS. Thus, reduced activity of the INS/Akt signaling leads to a marked increase in atrophic genes and muscle atrophy via the transcriptional activity of FoxO members (e.g., FoxO1 and FoxO3a) (Schiaffino and Mammucari, 2011). Due to the catabolic nature of the acute exercise, it has been shown that the rate of overall protein degradation and the induction of these atrophic genes also increases in human skeletal muscle immediately after the acute RE, likely to prevent the accumulation of damaged proteins and organelles (Phillips et al., 1997; Louis et al., 2007). However, the effect of a single bout of progressive RE-induced acute molecular changes in rodent skeletal muscle remain poorly explored. The present study shows that a single bout of RE performed until exhaustion did not affect the phosphorylation and activation/inactivation status of the major signaling pathways controlling muscle protein synthesis (i.e., Akt, ERK1/2, and their downstream targets GSK-3 and mTOR), and protein degradation (i.e., FoxO), when assessed immediately after exercise session. These data are controversial because published data have not been consistent on whether these signaling are increased or unaffected in response to acute RE (Bolster et al., 2003; Hamilton et al., 2010; Kido et al., 2016; McIntosh et al., 2023). Again, these conflicting results may be due to differences in the experimental approaches,

including exercise protocol, species (rodents *versus* humans), and time point of sample collecting after the exercise bout, among others.

It is well known that the SNS also contributes to the establishment of a catabolic state during acute exercise since catecholamines stimulates the catabolism of glycogen and intramuscular triglyceride through  $\beta_2$ -AR//PKA/CREB signaling in skeletal muscle (Egan and Zierath, 2013; Bruno et al., 2014). Conversely, we and others have consistently shown that both catecholamines and  $\beta_2$ -AR agonists exert anabolic and anticatabolic actions on protein metabolism, contributing to the maintenance of skeletal muscle mass under basal and catabolic conditions (Navegantes et al., 1999; Baviera et al., 2008; Graça et al., 2013; Khan et al., 2016; Gonçalves et al., 2019; Rodrigues et al., 2019). Thus, we hypothesized that the activation of  $\beta_2$ -ARs during acute RE could inhibit the expression of atrophic genes in the skeletal muscle of exercised mice. Accordingly, we demonstrate that performing acute RE under  $\beta_2$ -AR blockade upregulates the expression of the Ub-ligases *Murf1/Trim63* and *Atrogin-1/Mafbx32*. Although the exact molecular mechanisms cannot be accounted for in the present study, treatment with  $\beta_2$ -agonists has been suggested to inhibit atrophic gene expression by stimulating Akt/FoxO signaling pathway (Gonçalves et al., 2019; Lynch and Ryall, 2008). Our findings are in partial agreement with other studies showing that chemical (Baviera et al., 2008) or surgical (Graça et al., 2013) sympathectomy exacerbates atrophic genes during type I diabetes and fasting, respectively, apparently by a further decrease in Akt stimulation. An alternative possibility to the suppressive action of SNS in atrophic genes during RE is that cAMP/PKA signaling could mediate such an effect. In agreement with this notion, we have recently shown that muscle-specific overexpression of PKI (PKA inhibitor peptide) decreased the phosphorylation levels of CREB and upregulated the transcriptional activity of FoxO and the mRNA expression of *Atrogin-1* and *MURF1*, resulting in myofiber atrophy (Silveira et al., 2020). More important, the muscle-specific activating of PKA by the overexpression of PKA catalytic subunit suppressed FoxO transcriptional activity by multiple mechanisms *in vivo*, in addition to FoxO phosphorylation, acetylation, and nuclear export (Silveira et al., 2020), raising the possibility that this cAMP/PKA/FoxO signaling may have mediate this anti-catabolic action. Further studies are required to confirm this hypothesis.

An interesting finding of this study was that performing acute RE under  $\beta_2$ -AR blockade amplified the expression of *Mstn* mRNA in exercised muscles, suggesting that the  $\beta_2$ -AR signaling restrains *Mstn* overexpression during RE. Since it has been shown that the expression of a constitutively active form of FoxO1 may upregulate *Mstn* mRNA in differentiated C2C12 myotubes (Allen and Unterman, 2007), it is reasonable to speculate that prevention of the activation of PKA/CREB signaling by ICI in the muscles of exercised mice could exacerbate FoxO activity and consequently *Mstn* expression. Additionally, it is also possible that the downregulation of *Nr4a3* expression by  $\beta_2$ -AR blockade could further upregulate the *Mstn* mRNA levels in muscles of exercised mice. This hypothesis is based on findings that reduced endogenous *Nr4a3* mRNA levels induced by stable expression of a NOR-1 (*i.e.*, NR4A3) small interfering RNA in C2C12 cells led to a dramatic upregulation of *Mstn* mRNA expression, whereas *NR4a3* overexpression induced by an expression vector (pSG5-NOR-1) repressed *Mstn* promoter activity and gene expression (Pearen et al., 2009; Goode et al., 2016). Although these findings suggest that  $\beta_2$ -AR/PKA/CREB signaling may contribute to the adaptive anabolic pathways in response to RE, further experiments are needed to confirm all these hypotheses.

In summary, the present data suggest that  $\beta_2$ -AR stimulation during acute RE upregulates the expression of the hypertrophic gene *Nr4a3* and restrains the atrophic genes *Mstn*, *Murf1/Trim63*, *Atrogin-1/Mafbx32* and *Bnip3* in skeletal muscle. These effects may be physiologically important for preventing excessive protein breakdown during muscle contractions and for establishing the anabolic state observed during recovery from acute RE, which may contribute, at least in part, to the muscle adaptations in response to regular RE training.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was approved by the Commission of Ethics in Animal Research from Ribeirão Preto Medical School of the University of São Paulo. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

RA-S: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing—original draft, Writing—review and editing. GZ: Investigation, Methodology, Writing—review and editing. NL: Investigation, Methodology, Supervision, Writing—review and editing. AS: Investigation, Writing—review and editing, Conceptualization, Formal Analysis, Validation. LH: Investigation, Methodology, Writing—review and editing. DG: Conceptualization, Supervision, Writing—review and editing, Data curation, Investigation, Methodology. IK: Conceptualization, Supervision, Writing—review and editing, Data curation. LN: Conceptualization, Data curation, Supervision, Writing—review and editing. WS: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing—original draft, Writing—review and editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants to NL (FAPESP 2019/10420-3); DG (FAPEMIG APQ-01268-21 and APQ-02960-22); IK (FAPESP 2018/10089-2); and LN (PQ CNPq: 302396/2022-5; CAPES/PROEX).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

- Akimoto, T., Li, P., and Yan, Z. (2008). Functional interaction of regulatory factors with the *Pgc-1*  $\alpha$  promoter in response to exercise by *in vivo* imaging. *Am. J. Physiology-Cell Physiology* 295, C288–C292. doi:10.1152/ajpcell.00104.2008
- Allen, D. L., and Unterman, T. G. (2007). Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. *Am. J. Physiology-Cell Physiology* 292, C188–C199. doi:10.1152/ajpcell.00542.2005
- Altarejos, J. Y., and Montminy, M. (2011). CREB and the CRTG co-activators: sensors for hormonal and metabolic signals. *Nat. Rev. Mol. Cell Biol.* 12, 141–151. doi:10.1038/nrm3072
- Athanasiou, N., Bogdanis, G. C., and Mastorakos, G. (2023). Endocrine responses of the stress system to different types of exercise. *Rev. Endocr. Metabolic Disord.* 24, 251–266. doi:10.1007/s11154-022-09758-1
- Aweida, D., and Cohen, S. (2021). Breakdown of filamentous myofibrils by the UPS-step by step. *Biomolecules* 11, 110. doi:10.3390/biom11010110
- Azevedo Voltarelli, V., Coronado, M., Gonçalves Fernandes, L., Cruz Campos, J., Jannig, P. R., Batista Ferreira, J. C., et al. (2021).  $\beta$ 2-Adrenergic signaling modulates mitochondrial function and morphology in skeletal muscle in response to aerobic exercise. *Cells* 10, 146. doi:10.3390/cells10010146
- Baviera, A. M., Zanon, N. M., Navegantes, L. C. C., Migliorini, R. H., and Kettelhut, I. C. (2008). Chemical sympathectomy further increases muscle protein degradation of acutely diabetic rats. *Muscle Nerve* 38, 1027–1035. doi:10.1002/mus.21018
- Bengal, E., Aviram, S., and Hayek, T. (2020). p38 MAPK in glucose metabolism of skeletal muscle: beneficial or harmful? *Int. J. Mol. Sci.* 21, 6480. doi:10.3390/ijms21186480
- Berdeaux, R., Goebel, N., Banaszynski, L., Takemori, H., Wandless, T., Shelton, G. D., et al. (2007). SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nat. Med.* 13, 597–603. doi:10.1038/nm1573
- Berdeaux, R., and Stewart, R. (2012). cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *Am. J. Physiology-Endocrinology Metabolism* 303, E1–E17. doi:10.1152/ajpendo.00555.2011
- Bolster, D. R., Kubica, N., Crozier, S. J., Williamson, D. L., Farrell, P. A., Kimball, S. R., et al. (2003). Immediate response of mammalian target of rapamycin (mTOR)-Mediated signalling following acute resistance exercise in rat skeletal muscle. *J. Physiol.* 553, 213–220. doi:10.1113/jphysiol.2003.047019
- Bruno, N. E., Kelly, K. A., Hawkins, R., Bramah-Lawani, M., Amelio, A. L., Nwachukwu, J. C., et al. (2014). Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. *EMBO J.* 33, 1027–1043. doi:10.1002/embj.201386145
- Cairns, S. P., and Borrani, F. (2015).  $\beta$ -Adrenergic modulation of skeletal muscle contraction: key role of excitation-contraction coupling. *J. Physiol.* 593, 4713–4727. doi:10.1113/jp270909
- Cui, D., Drake, J. C., Wilson, R. J., Shute, R. J., Lewellen, B., Zhang, M., et al. (2020). A novel voluntary weightlifting model in mice promotes muscle adaptation and insulin sensitivity with simultaneous enhancement of autophagy and mTOR pathway. *FASEB J.* 34, 7330–7344. doi:10.1096/fj.201903055R
- Durlo, F. v., Castro, M., Elias, L. L. K., and Antunes-Rodrigues, J. (2004). Interaction of prolactin, ANPergic, oxytocinergic and adrenal systems in response to extracellular volume expansion in rats. *Exp. Physiol.* 89, 541–548. doi:10.1113/expphysiol.2004.027243
- Egan, B., and Sharples, A. P. (2023). Molecular responses to acute exercise and their relevance for adaptations in skeletal muscle to exercise training. *Physiol. Rev.* 103, 2057–2170. doi:10.1152/physrev.00054.2021
- Egan, B., and Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell metab.* 17, 162–184. doi:10.1016/j.cmet.2012.12.012
- Elfellah, M., Dalling, R., Kantola, I., and Reid, J. (1989). Beta-adrenoceptors and human skeletal muscle characterisation of receptor subtype and effect of age. *Br. J. Clin. Pharmacol.* 27, 31–38. doi:10.1111/j.1365-2125.1989.tb05332.x
- Fatouros, I., Chatzizakoulou, A., Paltoglou, G., Petridou, A., Avloniti, A., Jamurtas, A., et al. (2010). Acute resistance exercise results in catecholaminergic rather than hypothalamic-pituitary-adrenal axis stimulation during exercise in young men. *Stress* 13, 461–468. doi:10.3109/10253891003743432
- Figueiredo, V. C., and Markworth, J. F. (2015). Mechanisms of protein synthesis activation following exercise: new pieces to the increasingly complex puzzle. *J. Physiol.* 593, 4693–4695. doi:10.1113/jp271431
- Fisher, J. P., Young, C. N., and Fadel, P. J. (2015). "Autonomic adjustments to exercise in humans," in *Comprehensive physiology* (New Jersey, United States: Wiley), 475–512. doi:10.1002/cphy.c140022
- Fracomano, F. T., Kannen, V., Deminice, R., Geraldino, T. H., Pperea-da-Silva, G., Uyemura, S. A., et al. (2015). Aerobic training activates interleukin 10 for colon anticarcinogenic effects. *Med. Sci. Sports Exerc* 47, 1806–1813. doi:10.1249/MSS.0000000000000623
- Garofalo, M. A. R., Kettelhut, I. C., Roselino, J. S., and Migliorini, R. H. (1996). Effect of acute cold exposure on norepinephrine turnover rates in rat white adipose tissue. *J. Auton. Nerv. Syst.* 60, 206–208. doi:10.1016/0165-1838(96)00037-9
- Gonçalves, D. A., Silveira, W. A., Manfredi, L. H., Graça, F. A., Armani, A., Bertaggia, E., et al. (2019). Insulin/IGF1 signalling mediates the effects of  $\beta$ 2-adrenergic agonist on muscle proteostasis and growth. *J. cachexia, sarcopenia muscle* 10, 455–475. doi:10.1002/jcsm.12395
- Gonçalves, D. A. P., Silveira, W. A., Lira, E. C., Graça, F. A., Paula-Gomes, S., Zanon, N. M., et al. (2012). Clenbuterol suppresses proteasomal and lysosomal proteolysis and atrophy-related genes in denervated rat soleus muscles independently of Akt. *Am. J. Physiology. Endocrinol. metabolism* 302, E123–E133. doi:10.1152/ajpendo.00188.2011
- Goode, J. M., Pearen, M. A., Tuong, Z. K., Wang, S.-C. M., Oh, T. G., Shao, E. X., et al. (2016). The nuclear receptor, nor-1, induces the physiological responses associated with exercise. *Mol. Endocrinol.* 30, 660–676. doi:10.1210/me.2015-1300
- Goto, K., Takahashi, K., Yamamoto, M., and Takamatsu, K. (2008). Hormone and recovery responses to resistance exercise with slow movement. *J. Physiological Sci.* 58, 7–14. doi:10.2170/physiolsci.RP003107
- Graça, F. A., Gonçalves, D. A. P., Silveira, W. A., Lira, E. C., Chaves, V. E., Zanon, N. M., et al. (2013). Epinephrine depletion exacerbates the fasting-induced protein breakdown in fast-twitch skeletal muscles. *Am. J. Physiol. Endocrinol. Metab.* 305, 1483–1494. doi:10.1152/ajpendo.00267.2013
- Graham, Z. A., Lavin, K. M., O'Bryan, S. M., Thalacker-Mercer, A. E., Buford, T. W., Ford, K. M., et al. (2021). Mechanisms of exercise as a preventative measure to muscle wasting. *Am. J. Physiology-Cell Physiology* 321, C40–C57. doi:10.1152/ajpcell.00056.2021
- Greife, J. S., Hickner, R. C., Shah, S. D., Cryer, P. E., and Holloszy, J. O. (1999). Norepinephrine response to exercise at the same relative intensity before and after endurance exercise training. *J. Appl. Physiol.* 86, 531–535. doi:10.1152/jappl.1999.86.2.531
- Grumati, P., Coletto, L., Schiavinato, A., Castagnaro, S., Bertaggia, E., Sandri, M., et al. (2011). Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for collagen VI-deficient muscles. *Autophagy* 7, 1415–1423. doi:10.4161/auto.7.12.17877
- Hamilton, D. L., Philp, A., MacKenzie, M. G., and Baar, K. (2010). A limited role for PI(3,4,5)P3 regulation in controlling skeletal muscle mass in response to resistance exercise. *PLoS One* 5, e11624. doi:10.1371/journal.pone.0011624
- Harcourt, L. J., Schertzer, J. D., Ryall, J. G., and Lynch, G. S. (2007). Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul. Disord. NMD* 17, 47–55. doi:10.1016/j.nmd.2006.08.012
- Hornberger, T. A., and Farrar, R. P. (2004). Physiological hypertrophy of the FHL muscle following 8 Weeks of progressive resistance exercise in the rat. *Can. J. Appl. Physiology* 29, 16–31. doi:10.1139/h04-002
- Hu, Z., Wang, H., Lee, I. H., Du, J., and Mitch, W. E. (2009). Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. *J. Clin. Investigation* 119, 3059–3069. doi:10.1172/JCI38770
- Katayama, K., and Saito, M. (2019). Muscle sympathetic nerve activity during exercise. *J. Physiological Sci.* 69, 589–598. doi:10.1007/s12576-019-00669-6
- Khan, M. M., Lustrino, D., Silveira, W. A., Wild, F., Straka, T., Issop, Y., et al. (2016). Sympathetic innervation controls homeostasis of neuromuscular junctions in health and disease. *Proc. Natl. Acad. Sci.* 113, 746–750. doi:10.1073/pnas.1524272113
- Kido, K., Ato, S., Yokokawa, T., Makanae, Y., Sato, K., and Fujita, S. (2016). Acute resistance exercise-induced IGF1 expression and subsequent GLUT4 translocation. *Physiol. Rep.* 4, e12907. doi:10.14814/phy2.12907
- Kim, J., Grotegut, C. A., Wisler, J. W., Li, T., Mao, L., Chen, M., et al. (2018).  $\beta$ -arrestin 1 regulates  $\beta$ 2-adrenergic receptor-mediated skeletal muscle hypertrophy and contractility. *Skelet. Muscle* 8, 39. doi:10.1186/s13395-018-0184-8
- Kim, J., Yang, G., Kim, Y., Kim, J., and Ha, J. (2016). AMPK activators: mechanisms of action and physiological activities. *Exp. Mol. Med.* 48, 2244–e312. doi:10.1038/emmm.2016.16

- Kim, Y. S., and Sainz, R. D. (1992). Beta-adrenergic agonists and hypertrophy of skeletal muscles. *Life Sci.* 50, 397–407. doi:10.1016/0024-3205(92)90374-x
- Kline, W. O., Panaro, F. J., Yang, H., and Bodine, S. C. (2007). Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. *J. Appl. physiology (Bethesda, Md., 1985)* 102, 740–747. doi:10.1152/jappphysiol.00873.2006
- Koopman, R., Gehrig, S. M., Léger, B., Trieu, J., Walrand, S., Murphy, K. T., et al. (2010). Cellular mechanisms underlying temporal changes in skeletal muscle protein synthesis and breakdown during chronic [beta]-adrenoceptor stimulation in mice. *J. physiology* 588, 4811–4823. doi:10.1113/jphysiol.2010.196725
- Kraemer, W. J., and Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med. Auckl. N.Z.* 35, 339–361. doi:10.2165/00007256-200535040-00004
- Louis, E., Raue, U., Yang, Y., Jemiolo, B., and Trappe, S. (2007). Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J. Appl. Physiol.* 103, 1744–1751. doi:10.1152/jappphysiol.00679.2007
- Lourenço, Í., Krause Neto, W., Santos Portella Amorim, L., Moraes Munhoz Ortiz, V., Lopes Geraldo, V., Henrique da Silva Ferreira, G., et al. (2020). Muscle hypertrophy and ladder-based resistance training for rodents: a systematic review and meta-analysis. *Physiol. Rep.* 8, 1–25. doi:10.14814/phy2.14502
- Lynch, G. S., and Ryall, J. G. (2008). Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. *Physiol. Rev.* 88, 729–767. doi:10.1152/physrev.00028.2007
- Macedo, A. G., Almeida, T. A. F., Massini, D. A., De Paula, V. F., De Oliveira, D. M., and Pessôa Filho, D. M. (2023). Effects of exercise training on glucocorticoid-induced muscle atrophy: literature review. *Steroids* 195, 109240. doi:10.1016/j.steroids.2023.109240
- Mathur, S., Brooks, D., and Carvalho, C. R. F. (2014). Structural alterations of skeletal muscle in copd. *Front. Physiology* 5, 104. doi:10.3389/fphys.2014.00104
- McIntosh, M. C., Sexton, C. L., Godwin, J. S., Ruple, B. A., Michel, J. M., Plotkin, D. L., et al. (2023). Different resistance exercise loading paradigms similarly affect skeletal muscle gene expression patterns of myostatin-related targets and mTORC1 signaling markers. *Cells* 12, 898. doi:10.3390/cells12060898
- Mersmann, H. J. (1998). Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J. Animal Sci.* 76, 160–172. doi:10.2527/1998.761160x
- Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.-H., Frasson, L., et al. (2015). Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat. Commun.* 6, 6670. doi:10.1038/ncomms7670
- Miyazaki, M., and Takemasa, T. (2017). TSC2/Rheb signaling mediates ERK-dependent regulation of mTORC1 activity in C2C12 myoblasts. *FEBS Open Bio* 7, 424–433. doi:10.1002/2211-5463.12195
- Mounier, R., Cavalié, H., Lac, G., and Clottes, E. (2007). Molecular impact of clenbuterol and isometric strength training on rat EDL muscles. *Pflugers Archiv Eur. J. physiology* 453, 497–507. doi:10.1007/s00424-006-0122-1
- Nader, G. A., von Walden, F., Liu, C., Lindvall, J., Gutmann, L., Pistilli, E. E., et al. (2014). Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. *J. Appl. Physiol.* 116, 693–702. doi:10.1152/jappphysiol.01366.2013
- Navegantes, L. C. C., Resano, N. M. Z., Migliorini, R. H., and Kettelhut, I. C. (1999). Effect of guanethidine-induced adrenergic blockade on the different proteolytic systems in rat skeletal muscle. *Am. J. Physiology-Endocrinology Metabolism* 277, E883–E889. doi:10.1152/ajpendo.1999.277.5.E883
- Osman, A. A., Pendergrass, M., Koval, J., Maezono, K., Cusi, K., Pratipanawatr, T., et al. (2000). Regulation of MAP kinase pathway activity *in vivo* in human skeletal muscle. *Am. J. Physiology-Endocrinology Metabolism* 278, E992–E999. doi:10.1152/ajpendo.2000.278.6.E992
- Padilha, C. S., Borges, F. H., Costa Mendes da Silva, L. E., Frajacom, F. T. T., Jordao, A. A., Duarte, J. A., et al. (2017). Resistance exercise attenuates skeletal muscle oxidative stress, systemic pro-inflammatory state, and cachexia in Walker-256 tumor-bearing rats. *Appl. Physiology, Nutr. Metabolism* 42, 916–923. doi:10.1139/apnm-2016-0436
- Padilha, C. S., Cella, P. S., Chimin, P., Voltarelli, F. A., Marinello, P. C., De Jesus Testa, M. T., et al. (2021). Resistance training's ability to prevent cancer-induced muscle atrophy extends anabolic stimulus. *Med. Sci. Sports Exerc* 53, 1572–1582. doi:10.1249/MSS.0000000000002624
- Padilha, C. S., Cella, P. S., Ribeiro, A. S., Voltarelli, F. A., Testa, M. T. J., Marinello, P. C., et al. (2019). Moderate vs high-load resistance training on muscular adaptations in rats. *Life Sci.* 238, 116964. doi:10.1016/j.lfs.2019.116964
- Pearen, M. A., Ryall, J. G., Lynch, G. S., and Muscat, G. E. (2009). Expression profiling of skeletal muscle following acute and chronic beta2-adrenergic stimulation: implications for hypertrophy, metabolism and circadian rhythm. *BMC Genomics* 10, 448. doi:10.1186/1471-2164-10-448
- Pedersen, B. K. (2013). Muscle as a secretory organ. *Compr. Physiol.* 3, 1337–1362. doi:10.1002/cphy.c120033
- Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., and Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am. J. Physiology-Endocrinology Metabolism* 273, E99–E107. doi:10.1152/ajpendo.1997.273.1.E99
- Piccirillo, R. (2019). Exercise-induced myokines with therapeutic potential for muscle wasting. *Front. Physiology* 10, 287. doi:10.3389/fphys.2019.00287
- Pillon, N. J., Gabriel, B. M., Dollet, L., Smith, J. A. B., Sardón Puig, L., Botella, J., et al. (2020). Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. *Nat. Commun.* 11, 470. doi:10.1038/s41467-019-13869-w
- Raastad, T., Bjørø, T., and Hallén, J. (2000). Hormonal responses to high- and moderate-intensity strength exercise. *Eur. J. Appl. Physiol.* 82, 121–128. doi:10.1007/s004210050661
- Risling, T. E., Caulkett, N. A., and Florence, D. (2012). Open-drop anesthesia for small laboratory animals. *Can. veterinary J. = La revue vétérinaire Can.* 53, 299–302.
- Rodrigues, A. C. Z., Messi, M. L., Wang, Z., Abba, M. C., Pereyra, A., Birbrair, A., et al. (2019). The sympathetic nervous system regulates skeletal muscle motor innervation and acetylcholine receptor stability. *Acta Physiol.* 225, e13195. doi:10.1111/apha.13195
- Rommel, C., Bodine, S. C., Clarke, B. A., Rossman, R., Nunez, L., Stitt, T. N., et al. (2001). Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat. Cell Biol.* 3, 1009–1013. doi:10.1038/ncb1101-1009
- Rose, A. J., Kiens, B., and Richter, E. A. (2006). Ca<sup>2+</sup>-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J. Physiol.* 574, 889–903. doi:10.1113/jphysiol.2006.111757
- Rowell, L. B., and O'Leary, D. S. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J. Appl. Physiol.* 69, 407–418. doi:10.1152/jappphysiol.1990.69.2.407
- Ruas, J. L., White, J. P., Rao, R. R., Kleiner, S., Brannan, K. T., Harrison, B. C., et al. (2012). A PGC-1 $\alpha$  isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell* 151, 1319–1331. doi:10.1016/j.cell.2012.10.050
- Ryall, J. G., Schertzer, J. D., and Lynch, G. S. (2007). Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: therapeutic implications for sarcopenia. *Journals gerontology. Ser. A, Biol. Sci. Med. Sci.* 62, 813–823. doi:10.1093/geronol/62.8.813
- Santana, F. M., Domiciano, D. S., Gonçalves, M. A., Machado, L. G., Figueiredo, C. P., Lopes, J. B., et al. (2019). Association of appendicular lean mass, and subcutaneous and visceral adipose tissue with mortality in older Brazilians: the São Paulo ageing and health study. *J. Bone Mineral Res.* 34, 1264–1274. doi:10.1002/jbmr.3710
- Sartori, R., Romanello, V., and Sandri, M. (2021). Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. *Nat. Commun.* 12, 330–412. doi:10.1038/s41467-020-20123-1
- Schiaffino, S., and Mammucari, C. (2011). Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skelet. Muscle* 1, 4. doi:10.1186/2044-5040-1-4
- Schmittgen, T. D., and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* 3, 1101–1108. doi:10.1038/nprot.2008.73
- Schnyder, S., and Handschin, C. (2015). Skeletal muscle as an endocrine organ: PGC-1 $\alpha$ , myokines and exercise. *Bone* 80, 115–125. doi:10.1016/j.bone.2015.02.008
- Shaywitz, A. J., and Greenberg, M. E. (1999). CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* 68, 821–861. doi:10.1146/annurev.biochem.68.1.821
- Silveira, W. A., Gonçalves, D. A., Graça, F. A., Andrade-Lopes, A. L., Bergantin, L. B., Zanon, N. M., et al. (2014). Activating cAMP/PKA signaling in skeletal muscle suppresses the ubiquitin-proteasome-dependent proteolysis: implications for sympathetic regulation. *J. Appl. Physiol.* 117, 11–19. doi:10.1152/jappphysiol.01055.2013
- Silveira, W. A., Gonçalves, D. A., Machado, J., Lautherbach, N., Lustrino, D., Paula-Gomes, S., et al. (2020). cAMP-dependent protein kinase inhibits FoxO activity and regulates skeletal muscle plasticity in mice. *FASEB J.* 34, 12946–12962. doi:10.1096/fj.201902102RR
- Silvestre, J. G. O., Speretta, G. F. F., Fabrizzi, F., Moraes, G., and Duarte, A. C. G. de O. (2017). Acute effects of Resistance exercise performed on ladder on energy metabolism, stress, and muscle damage in rats. *Mot. Rev. Educ. Física* 23. doi:10.1590/s1980-6574201700si010
- Takemori, H., Katoh Hashimoto, Y., Nakae, J., Olson, E. N., and Okamoto, M. (2009). Inactivation of HDAC5 by SIK1 in AICAR-treated C2C12 myoblasts. *Endocr. J.* 56, 121–130. doi:10.1507/endocrj.K08E-173
- Testa, M. T. de J., Cella, P. S., Marinello, P. C., Frajacom, F. T. T., Padilha, C. de S., Perandini, P. C., et al. (2022). Resistance training attenuates activation of STAT3 and muscle atrophy in tumor-bearing mice. *Front. Oncol.* 12, 880787. doi:10.3389/fonc.2022.880787
- Thomson, D. M., Herway, S. T., Fillmore, N., Kim, H., Brown, J. D., Barrow, J. R., et al. (2008). AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J. Appl. Physiol.* 104, 429–438. doi:10.1152/jappphysiol.00900.2007

- Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., et al. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J. Physiol.* 586, 3701–3717. doi:10.1113/jphysiol.2008.153916
- Wojtaszewski, J. F. P., Nielsen, P., Hansen, B. F., Richter, E. A., and Kiens, B. (2000). Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. *J. Physiol.* 528, 221–226. doi:10.1111/j.1469-7793.2000.t01-1-00221.x
- Xiao, R. P. (2001). Beta-adrenergic signaling in the heart: dual coupling of the beta2-adrenergic receptor to G(s) and G(i) proteins. *Science's STKE signal Transduct. Knowl. Environ.* 2001, re15. doi:10.1126/stke.2001.104.re15
- Yang, J., Zong, C. S., Xia, W., Yamaguchi, H., Ding, Q., Xie, X., et al. (2008). ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat. Cell Biol.* 10, 138–148. doi:10.1038/ncb1676
- Yimlamai, T., Dodd, S. L., Borst, S. E., and Park, S. (2005). Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin-proteasome pathway. *J. Appl. physiology (Bethesda, Md. 1985)* 99, 71–80. doi:10.1152/jappphysiol.00448.2004
- Zhou, H.-H., Liao, Y., Peng, Z., Liu, F., Wang, Q., and Yang, W. (2023). Association of muscle wasting with mortality risk among adults: a systematic review and meta-analysis of prospective studies. *J. cachexia, sarcopenia muscle* 14, 1596–1612. doi:10.1002/jcsm.13263