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Efeitos do treinamento físico resistido sobre a atrofia muscular em modelo de hipertensão arterial pulmonar

Sebastião Felipe Ferreira Costa
Magister Scientiae

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SEBASTIÃO FELIPE FERREIRA COSTA

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Educação Física, para obtenção do título de *Magister Scientiae*.

Orientador: Antonio Jose Natali

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APROVADA: 25 de julho de 2025.

Assentimento:

Sebastião Felipe Ferreira Costa
Autor

Antonio Jose Natali
Orientador

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“Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana.”

Carl Jung

RESUMO

COSTA, Sebastião Felipe Ferreira, M.Sc., Universidade Federal de Viçosa, julho de 2025. **Efeitos do treinamento físico resistido sobre a atrofia muscular em modelo de hipertensão arterial pulmonar**. Orientador: Antonio Jose Natali.

O objetivo do presente estudo foi investigar os efeitos de um programa de treinamento resistido (RT) aplicado durante o desenvolvimento da HAP induzida por MCT sobre a atrofia da musculatura esquelética em ratos. O primeiro capítulo apresenta uma revisão narrativa sobre os potenciais mecanismos celulares e moleculares que podem contribuir para o surgimento da atrofia muscular em pacientes com HAP, assim como os efeitos do exercício físico sobre a musculatura esquelética nessa condição. Esta revisão discute os principais mecanismos envolvidos na atrofia muscular na HAP, destacando a ativação de vias de proteólise muscular, como o sistema ubiquitina-proteassoma, autofagia e calpaínas, bem como a mudança no tipo de fibra muscular favorecendo a atrofia muscular nessa patologia. Além disso, aborda-se a possível inibição das vias de hipertrofia muscular – antagônicas à degradação proteica. Por fim, os efeitos do exercício físico sobre essa condição são discutidos com base em estudos anteriores que investigaram adaptações musculares esqueléticas, e o exercício se mostra uma intervenção não farmacológica promissora para o manejo da HAP. O segundo capítulo apresenta a investigação dos efeitos do RT realizado durante o desenvolvimento da HAP induzida por MCT sobre a atrofia muscular em ratos. Para isso, quarenta e dois ratos Wistar (~200 g de peso corporal (BW)) foram distribuídos aleatoriamente em três grupos experimentais (n = 14 por grupo): Controle Sedentário (SC), Hipertenso Sedentário (SH) e Hipertenso Treinado (TH). A HAP foi induzida nos grupos SH e TH por meio de uma única injeção intraperitoneal de MCT (60 mg/kg), enquanto o grupo SC recebeu o mesmo volume de solução salina. Os ratos do grupo TH foram submetidos ao RT (subida em escada vertical; 15 subidas com intervalo de 1 minuto; 60% da carga máxima suportada), uma sessão/dia, 5 dias/semana, por aproximadamente 3 semanas. No 23º dia pós-administração de MCT, foi realizada ecocardiografia para caracterização da HAP e avaliação da função ventricular direita. No 24º dia, todos os animais foram eutanasiados e, em seguida, os bíceps braquiais foram removidos, processados e destinados às análises subsequentes. O bíceps esquerdo foi destinado às análises histológicas, enquanto o bíceps direito foi utilizado para análises de estresse oxidativo, expressão gênica e expressão proteica. Foi observado que o RT aumentou a tolerância ao esforço físico nos animais com HAP

induzida (ou seja, carga máxima suportada 21 dias após a aplicação de MCT: TH = 1,73 ± 0,13 g/BW; SC = 1,19 ± 0,06 g/BW; SH = 0,97 ± 0,08 g/BW; $p < 0,05$). Em relação ao músculo esquelético, o RT preveniu o seu remodelamento adverso estrutural ao preservar a porcentagem de número de miócitos (SC = 92,84 ± 1,18 %; SH = 88,52 ± 1,92 %; TH = 91,33 ± 1,11 %, $p < 0,05$), a área da secção transversal (CSA; SC = 6,446,0 ± 533,8 μm^2; SH = 4,016,0 ± 500,4 μm^2; TH = 5,430,0 ± 282,2 μm^2, $p < 0,05$) e a deposição total de colágeno (SC = 2,67 ± 0,45 %; SH = 6,33 ± 0,78 %; TH = 3,64 ± 0,72 %, $p < 0,05$). Uma forte correlação negativa foi observada entre CSA e colágeno total ($r = -0,89$; IC 95% = [(-0,9534)-(-0,7369)]; $R^2 = 0,7860$; $p < 0,05$). Além disso, o RT atenuou a expressão gênica de MuRF1 (SC = 1,12 ± 0,18 fold change; SH = 1,83 ± 0,20 fold change; TH = 1,41 ± 0,62 fold change, $p < 0,05$), atrogin-1 (SC = 1,33 ± 0,29 fold change; SH = 4,66 ± 1,02 fold change; TH = 2,60 ± 0,43 fold change, $p < 0,05$) e miostatina (SC = 0,43 ± 0,13 fold change; SH = 1,17 ± 0,35 fold change; TH = 0,78 ± 0,09 fold change, $p < 0,05$), mas não foi capaz de minimizar a expressão de calpaína (SC = 0,99 ± 0,37 fold change; SH = 2,65 ± 0,90 fold change; TH = 2,16 ± 0,90 fold change, $p > 0,05$). O RT também mitigou o desequilíbrio redox ao preservar a atividade da CAT (SC = 213,20 ± 11,00 U.mg.ptn?¹; SH = 163,20 ± 24,50 U.mg.ptn?¹; TH = 230,00 ± 39,50 U.mg.ptn?¹, $p < 0,05$) e as concentrações de NO (SC = 4,31 ± 0,88 µmol/L; SH = 1,43 ± 0,39 µmol/L; TH = 2,70 ± 0,40 µmol/L, $p < 0,05$), bem como as concentrações de PC (SC = 2,14 ± 0,38 nmol/mL; SH = 3,02 ± 0,36 nmol/mL; TH = 2,18 ± 0,27 nmol/mL, $p < 0,05$). No entanto, o RT não preveniu a redução da atividade da SOD (SC = 62,52 ± 9,14 U.mg.ptn?¹; SH = 37,96 ± 4,91 U.mg.ptn?¹; TH = 46,36 ± 10,80 U.mg.ptn?¹, $p > 0,05$). Não foram encontrados efeitos da HAP ou do RT sobre a atividade da GST e as concentrações de MDA ($p > 0,05$). Quanto à via de hipertrofia muscular, nem a HAP nem o RT afetaram essa via (ou seja, Akt-1 e eIF4E) neste modelo experimental ($p > 0,05$). Em conclusão, o RT realizado durante o desenvolvimento da HAP induzida por MCT protege contra a atrofia muscular ao atenuar o remodelamento estrutural adverso por meio da modulação da proteólise e do desequilíbrio redox. Esses achados destacam o potencial terapêutico do RT como uma estratégia não farmacológica para preservar a funcionalidade muscular e a tolerância ao esforço físico em condições de HAP.

Palavras-chave: treinamento físico; proteólise; monocrotalina; intolerância ao exercício; músculo esquelético

ABSTRACT

COSTA, Sebastião Felipe Ferreira, M.Sc., Universidade Federal de Viçosa, July, 2025. **Effects of resistance training on muscle atrophy in a model of pulmonary arterial hypertension**. Adviser: Antonio Jose Natali.

The objective of the present study was to investigate the effects of a resistance exercise training (RT) program applied during the development of MCT-induced PAH on skeletal muscle atrophy in rats. The first chapter presents a narrative review on the potential cellular and molecular mechanisms that may contribute to the onset of muscle atrophy in PAH patients, as well as the effects of physical exercise on skeletal muscle in this condition. This review discusses the main mechanisms involved in muscle atrophy in PAH, highlighting the activation of muscle proteolysis pathways, such as the ubiquitin-proteasome system, autophagy and calpains, as well as the shift in muscle fiber type favoring muscle atrophy in this pathology. Additionally, the possible inhibition of muscle hypertrophy pathways – antagonistic to protein degradation – is addressed. Finally, the effects of physical exercise on this condition are discussed using previous studies investigating skeletal muscle adaptations, and it appears to be a promising non-pharmacological intervention for the management of PAH. The second chapter shows the investigation of the effects of RT on muscle atrophy during the development of PAH induced by MCT in rats. For this purpose, forty-two Wistar rats (~200 g body weight (BW)) were randomly assigned to three experimental groups (n = 14 per group): Sedentary Control (SC), Sedentary Hypertensive (SH), and Trained Hypertensive (TH). PAH was induced in the SH and TH groups through a single intraperitoneal injection of MCT (60 mg/kg), while the SC group received the same volume of saline solution. The rats in TH group underwent RT (vertical ladder climbing; 15 climbs with a 1-minute interval; 60% of the maximum supported load), one session/day, 5 days/week, for approximately 3 weeks. On the 23rd day post-MCT administration, echocardiography was performed to characterize PAH and to evaluate the right ventricular function. On the 24th day, all animals were euthanized, and subsequently, the brachial biceps were removed, processed, and allocated for further analyses. The left biceps were designated for histological analyses, while the right biceps were used for oxidative stress, gene expression, and protein expression analyses. It was found that RT increased physical effort tolerance in PAH-induced animals (i.e., maximum supported load 21 days post-MCT application: TH = 1.73 ± 0.13 g/BW; SC = 1.19 ± 0.06 g/BW; SH = 0.97 ± 0.08 g/BW; p <

0.05). Concerning skeletal muscle, RT prevented its structural adverse remodeling by preserving the percentage of myocyte number (SC = 92.84 ± 1.18 %; SH = 88.52 ± 1.92 %; TH = 91.33 ± 1.11 %, $p < 0.05$), the cross-sectional area (CSA; SC = 6,446.0 ± 533.8 µm2; SH = 4,016.0 ± 500.4 µm2; TH = 5,430.0 ± 282.2 µm2, $p < 0.05$), and the total collagen deposition (SC = 2.67 ± 0.45 %; SH = 6.33 ± 0.78 %; TH = 3.64 ± 0.72 %, $p < 0.05$). A strong negative correlation was observed between CSA and total collagen ($r = -0.89$; 95% CI = [(-0.9534)-(-0.7369)]; $R2 = 0.7860$; $p < 0.05$). In addition, RT attenuated the gene expression of MuRF1 (SC = 1.12 ± 0.18 fold change; SH = 1.83 ± 0.20 fold change; TH = 1.41 ± 0.62 fold change, $p < 0.05$), atrogin-1 (SC = 1.33 ± 0.29 fold change; SH = 4.66 ± 1.02 fold change; TH = 2.60 ± 0.43 fold change, $p < 0.05$), and myostatin (SC = 0.43 ± 0.13 fold change; SH = 1.17 ± 0.35 fold change; TH = 0.78 ± 0.09 fold change, $p < 0.05$), but was not able to minimize calpain expression (SC = 0.99 ± 0.37 fold change; SH = 2.65 ± 0.90 fold change; TH = 2.16 ± 0.90 fold change, $p > 0.05$). Moreover, RT mitigated redox imbalance by preserving the CAT activity (SC = 213.20 ± 11.00 U.mg.ptn-1; SH = 163.20 ± 24.50 U.mg.ptn-1; TH = 230.00 ± 39.50 U.mg.ptn-1, $p < 0.05$) and the NO concentrations (SC = 4.31 ± 0.88 µmol/L; SH = 1.43 ± 0.39 µmol/L; TH = 2.70 ± 0.40 µmol/L, $p < 0.05$), as well as the PC concentrations (SC = 2.14 ± 0.38 nmol/mL; SH = 3.02 ± 0.36 nmol/mL; TH = 2.18 ± 0.27 nmol/mL, $p < 0.05$). However, RT did not prevent the reduction in the SOD activity (SC = 62.52 ± 9.14 U.mg.ptn-1; SH = 37.96 ± 4.91 U.mg.ptn-1; TH = 46.36 ± 10.80 U.mg.ptn-1, $p > 0.05$). No effects of either PAH or RT were found on the GST activity and MDA concentrations ($p > 0.05$). Regarding the muscle hypertrophy pathway, neither PAH nor RT affected the muscle hypertrophy pathway (i.e., Akt-1 and eIF4E) in this experimental model ($p > 0.05$). In conclusion, the RT applied during the development of MCT-induced PAH protects against skeletal muscle atrophy, by mitigating adverse structural remodeling and muscle atrophy through proteolysis modulation and attenuation of redox imbalance. These findings highlight the therapeutic potential of RT as a non-pharmacological strategy to preserve muscle functionality and physical effort tolerance in PAH conditions.

Keywords: physical exercise ; proteolysis; monocrotaline; exercise intolerance; skeletal muscle

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LISTA DE ABREVIAÇÃOES

µg. *Microgram*
µL. *Microliter*
µm. *Micrometer*
ActRIIB. *Activin type II receptor*
Akt. *Protein Kinase B*
AMP. *Adenosine monophosphate*
AMPK. *AMP-activated protein kinase*
ANOVA. *Analysis of variance*
AT. *Acceleration time*
ATP. *Adenosine triphosphate*
BNIP3. *BCL2 and adenovirus E1B 19-kDa-interacting protein 3*
BNP. *B-type natriuretic peptide*
BW. *Body weight*
Ca^{2+}. *Calcium ion*
CaMKK. *Calcium/calmodulin-dependent protein kinase*
CAT. *Catalase*
cDNA. *Complementary DNA*
CDNB. *Formadinitrochlorobenzene*
cm. *Centimeters*
CO. *Cardiac output*
COPD. *Chronic obstructive pulmonary disease*
CP. *Carbonyl protein*
CSA. *Cross-sectional area*
DNA. *Deoxyribonucleic acid*
dNTPs. *Nucleotides*
ECM. *Extracellular matrix*
eIF4E. *Eukaryotic translation initiation factor 4E*
ET. *Ejection time*
FoxO. *Forkhead box O*
g. *grams*
GST. *Glutathione S-transferase*
h. *Hours*
H&E. *Hematoxylin and eosin*
H_2O_2. *Hydrogen peroxide*

HCl. *Hydrochloric acid*

HIF-1α. *Hypoxia-inducible factor 1-alpha*

HIIT. *High intensity interval training*

HIV. *Human immunodeficiency virus*

IGF-1. *Insulin-like growth fator 1*

IKK. *IkappaB kinase*

IL-6. *Interleukin 6*

iPAH. *Idiopathic pulmonary arterial hypertension*

IRS1. *Insulin receptor substrate 1*

JAK. *Janus kinase*

JUNK. *Jun N-terminal kinase*

LC3. *Microtubule-associated protein 1A/1B-light chain 3*

LDH. *Lactate dehydrogenase*

MAFbx/atrogin-1/Fbx32. *Muscle atrophy F-box*

MAPK. *Mitogen-activated protein kinase*

MDA. *Malondialdehyde*

mg. *Milligram*

$MgCl_2$. *Magnesium chloride*

min. *Minutes*

mM. *Millimolar*

mmHg. *Consulte*, *millimeters of mercury*

mPAP. *Pulmonary arterial pressure*

MSTN. *Myostatin*

mTOR. *Consulte*, *Mechanistic Target of Rapamycin*

MuRF1/TRIM63. *Muscle ring finger-1*

MyHC. *Myosin heavy chain*

MyoD. *Myoblast determination protein 1*

NaCl. *Sodium chloride*

NCX. *Sodium-calcium exchanger*

NF-κB. *Nuclear transcription factor kappa B*

NO. *Nitric oxide*

NT-proBNP. *N-terminal pro B-type natriuretic peptide*

O^{-2}. *Superoxide*

O_2. *Oxigen*

ORM. *One repetition maximum*

p38. *p38 mitogen-activated protein kinase*

Pax7. *Paired box protein 7*

PCR. *Polymerase chain reaction*

PDK. *Pyruvate dehydrogenase kinase*

pH. *Hydrogen potential*

PH. *Pulmonary hypertension*

PI3K. *Phosphoinositide 3-kinase*

PVR. *Pulmonary vascular resistance*, *Pulmonary vascular resistance*

RNA. *Ribonucleic acid*

ROS. *Reactive oxygen species*

RT. *Resistance training*

RT-PCR. *Real-Time polymerase chain reaction*

RV. *Right ventricular*

RyR. *Ryanodine receptor*

SC. *Sedentary control*

SD. *Standard deviation*

^{Ser}. *Serine*

SERCA2a. *Sarcoplasmic reticulum* Ca^{2+}*-ATPase*

SH. *Sedentary hypertensive*

SMAD. *SMAD family member*

SOD. *Superoxide dismutase*

STAT3. *Signal transducer and activator of transcription 3*

SV. *Stroke volume*

TAPSE. *Tricuspid valve annular plane systolic excursion*

TGF-β. *Transforming growth factor beta*

TH. *Trained hypertensive*

TNFR. *Tumor necrosis factor alpha receptor*

TNF-α. *Tumor necrosis factor alpha*

ULK-1. *Unc-51 like autophagy activating kinase 1*

UPS. *Ubiquitin-proteasome system*

VEGF. *Vascular endothelial growth factor*

WSPH. *World Symposium on Pulmonary Hypertension*

SUMÁRIO

1. INTRODUÇÃO

A hipertensão pulmonar (HP) é um distúrbio multifatorial associado a disfunções cardiovasculares e respiratórias. É caracterizada por um aumento da pressão arterial pulmonar média (mPAP) acima de 20 mmHg em repouso [1,2]. Originalmente, sua definição considerava um valor de mPAP ≥ 25 mmHg, estabelecido de forma arbitrária durante o 1º Simpósio Mundial sobre Hipertensão Pulmonar (WSPH) em 1973 [3].

De acordo com sua etiologia, a HP é classificada em cinco grupos: 1) Hipertensão arterial pulmonar (HAP); 2) HP associada a doenças do coração esquerdo (ex.: insuficiência cardíaca); 3) HP resultante de doenças pulmonares e/ou hipóxia (ex.: doença pulmonar obstrutiva crônica – DPOC); 4) HP causada por obstruções da artéria pulmonar (ex.: HP tromboembólica crônica); e 5) HP de mecanismos indefinidos e/ou multifatoriais (ex.: distúrbios hematológicos e doenças inflamatórias sistêmicas) [1,4].

A prevalência da HP é de aproximadamente 1% da população mundial, sendo mais elevada em indivíduos com mais de 65 anos, devido ao aumento da incidência de doenças cardíacas e pulmonares nessa faixa etária [5,6].

Com relação ao Grupo 1 da HP, a hipertensão arterial pulmonar (HAP) é caracterizada por alterações primárias na vasculatura, sem associação direta com outras doenças cardiopulmonares [7]. A HAP é caracterizada por uma mPAP superior a 20 mmHg, resistência vascular pulmonar (RVP) acima de 2 unidades Wood e pressão de oclusão da artéria pulmonar ≤ 15 mmHg, mensurado por meio de cateterismo direto no coração direito [8]. É uma doença crônica e progressiva, com uma incidência global estimada entre 2,0 e 7,6 casos por milhão de adultos por ano e uma prevalência que varia de 11 a 55 casos por milhão de adultos [9,10].

A etiologia da HAP inclui formas idiopáticas e hereditárias, casos induzidos por drogas ou toxinas e associações com outras doenças (por exemplo, HIV, hipertensão portal, cardiopatias congênitas e esquistossomose). Também está relacionada ao uso prolongado de bloqueadores dos canais de cálcio, ao comprometimento venoso/capilar e à síndrome neonatal persistente [4,7].

O diagnóstico da HAP é um processo que envolve múltiplas abordagens e métodos para uma avaliação abrangente dessa condição clínica. Inicialmente, realiza-se a investigação clínica por meio da anamnese, analisando sintomas como dispneia

e fadiga, bem como a presença de fatores de risco associados. Testes de esforço, como o teste de caminhada de seis minutos, são realizados para avaliar a capacidade funcional do paciente. Além disso, biomarcadores como BNP (peptídeo natriurético tipo B) e NT-proBNP (fragmento N-terminal do Pró-BNP) auxiliam na avaliação cardíaca. Testes pulmonares ajudam a diferenciar a HAP de outras doenças respiratórias. Durante a fase de triagem, o ecocardiograma transtorácico é amplamente utilizado, mas o padrão-ouro para a confirmação definitiva do diagnóstico de HAP é o cateterismo do coração direito. Outros exames de imagem (como a tomografia computadorizada e a cintilografia ventilação-perfusão) podem ser utilizados para complementar a investigação e garantir um diagnóstico mais preciso [8,11].

A fisiopatologia da HAP envolve um remodelamento vascular adverso das artérias e arteríolas pulmonares, caracterizado por proliferação descontrolada das células endoteliais, hipertrofia da camada média e deposição excessiva de matriz extracelular [10,12]. Como resultado, há um aumento progressivo da RVP. Esse fenômeno patológico sobrecarrega o ventrículo direito (VD), levando a alterações adversas em sua estrutura morfológica e molecular, como a hipertrofia patológica caracterizada pela proliferação de tecido fibrótico, desequilíbrio redox, inflamação, disfunção mitocondrial e disfunção contrátil [13–15]. À medida que a doença progride, o débito cardíaco é comprometido, reduzindo a oferta de oxigênio aos tecidos periféricos e limitando a capacidade funcional dos pacientes. Nos estágios avançados, desenvolve-se insuficiência cardíaca direita, tornando-se a principal causa de morbidade e mortalidade na HAP [15,16].

As manifestações clínicas da HAP nos pacientes incluem dispneia, fadiga, falta de ar durante o esforço físico, letargia, retenção de líquidos, edema de membros inferiores e pré-síncope/síncope, sendo os dois primeiros sintomas os mais frequentemente observados. Essas alterações refletem adaptações cardiovasculares adversas [17,18]. Esses sintomas contribuem para a redução da tolerância ao exercício, uma característica marcante da doença [19].

A intolerância ao exercício na HAP é causada pela interação entre fatores centrais e periféricos. Em nível central, a disfunção do ventrículo direito e a ineficiência ventilatória limitam a capacidade do coração de bombear sangue oxigenado para os tecidos. Em nível periférico, a rarefação capilar (redução na densidade dos vasos sanguíneos) e a diminuição da perfusão sanguínea no músculo esquelético

prejudicam a oferta de oxigênio e nutrientes, levando à disfunção muscular [20,21]. Esses processos podem desencadear a ativação de mecanismos de degradação proteica, como o sistema ubiquitina-proteassoma (UPS). Além disso, a disfunção mitocondrial, o desequilíbrio redox, o aumento da inflamação e as alterações metabólicas podem contribuir para a atrofia muscular em pacientes com HAP [22–25].

Apesar dos avanços significativos na compreensão da fisiopatologia da HAP, sua gênese ainda não está totalmente elucidada na literatura, sendo necessários estudos em animais para uma melhor compreensão da doença. Nesse contexto, o modelo animal de HAP induzida por monocrotalina (MCT) é um dos mais utilizados para investigação da doença. A MCT é um alcaloide derivado da planta *Crotalaria spectabilis* e mimetiza a HAP devido à sua capacidade de reproduzir as características clínicas e patológicas da enfermidade, como o aumento da pressão arterial pulmonar, a hipertrofia do VD e o remodelamento vascular [26,27].

As terapias atuais para a HAP concentram-se em três principais vias fisiopatológicas: a via do óxido nítrico, a via da prostaciclina e a via da endotelina. Essas abordagens farmacológicas têm como objetivo restaurar o equilíbrio entre os mediadores vasoativos, promovendo a vasodilatação pulmonar e reduzindo a resistência vascular. No entanto, embora o transplante pulmonar continue sendo a única intervenção curativa para os casos graves de HAP, diversas terapias inovadoras estão atualmente em investigação, com potencial para reduzir a morbimortalidade e melhorar significativamente os desfechos dos pacientes [8].

Nesse sentido, juntamente com as intervenções farmacológicas, estratégias não farmacológicas têm ganhado destaque no manejo da HAP. O exercício físico, em particular, tem sido recomendado como uma abordagem complementar promissora [28,29]. Estudos clínicos demonstraram que o exercício pode melhorar a capacidade funcional e a qualidade de vida de pacientes com HAP [30–32]. De forma semelhante, pesquisas em modelos animais com HAP induzida por MCT corroboram esses benefícios, mostrando que o exercício físico atenua a progressão da doença e melhora parâmetros cardiovasculares e metabólicos [33–36]. No entanto, apesar dos avanços, ainda não está claro como o treinamento resistido (TR) influencia os parâmetros de atrofia muscular esquelética na HAP. Essa lacuna na literatura destaca a necessidade de mais pesquisas para elucidar os efeitos do TR na preservação da massa muscular esquelética associada à doença.

2. OBJETIVOS

2.1. Geral

Investigar os efeitos do treinamento resistido (TR) sobre a atrofia muscular durante o desenvolvimento da HAP induzida por MCT em ratos.

2.2. Específico

2.2.1. Revisar a literatura sobre os possíveis mecanismos moleculares envolvidos na perda de massa muscular na HAP e os potenciais efeitos do exercício físico nessa condição.

2.2.2. Avaliar se o programa de exercício resistido aplicado durante o desenvolvimento da HAP afeta os seguintes parâmetros em ratos:

- Tolerância ao esforço físico;
- Morfometria e estereologia do músculo esquelético;
- Estresse oxidativo no músculo esquelético; e
- Expressão gênica e proteica relacionada à atrofia do músculo esquelético.

Para tanto, este trabalho foi estruturado em dois capítulos: o primeiro consiste em uma revisão narrativa para atender ao objetivo 2.2.1; e o segundo, para atender ao objetivo 2.2.2, foi realizado um estudo experimental.

3. HIPÓTESES

H0: O treinamento resistido implementado durante o desenvolvimento da HAP induzida por MCT não terá efeito sobre a atrofia muscular esquelética em ratos.

H1: O treinamento resistido implementado durante o desenvolvimento da HAP induzida por MCT ter efeito sobre a atrofia muscular esquelética em ratos.

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Capítulo 1: SKELETAL MUSCLE ATROPHY IN PULMONARY ARTERIAL HYPERTENSION: POTENTIAL MECHANISMS AND EFFECTS OF PHYSICAL EXERCISE

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Keywords: exercise intolerance; inflammation; PAH; pathological remodeling; protein degradation.

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by pathological remodeling of the pulmonary arteries, resulting in increased pulmonary vascular resistance and right ventricular overload. This condition triggers common symptoms such as dyspnea and exercise intolerance, compromising thus the quality of life of individuals affected by this pathology. Skeletal muscle atrophy is one of the main determinants of these symptoms, which is mediated by an imbalance between protein synthesis and degradation, triggered by adverse systemic adaptations promoted by PAH, such as decreased blood perfusion and increased inflammation. This review addresses the main cellular and molecular mechanisms that potentially trigger or inhibit protein degradation pathways, and how they interact in the context of PAH. Furthermore, we focus on physical exercise as a non-pharmacological approach capable of modulating muscle atrophy induced by PAH.

KEYWORDS: exercise intolerance; inflammation; PAH; pathological remodeling; protein degradation.

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare disease characterized by a mean resting pulmonary arterial pressure above 20 mmHg [1,2] and its worldwide prevalence is around 2.0 to 7.6 cases per million people. The pathophysiology of PAH involves adverse remodeling of the pulmonary artery and arterioles (vasculopathy), resulting in a progressive increase in pulmonary vascular resistance (PVR) [3].

Chronic increases in PVR lead to increased right ventricular (RV) afterload. This overload generates an adaptive response or adverse RV remodeling, resulting in pathological hypertrophy characterized by fibrotic tissue proliferation, redox imbalance, inflammation, mitochondrial dysfunction, and contractile dysfunction [4–6]. Such framework leads to RV failure characterized by reduced stroke volume (SV) and cardiac output (CO) and ultimately leads to patient's death [6,7].

The clinical manifestations commonly observed in patients with PAH (i.e., dyspnea and fatigue) result from the adverse cardiovascular adaptations [8,9]. These symptoms contribute to reduced tolerance to physical exertion, a striking feature in individuals with PAH [10].

Intolerance to physical effort in PAH is the result of a complex interaction between central factors (e.g. reduced CO and ventilatory inefficiency) and peripheral factors (e.g. rarefaction of skeletal muscle capillarization and reduced blood perfusion) that limit the adequate supply of oxygen and nutrients to skeletal muscles [11,12] which contributes directly to muscle atrophy.

Muscle atrophy is a phenomenon characterized by the progressive loss of muscle mass and function, resulting from an imbalance between the synthesis and degradation of muscle proteins [13,14]. Such process is driven by an increase in the activity of proteolytic pathways, as well as the inhibition of anabolic and myogenic pathways [15–17]. Hyperactivation of proteolytic pathways, associated with the suppression of protein synthesis mechanisms, results in progressive loss of muscle mass and strength [18].

While the molecular mechanisms underlying skeletal muscle atrophy and exercise intolerance have been extensively investigated in cardiovascular diseases, as chronic heart failure (HF), revealing key roles for neurohormonal dysregulation, systemic inflammation, and impaired mitochondrial function [19–22], the precise pathways in PAH are less well-defined and represent an area of ongoing research. Despite the

more limited understanding in PAH, evidence indicates the involvement of similar downstream effectors of muscle wasting, such as the activation of the ubiquitin-proteasome system and alterations in autophagy [23,24], highlighting potential shared pathways contributing to this debilitating feature in both conditions.

In fact, there are studies on both humans [23,25] and experimental models [24,26,27] showing that PAH leads to dysfunctions in skeletal muscle tissue, suggesting a condition of muscle atrophy. Thus, together with central dysfunctions, the atrophy process may play a central role among the factors that contribute to intolerance to physical effort in patients with PAH [23,24], and the understanding of underlying molecular mechanisms are of interest to face such a lethal disease.

Therefore, this review aims to explore the main molecular pathways involved in the development of skeletal muscle atrophy in PAH, highlighting critical mechanisms in the progression of such condition. Moreover, we analyze the therapeutic potential of physical exercise as a non-pharmacological intervention, focusing on its modulation of the molecular processes involved.

2. THE ACTIVATION OF UBIQUITIN-PROTEASOME SYSTEM IN PAH

The ubiquitin-proteasome system (UPS) is a crucial cellular mechanism responsible for the selective degradation of proteins, thus maintaining protein homeostasis and regulating several cellular processes. The UPS acts to control protein quality by degrading damaged or dysfunctional proteins, thus ensuring cellular integrity and protein functionality within the cell [28]. To this end, this system involves the marking of proteins to be degraded by ubiquitin ligases (E3 ligases), such as muscle ring finger-1 (MuRF1/TRIM63) and muscle atrophy F-box (MAFbx/atrogin-1/Fbx32). Subsequently, the ubiquitinated proteins are directed to the proteasome, a proteolytic enzyme complex responsible for protein degradation [29]. In skeletal muscle, the UPS contributes to the maintenance of cellular homeostasis by promoting the selective degradation of damaged, misfolded, or dysfunctional proteins. This process ensures protein quality and preserves the structural and functional integrity of muscle tissue [30].

In the context of PAH, the UPS is one of the possible mechanisms that may be involved in the development of muscle atrophy in this population [23,31]. Factors such as hypoxia, chronic inflammation, and oxidative stress, frequently present in patients with

PAH, can trigger excessive activation of the UPS, resulting in increased degradation of muscle proteins and, consequently, contributing to the loss of muscle mass and strength observed in this condition (Fig. 1).

Understanding how these factors interact is essential for the development of effective therapeutic strategies. The implications of inflammation, oxidative stress, myostatin, Forkhead box O (FoxO), and inhibition of Phosphoinositide 3-kinase/ Protein Kinase B/ Mechanistic Target of Rapamycin pathway (PI3K/Akt/mTOR), and AMP-activated protein kinase (AMPK) and their contributions to disease progression will be discussed below.

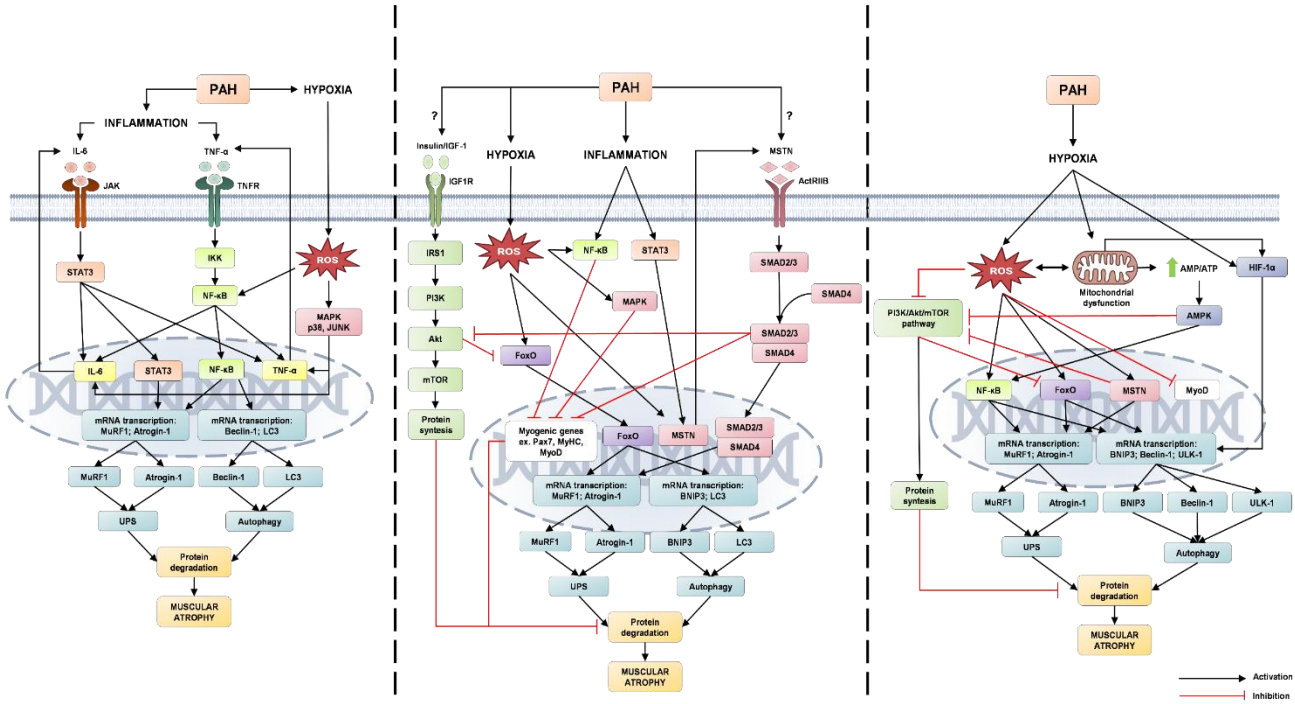

Figure 1. Pulmonary arterial hypertension-induced activation of ubiquitin-proteasome system and autophagy pathways in skeletal muscle. The scheme illustrates the possible molecular pathways for the activation of these proteolytic systems, highlighting systemic inflammation, tissue hypoxia, oxidative stress, mitochondrial dysfunction and the role of myostatin. Black arrows indicate activation, while red lines indicate inhibition. ActRIIB, activin type II receptor; Akt, protein kinase B; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; Atrogin-1, muscle atrophy F-box; BNIP3, BCL2 and adenovirus E1B 19-kDa-interacting protein 3; FoxO, forkhead box O; HIF-1α, hypoxia-inducible factor 1-alpha; IGF-1, insulin-like growth fator 1; IKK, IkappaB kinase; IL-6, interleukin 6; IRS1, insulin receptor substrate 1; JAK, Janus kinase; JUNK, Jun N-terminal kinase; LC3, microtubule-associated protein 1A/1B-light chain 3; MAPK, mitogen-activated protein kinase; MSTN, myostatin; mTOR, mechanistic target of rapamycin; MuRF1, muscle ring finger 1; MyHC, myosin heavy chain; MyoD, myoblast determination protein 1; NF-κB, nuclear transcription factor kappa B; p38, p38 mitogen-activated protein kinase; Pax7, paired box protein 7; PI3K, phosphoinositide 3-kinase; PAH,

pulmonary arterial hypertension; ROS, reactive oxygen species; SMAD, SMAD family member; STAT3, signal transducer and activator of transcription 3; TNFR, tumor necrosis factor alpha receptor; TNF-α, tumor necrosis factor alpha; ULK-1, Unc-51 like autophagy activating kinase 1; UPS, ubiquitin-proteasome system.

2.1. Inflammation

Patients with PAH generally present a chronic inflammatory condition, characterized by an increase in circulating pro-inflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) [32–34], in response to systemic and central dysfunctions (i.e., reduced blood perfusion, oxidative stress and abnormal immune cell signaling) caused by the disease [35] (Fig.1).

In this context, TNF-α plays a central role in inducing the inflammatory response, which in turn contributes to muscle atrophy. By binding to its specific receptor, TNF-α activates the nuclear transcription factor kappa B (NF-κB) pathway, mediated by IKK (IkappaB kinase). Activation of NF-κB, in turn, stimulates the transcription of genes encoding E3 ligases, such as MuRF1 and atrogin-1, which are essential for the activation of muscle proteolytic processes via the UPS [36,37].

The activation of UPS by inflammatory cytokines may also involve the pathway mediated by the signal transducer and activator of transcription 3 (STAT3), which is activated by the cytokines IL-6 and TNF-α. These cytokines induce the activation of Janus kinase (JAK), which in turn phosphorylate STAT3, leading to its activation. STAT3 hyperactivation then upregulates the expression of E3 ubiquitin ligases, promoting UPS activation [17,38]. Although this pathway has been shown to be active in cardiac tissue in a PAH model [39], it was not observed in skeletal muscle. Additional studies are needed to elucidate these speculations, especially considering the increases in IL-6 and TNF-α observed in PAH.

Hyperactivity of the transcription factors NF-κB and STAT3 not only regulates the UPS proteolytic pathway but also contributes to the perpetuation of the inflammatory environment. By activating inflammatory genes, such as TNF-α and IL-6, transcription factors establish a positive feedback loop, exacerbating inflammation and increasing the expression of pro-inflammatory cytokines that act systemically and locally [40–42]. Such chronic inflammatory cycle amplifies UPS activation, which may contribute to the progression of muscle atrophy in patients with PAH. Thus, processes modulated by inflammatory agents culminate in an increase in muscle protein

degradation, which may contribute to muscle atrophy in patients with PAH.

2.2. Myostatin

Myostatin (MSTN) or growth and differentiation factor-8 (GDF-8) is a protein of the transforming growth factor beta (TGF-β) family that acts as a negative modulator of muscle cell proliferation and differentiation in an autocrine and paracrine manner, suppressing anabolic pathways and promoting muscle proteolysis [43–45]. Under physiological conditions, skeletal muscle produces MSTN to maintain the balance between muscle protein synthesis and degradation. However, in PAH, studies show that there is hyperactivation of this protein, leading to exacerbated activation of proteolytic pathways, such as the UPS [24,27].

The MSTN activation triggers a signaling cascade that involves the activation of specific receptors on the surface of muscle cells, such as the activin type II receptor (ActRIIB). Such interaction, in turn, activates the SMAD (SMAD family member) signaling pathway, in particular the SMAD2/3 complex, which translocate to the nucleus and regulate the expression of atrophic genes (e.g. MuRF1 and atrogin-1) that activate the UPS [17,46]. Furthermore, the inflammatory environment characteristic of PAH [47] is capable of positively modulating MSTN expression through the IL-6/JAK/STAT3 pathway [48] (Fig. 1).

Concomitantly with these events, MSTN via SMAD2/3/4 and MAPK (mitogen-activated protein kinase) in synergistic action with NF-κB suppress genes involved in myogenesis, such as Pax7 (Paired box protein Pax-7), MyoD (myoblast determination protein 1) and MyHC (Myosin Heavy Chain), essential for muscle recruitment, proliferation and regeneration [49–52].

These mechanisms highlight the central role of myostatin in the regulation of muscle protein balance and in the suppression of myogenic regeneration, especially in pathological conditions such as PAH. In this context, understanding in detail the molecular pathways mediated by myostatin and its modulators becomes crucial for the development of therapeutic interventions capable of attenuating muscle atrophy and improving musculoskeletal functionality in these patients. Future studies exploring these mechanisms could contribute significantly to advances in the treatment of pathological conditions related to this system.

2.3. FoxO and inhibition of PI3K/Akt/mTOR pathway

Forkhead box O (FoxO) is a regulatory protein involved in the control of several cellular processes, including metabolism, stress resistance, cell proliferation and apoptosis [53]. The FoxO family is composed of four main members: FoxO1, FoxO3, FoxO4 and FoxO6, and each one exerts distinct functions and regulatory mechanisms. Understanding these proteins is fundamental to exploring their implications in health and disease. For example, FoxO1, mainly involved in the regulation of pancreatic beta cell function and glucose metabolism, protects against oxidative stress and plays a crucial role in the pathogenesis of diabetes [54]. FoxO3 is known for its role in apoptosis and longevity, being associated with several types of cancer and immune responses [55,56]. FoxO4 is related to cellular responses to stress and has been linked to aging and cancer [55]. FoxO6, although less studied, presents emerging evidence suggesting its influence on metabolic processes and cell survival [55].

In muscle tissue, FoxO family factors play a central role in maintaining cellular homeostasis, standing out in the regulation of protein balance [57]. Specifically, FoxO3 controls protein degradation by activating atrogenic genes, such as MuRF1 and Atrogin-1, which promote proteolysis via systems such as the UPS and lysosomal autophagy [58,59]. Such function is particularly important in conditions of metabolic stress, muscle disuse, or chronic diseases, where the imbalance between protein synthesis and degradation rules muscle atrophy.

FoxO activity is regulated by several signaling pathways, highlighting its interaction with the PI3K/Akt/mTOR anabolic pathway, which promotes the growth and maintenance of muscle mass. Phosphorylation of the Akt protein results in the inactivation of FoxO, preventing the activation of genes associated with protein degradation. Furthermore, FoxO also antagonizes the mTOR pathway, exacerbating muscle mass loss in pathological situations [58,60]. These interactions highlight the multifunctional role of FoxO, which integrates signaling processes in different tissues, with a specific relevance in skeletal muscle, where it regulates both preservation and structural remodeling in response to internal and external stimuli [17,61,62].

In certain pathological conditions, such as PAH, FoxO activity in muscle tissue may be exacerbated. This increase in FoxO activation may be triggered by the deleterious consequences of the disease, such as increased activity and expression of inflammatory cytokines and oxidative stress [33,63]. These factors contribute to Akt

inhibition and, consequently, lead to dysregulation of the PI3K/Akt/mTOR pathway, resulting in FoxO activation [37]. Furthermore, the presence of these factors may act directly in the upregulation of FoxO expression and activity, independently of the inhibition of the PI3K/Akt/mTOR pathway, highlighting the potential impact of these pathological conditions in the modulation of FoxO activity [38] (Fig. 1).

In summary, FoxO regulation in the pathological context highlights its crucial role in modulating protein homeostasis and stress response in muscle tissue. In this sense, studies evaluating this pathway in conditions such as PAH are essential to understand how FoxO dysfunction can contribute to muscle mass loss and worsening of the clinical manifestations of the disease. Although further investigation is still needed, it is possible that modulation of this pathway may offer new therapeutic perspectives to mitigate muscle atrophy and improve the quality of life of patients with PAH. Exploring these interactions could pave the way for the development of treatment strategies aimed at restoring protein balance and promoting muscle regeneration in pathological contexts.

2.4. Oxidative stress

Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant systems to neutralize them [64]. This phenomenon, frequently observed in the cardiac, pulmonary and skeletal muscle tissue of patients and animals with PAH [65–70], triggers a series of biochemical and cellular events that result in damage and dysfunction in various tissues, aggravating the complications of the disease [35].

Increased oxidative stress in peripheral tissues of patients with PAH, including skeletal muscle, is associated with decreased blood perfusion resulting from cardiovascular changes caused by the disease. Such impairment contributes to tissue hypoxia, aggravating skeletal muscle damage [35,71]. For example, tissue hypoxia induces dysfunction and damage to mitochondrial organelles [71], resulting in increased pro-oxidative agents (i.e., superoxide, hydroxyl, hydrogen peroxide), and in decreased activity and expression of antioxidant agents [i.e., superoxide dismutase (SOD) and catalase (CAT)] [72–74] (Fig. 1).

The literature suggests that redox imbalance can positively modulate the activity and expression of transcription factors, such as NF-κB, FoxO, and MSTN, in muscle

tissue, promoting UPS activation and, consequently, leading to protein degradation [75,76]. Additionally, the activation of these transcription factors, together with oxidative stress, contribute to the suppression of the PI3K/Akt/mTOR pathway and myogenic regulators such as MyoD, reducing thus the capacity for cell regeneration and proliferation in muscle tissue [76,77].

Furthermore, oxidative stress can amplify skeletal muscle atrophy by stimulating the production of pro-inflammatory cytokines, such as TNF-α and IL-6, via MAPKs, in particular p38 and JNK (Jun N-terminal kinase) proteins [76,78]. The increased inflammatory response not only activates the UPS system but also aggravates oxidative stress itself, creating a feedback loop in which the pathological processes reinforce each other [76,79,80].

Therefore, the redox imbalance propagated by PAH may contribute to the muscle atrophy associated with this pathological condition, by favoring the predominance of catabolic processes.

2.5. AMPK

The AMP-activated protein kinase (AMPK) is a heterotrimeric protein kinase composed of catalytic α and regulatory β and γ subunits. In mammals, its activation is mediated by interactions between the subunits, involving allosteric effects and covalent modifications that stimulate its kinase activity [81,82].

The main function of AMPK is to restore cellular energy balance. To this end, this kinase activates catabolic pathways that increase ATP production, such as glycolysis and fatty acid oxidation [81,82]. Simultaneously, it inhibits anabolic pathways that consume ATP, such as the PI3K/Akt/mTOR pathway [83]. In addition, AMPK positively modulates the expression of FoxO family transcription factors (1, 3 and 4), which play a crucial role in the activation of genes involved in protein degradation, specifically through the UPS proteolytic pathway [17,38].

In the context of PAH, AMPK may remain persistently hyperactive in response to mitochondrial dysfunctions associated with chronic tissue hypoxia and oxidative stress in this condition. These events compromise the efficiency of the tissue electron transport chain [84], possibly resulting in an alteration in the energy homeostasis of skeletal muscle. This condition may result in an increase in the AMP/ATP ratio, promoting the exacerbated activation of AMPK [81] (Fig. 1).

In this scenario, it is possible that AMPK plays an important role in inhibiting anabolic pathways, such as the PI3K/Akt/mTOR pathway, while activating protein degradation pathways, such as the UPS, thus favoring muscle atrophy. This imbalanced regulation between anabolic and catabolic processes may contribute to the loss of muscle mass observed in patients with PAH. Furthermore, AMPK may be implicated in the modulation of inflammation and oxidative stress, two critical factors in the progression of the disease. Therefore, AMPK emerges as a possible therapeutic target to mitigate the effects of muscle atrophy and improve musculoskeletal functionality in patients with PAH.

3. MUSCLE AUTOPHAGY IN PAH

Autophagy is an indispensable cellular process that orchestrates the degradation and recycling of intracellular components, ensuring the maintenance of homeostasis and the cellular ability to adapt to stress. Central to this process is the formation of autophagosomes, specialized structures that capture damaged organelles and misfolded proteins, subsequently directing them to lysosomes for degradation. Such highly regulated mechanism underpins a wide range of physiological functions and has far-reaching implications for health and disease, including cancer, neurodegeneration, and aging. Recent insights into the mechanisms and regulatory pathways of autophagy, such as macroautophagy, microautophagy, chaperone-mediated autophagy, and crinophagy, highlight its multifaceted role in cellular adaptation and survival [85–87].

The autophagy process can be dysregulated in response to the combined action of inflammatory agents, oxidative stress and hypoxia, factors widely associated with the complications in PAH [88–90]. Such dysfunctional interaction can lead to the exacerbated activation of autophagic genes in skeletal muscle tissue promoting protein degradation and, consequently, muscle atrophy (Fig. 1).

Inflammatory cytokines, such as TNF-α, play a central role in the activation of the IKK/NF-κB signaling pathway, which has been associated with increased expression of genes involved in the autophagic process, such as beclin-1 and microtubule-associated protein 1A/1B-light chain 3 (LC3) [91–93]. Such mechanism highlights the connection between chronic inflammation and the imbalanced regulation of autophagy that contributes to cellular alterations and dysfunctions observed in

pathological conditions.

Oxidative stress, intensified by ROS production, plays a central role in the activation of the AMPK protein. When activated, this enzyme can inhibit the PI3K/Akt/mTOR pathway, resulting in a reduction in cellular protein synthesis activity [83]. In addition, AMPK is capable of modulating the expression of autophagogens, such as ULK-1 (Unc-51 like autophagy activating kinase 1) and beclin-1 [94], promoting the activation of the autophagy process. The inhibition of the PI3K/Akt/mTOR anabolic pathway associated with ROS accumulation can also activate FoxO family transcription factors [37,38,76], which directly regulate the expression of autophagy-related genes, such as BNIP3 (BCL2 and adenovirus E1B 19-kDa-interacting protein 3) and LC3 [58]. These events highlight the close relationship between oxidative stress, metabolic regulation and autophagy in pathological conditions.

Tissue hypoxia induced by PAH leads to the activation of hypoxia-inducible factor 1-alpha (HIF-1α) [95,96], that can promote the expression of autophagic genes, such as BNIP3, a regulator of mitophagy (i.e., mitochondrial autophagy) [97].

Studies investigating the contribution of specific autophagy and mitophagy pathways in skeletal muscle tissue of patients with PAH are crucial to elucidating the molecular mechanisms underlying muscle atrophy in this condition. Furthermore, research exploring therapeutic interventions targeting the modulation of these pathways, such as selective inhibitors or strategies that restore the balance between protein synthesis and degradation, may offer new insights into minimizing muscle loss and improving the functional capacity of these individuals. Such approaches may pave the way for personalized treatments that address the challenges of PAH treatment, promoting better quality of life and tolerance to physical exertion.

4. POSSIBLE STIMULATION OF CALPAINS IN PAH

Calpains are a group of calcium (Ca^{2+})-dependent proteases that play a crucial role in the regulation of protein homeostasis and cellular remodeling. They are present in several tissues, including skeletal muscles. Within the calpain family, the most studied isoforms are calpain-1 (μ-calpain) and calpain-2 (m-calpain), which have distinct functions in protein degradation and in cellular response to different stimuli [98].

As highlighted previously, PAH triggers a series of adverse systemic changes,

including tissue hypoxia, chronic inflammation, oxidative stress and mitochondrial dysfunctions [35,99]. These anomalies may contribute to increased intracellular Ca^{2+} in peripheral tissues, like skeletal muscle [100]. Furthermore, anomalies in the excitation-contraction coupling mechanism of skeletal muscle, involving Ca^{2+} cycling regulatory proteins [e.g. ryanodine receptor (RyR), sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) and sodium-calcium exchanger (NCX)] associated with PAH [23] may intensify the Ca^{2+} overload (Fig. 2).

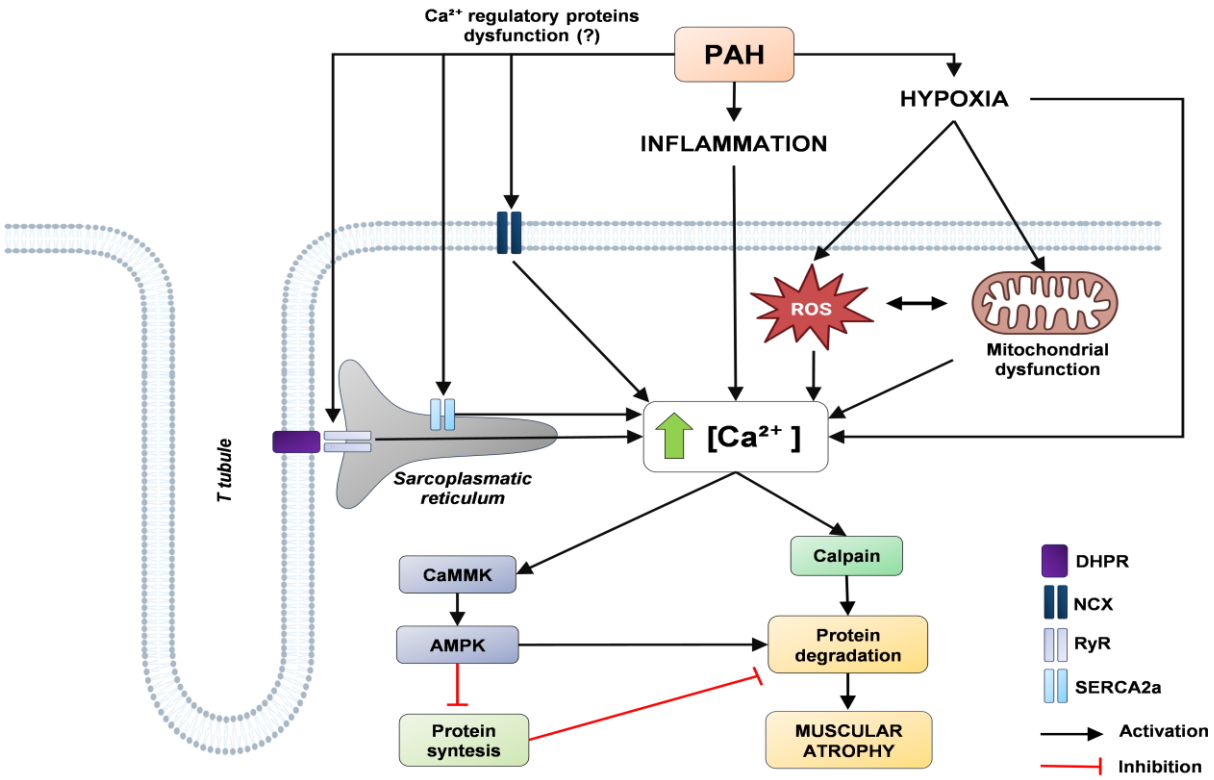

Figure 2. Calpain activation in skeletal muscle in pulmonary artery hypertension. The illustrative scheme presents the possible mechanisms for calpain activation contributing to muscle atrophy in PAH. Black arrows indicate activation, while red lines indicate inhibition. AMPK, AMP-activated protein kinase; CaMMK, calcium/calmodulin-dependent protein kinase; DHPR, dihydropyridine receptor; NCX, sodium-calcium exchanger; PAH, pulmonary arterial hypertension; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA2a, sarcoplasmic reticulum Ca^{2+}-ATPase.

Chronic elevation of intracellular Ca^{2+} concentration makes it cytotoxic, triggering a series of cellular dysfunctions, such as membrane rupture, activation of proteolytic pathways and apoptosis [101,102]. Among the proteolytic pathways activated by excessive Ca^{2+}, the calpain pathway stands out, which plays a fundamental role in protein degradation and in the regulation of the cellular response to damage [103].

Thus, excessive intracellular Ca^{2+} induced by pathological adaptations to PAH [35,99] can stimulate the activation of calpains, resulting in dysregulated cleavage of structural cytoskeletal proteins and muscle contractility regulators, such as troponin and tropomyosin. Such process alters the dynamics of muscle contraction and may contribute to the development of muscle atrophy in this clinical condition. Furthermore, increased intracellular Ca^{2+} can activate AMPK through calcium/calmodulin-dependent protein kinase (CaMKK) [104], further intensifying the process of muscle atrophy in patients with PAH.

Studies investigating the interaction between exaggerated intracellular Ca^{2+}, proteolytic pathways such as calpains, and AMPK activation in PAH models are essential to deepen our understanding of the mechanisms that contribute to muscle atrophy in this context. Understanding these processes may open new therapeutic perspectives, aiming at modulating the activity of these pathways and, thus, mitigating the loss of muscle mass observed in patients with PAH. Exploring these approaches may lead to the development of more effective strategies for the treatment and prevention of muscle complications associated with this debilitating clinical condition.

5. FIBER TYPE SHIFT AND MUSCLE MASS LOSS IN PAH

In patients with PAH, a transition from slow-twitch fibers (type I), which have high oxidative capacity and resistance, to fast-twitch fibers (type II), that are predominantly glycolytic and more vulnerable to fatigue, is commonly observed. This phenomenon reflects the adaptive responses of skeletal muscle to the adverse environment imposed by PAH, that affects both oxidative capacity and muscle resistance. As a result, an increase in intolerance to physical effort is observed, exacerbating the functional limitation and quality of life of patients [23,25,105]. The phenotypic change of muscle fibers may be an adaptive response to the chronic hypoxic environment, associated with increased oxidative stress and systemic inflammation, and mitochondrial dysfunctions induced by PAH [35,105] (Fig. 3).

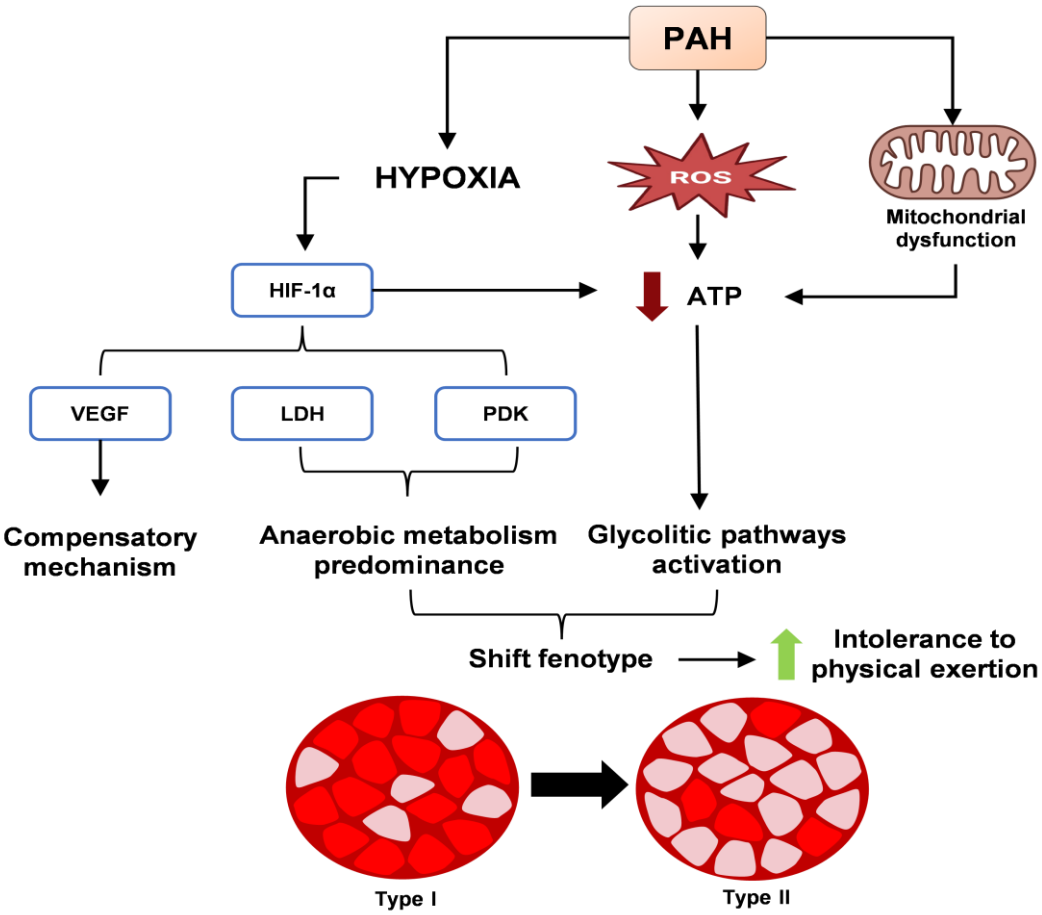

Figure 3. Skeletal muscle fiber type shifting in skeletal muscle in pulmonary arterial hypertension. Black arrows indicate activation; red arrow indicates decrease; green arrow indicates increase. ATP, adenosine triphosphate; HIF-1α, hypoxia-inducible factor 1-alpha; LDH, lactate dehydrogenase; PAH, pulmonary arterial hypertension; PDK, pyruvate dehydrogenase kinase; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

One of the cellular mechanisms that potentially drive fiber type shift is the action of HIF-1α. Under hypoxic conditions, HIF-1α is activated and it regulates the expression of genes that favor predominantly glycolytic metabolism [95,96]. For instance, the increased expression of enzymes that promote anaerobic glycolysis, such as PDK (pyruvate dehydrogenase kinase) and LDH (lactate dehydrogenase) [96,106], which may promote the predominance of type II fibers. Additionally, as a compensatory mechanism to the hypoxic environment, HIF-1α stimulates the production of vascular endothelial growth factor (VEGF) that stimulates angiogenesis [96,107]. However, such adaptations may not be sufficient to compensate for the metabolic impairments triggered by PAH.

Furthermore, it is possible that the action of HIF-1α together with the

mitochondrial detriments observed in PAH driven by hypoxia and oxidative stress result in the transition of muscle fibers, compromising the production of ATP by aerobic pathways [84]. As a consequence, skeletal muscle becomes predominantly dependent on glycolytic pathways for energy generation, a phenomenon similar to the Warburg effect, where there is an adaptive preference for anaerobic metabolism due to the limited availability of oxygen [84,108].

Thus, the transition from slow- (type I) to fast-twitch fibers (type II) observed in PAH makes individuals affected by this pathology more susceptible to muscle atrophy. Such occurrence is because type II fibers are more vulnerable to atrophy than type I fibers [109,110].

Initially, the change in fiber type may be a compensatory response to increase energy production in muscle tissue in face of the adverse conditions imposed by PAH. However, in the long term, this adaptation may increase vulnerability to muscle fatigue, where the predominance of type II fibers, associated with the activation of proteolytic pathways, causes the degradation of muscle proteins, which may contribute to the atrophy and exercise intolerance observed in patients with PAH.

Further studies evaluating how muscle fiber transition occurs in PAH may provide crucial information on the adaptive and pathological mechanisms that affect skeletal muscle in response to chronic hypoxia and oxidative stress. Understanding these processes may reveal important therapeutic targets to lessen the effects of muscle atrophy and loss of function in patients with PAH. Furthermore, the identification of molecular factors that regulate the transition from slow- to fast-twitch fibers may help in the development of treatment strategies that promote the preservation of muscle mass and improve the functional capacity of these patients, reducing thus intolerance to physical effort and improving quality of life.

6. MODULATION OF MUSCLE ATROPHY BY PHYSICAL EXERCISE IN PAH

Physical exercise exerts a profound influence on skeletal muscle by modulating various functions and signaling pathways, including the ubiquitin-proteasome system [111,112], inflammation [113], myostatin [114], PI3K/Akt/mTOR [115], FOXO3 and AMPK [116], oxidative stress [117], calpains [118], muscle fiber type transitions [119] and autophagy [120]. These adaptations are dependent on the type, intensity, and duration of exercise. In

the context of health and disease, such modulation plays a pivotal role in preserving and enhancing muscle function, countering atrophy, and improving overall muscle health. Understanding these mechanisms is particularly critical in pathological conditions like PAH, where skeletal muscle dysfunction is a prominent contributor to exercise intolerance and impaired quality of life. Elucidating the exercise-induced regulation of these pathways provides a foundation for developing targeted interventions to optimize muscle health in PAH and other chronic diseases.

Patients with PAH exhibit skeletal muscle dysfunctions characterized by diminished contractile capacity, mitochondrial abnormalities, elevated skeletal muscle proteolysis, and suppression of anabolic pathways associated with muscle hypertrophy. These alterations contribute to significant muscle atrophy and a marked reduction in exercise tolerance, further exacerbating the systemic impact of PAH [11,23,73,105]. In this context, physical exercise has emerged as a cornerstone in rehabilitation programs for PAH, demonstrating significant efficacy in enhancing exercise tolerance and improving the patients' quality of life. These benefits are attributed to the ability of exercise to counteract skeletal muscle atrophy and dysfunctions through the modulation of key cellular processes and signaling pathways [25,121]. Understanding the intricate mechanisms underlying these improvements is essential for optimizing exercise-based interventions and tailoring rehabilitation strategies to the unique physiological challenges posed by PAH (Table 1).

Table 1. Effects of physical exercise on skeletal muscle in pulmonary arterial hypertension.

Study	Sample	Exercise protocol	Main effects
Vieira et al. (2020)	• C57BL6/J mice (male) • n not informed • MCT model (80 mg/kg injected every 20 days)	Aerobic exercise (Treadmill): • 4 weeks • 60 minutes • 5 times/week • 60% of the maximum speed achieved in the progressive exercise test	↑ Exercise tolerance ⛊ CSA type IIX/B fibers
Drummond et al. (2023)	• Wistar rats (male) • n = 8 • MCT model (single injection of 60 mg/kg)	Aerobic exercise (voluntary running) • Free access rodent wheel throughout the experimental protocol	↑ Muscle fiber proportion ↓ ECM porportion
Soares et al. (2023)	• Wistar rats (male) • n = 8 • MCT model (single injection of 60 mg/kg)	Resistance exercise (ladder climbing): • 4 weeks • 5 times/week • 15 climbs with 1 minute rest • 55–65% calculated from the exercise tolerance test	↑ CSA ↓ Collagen deposition ↑ Exercise tolerance ⛊ Oxidative stress
Becker et al. (2020)	• Wistar rats (male) • n = 8 • MCT model (single injection of 60 mg/kg)	Aerobic exercise (Treadmill): • 4 weeks • 50 minutes • 5 times/week • 60% of the maximum speed achieved in the progressive exercise test	⛊ Oxidative stress ↓ Lipid peroxidation ↑ Antioxidant enzymes
Brown et al. (2017)	• Sprague-Dawley rats (male) • n = 7-8 (CExT and HIIT groups, respectively) • MCT model (single injection of 40 mg/kg)	Aerobic exercise (Treadmill): • CExT: 6 weeks; 30 minutes increasing to 60 minutes by the end of week 2; 5 times/week; 50% of the maximum speed achieved in the progressive exercise test • HIIT: 6 weeks; 6-min warm up at 50% and then proceeded into five 5-min cycles of alternatinghigh- and low-intensity intervals as follows: 2 min at 85–90% and 3 min at 30% (totaling 30 min)	↑ GLUT-1 expression (CExT) ↓ GLUT-1 expression (HIIT)
De Man et al. (2009)	• Patients with iPAH (both sex) • n = 19 (4 male and 15 female)	Combined exercise (aerobic + resistance) • 12 weeks • 3 times/week • Aerobic training: cycling; 50-75% VO_{2max}; 2-5 minutes with 2 minutes rest; 5-10 sets • Resistance training: 30-75% ORM; 12-60 repetitions with 1 minute rest; 3-6 sets	↑ Capillarization ↑ Activity of oxidative enzymes ↔ CSA

↑ indicates increases or improves; ↓ indicates reduces or decreases; ⛊ indicates mitigates, delay or protects; ↔ indicates unchanged. CExT, contínuos aerobic exercise training; CSA, cross-sectional area; ECM, extracellular matrix; HIIT, high intensity interval training; iPAH, idiopathic pulmonary arterial hypertension; MCT, monocrotaline; ORM, one repetition maximum.

In this context, Moreira-Gonçalves and colleagues [24] demonstrated, in a model of PAH induced by monocrotaline, a significant reduction in muscle mass and cross-sectional area (CSA) of the gastrocnemius muscle. Notably, the employed aerobic exercise not only mitigated these reductions but also improved exercise tolerance, highlighting its protective effects on skeletal muscle integrity [122]. Complementing these findings, Vieira and collaborators [122] reported that monocrotaline-induced PAH led to pronounced atrophy of type IIX/B skeletal muscle fibers in mice. Interestingly, animals subjected to treadmill-based aerobic exercise were spared from such reductions, further emphasizing the role of physical exercise in preserving muscle fiber composition and function under pathological conditions.

The study by Drummond et al. [123], further revealed that voluntary running

enhanced the structural integrity of the gastrocnemius muscle in rats with monocrotaline-induced PAH by increasing the proportion of muscle fibers and reducing extracellular matrix deposition, both of which were disrupted in PAH. Similarly, Soares and colleagues [69] reported compelling evidence that resistance exercise effectively mitigates adverse skeletal muscle remodeling, such as reductions in CSA and excessive collagen accumulation, induced by monocrotaline-induced PAH. Such remodeling attenuation was accompanied by an improvement in exercise tolerance, underscoring the therapeutic potential of resistance exercise. While the precise molecular mechanisms underlying skeletal muscle atrophy in these studies were not directly investigated, the findings collectively highlight the pivotal role of different exercise modalities in preserving skeletal muscle mass, structure, and functionality in the context of PAH.

In a related context, Becker and coworkers [124] demonstrated the critical role of aerobic exercise in modulating oxidative stress-related pathways in experimental PAH. Their study revealed that four weeks of aerobic exercise not only mitigated oxidative damage in the gastrocnemius muscle but also enhanced the redox balance, evidenced by a reduction in lipid peroxidation and an upregulation of key antioxidant enzymes, such as superoxide dismutase and catalase. These adaptations reflect the muscle's improved capacity to counteract oxidative insults, which are strongly implicated in inflammation, proteolysis, and impaired protein synthesis, key drivers of skeletal muscle atrophy [76]. Collectively, these findings underscore the therapeutic potential of resistance exercise in addressing oxidative stress and its downstream effects on skeletal muscle integrity and function in PAH models.

This metabolic shift highlights the glycolytic dominance in skeletal muscle as a hallmark of PAH-induced dysfunction, which is intricately linked to reduced oxidative capacity and exercise intolerance. Indeed, PAH is characterized by a shift in muscle fiber composition, where a greater proportion of glycolytic fibers and a reduced presence of oxidative fibers contributes to muscle atrophy [23,105]. In patients with PAH, a 12-week rehabilitation program including aerobic and resistance exercises improved cardiovascular response during exercise and reduced the proportion of type IIX fibers in the quadriceps [25]. In the rat model of monocrotaline-induced PAH, Brown and colleagues [125] reported an increase in glucose transporter 1 (GLUT-1) in soleus muscle, thereby promoting a shift to glycolytic metabolism. Although the applied aerobic exercise was not able to reduce such an increase compared with the not

exercised animals, the exercised rats showed similar levels when compared with the control animals without PAH.

It is well established that changes in fiber composition are linked to reduced peripheral blood flow, which impairs O_2 uptake and contributes to muscle dysfunction in PAH [23,126]. As demonstrated by De Man and colleagues [127], a rehabilitation program containing aerobic and resistance exercises increased capillarization and the activity of oxidative enzymes in the quadriceps, although CSA did not change. These findings are crucial as they help improving exercise tolerance and overall quality of life in patients with PAH [126].

Complementarily, Esposito et al. (2011) [128] in their study involving patients with chronic heart failure (CHF) demonstrated that a rehabilitation program involving aerobic exercise (i.e., cycle ergometer) and knee extension led to improvements in muscle structure and in the coupling of peripheral convective-diffusive O_2 transport, according to Wagner's diagram [129–132], thus, resulting in increased exercise tolerance in this condition. Given the cardiopathological nature of PAH and CHF, it is plausible that similar interventions may yield analogous benefits. Improved blood perfusion and oxygen delivery could negatively modulate previously described proteolytic pathways and the phenotypic transition of muscle fibers, potentially attenuating the tissue hypoxia observed in PAH.

Despite significant progress in understanding physical exercise effects on the structure, remodeling, and critical signaling pathways of skeletal muscle in PAH, several avenues warrant further exploration. Future research should aim at investigating the intricate interplay between pathways such as inflammation, oxidative stress, and glycolytic metabolism, rather than examining them separately. A more integrative approach could provide a more comprehensive understanding of how different types, intensities, and durations of exercise influence muscle remodeling in the context of PAH. Additionally, expanding research beyond animal models and small patient cohorts to include larger and more diverse clinical populations would improve the generalizability of findings and enhance their clinical applicability.

Comparative studies evaluating the effects of distinct exercise modalities (i.e., aerobic, resistance, high-intensity interval training (HIIT), or combined approaches) on multiple aspects of muscle remodeling and their underlying molecular pathways are also needed. Such investigations could inform the development of evidence-based interventions that maximize therapeutic outcomes for patients with PAH. Furthermore,

exploring how exercise modulates adaptive responses at various stages of PAH progression represents an important area for future inquiry. Understanding these stage-specific dynamics could enable the design of personalized exercise regimens tailored to the evolving physiological needs of patients throughout the course of the disease.

The existing body of research has provided valuable insights into the potential of physical exercise to modulate skeletal muscle structure, remodeling, and signaling pathways in PAH. These findings have laid a strong foundation for understanding the therapeutic role of exercise in counteracting muscle dysfunction and improving patient outcomes. By addressing the outlined research gaps, future studies can build upon this knowledge, driving innovation in exercise-based interventions and advancing the field toward more effective, personalized strategies for managing PAH and its systemic impacts.

Clinical studies from the past five years have shown that aerobic exercise [133,134], as well as inspiratory exercises [135,136] and combined exercise (i.e., aerobic and resistance) [137–139], have been shown to be effective in patients with PAH by promoting improvements in exercise tolerance, quality of life, and muscle strength, as well as potential beneficial effects on right ventricular function and pulmonary hemodynamics. However, due to the scarcity of reports, it is necessary to investigate the underlying mechanisms of muscle atrophy and the positive impact of different types of physical exercise in PAH.

Finally, the summarized impacts of PAH on skeletal muscle highlighting the pathophysiological events that can lead to muscle atrophy and dysfunctions as well as the potential effects of physical exercise are presented in figure 4. PAH features systemic inflammation and tissue hypoxia, which triggers redox imbalance, pathological remodeling of skeletal muscle, and changes in the muscle fiber profile, culminating in reduced tolerance to physical exertion.

In conclusion, physical exercise seems to be promising non-pharmacological intervention capable of modulating these deleterious processes, preserving muscle function and contributing to improving functional capacity in patients with PAH.

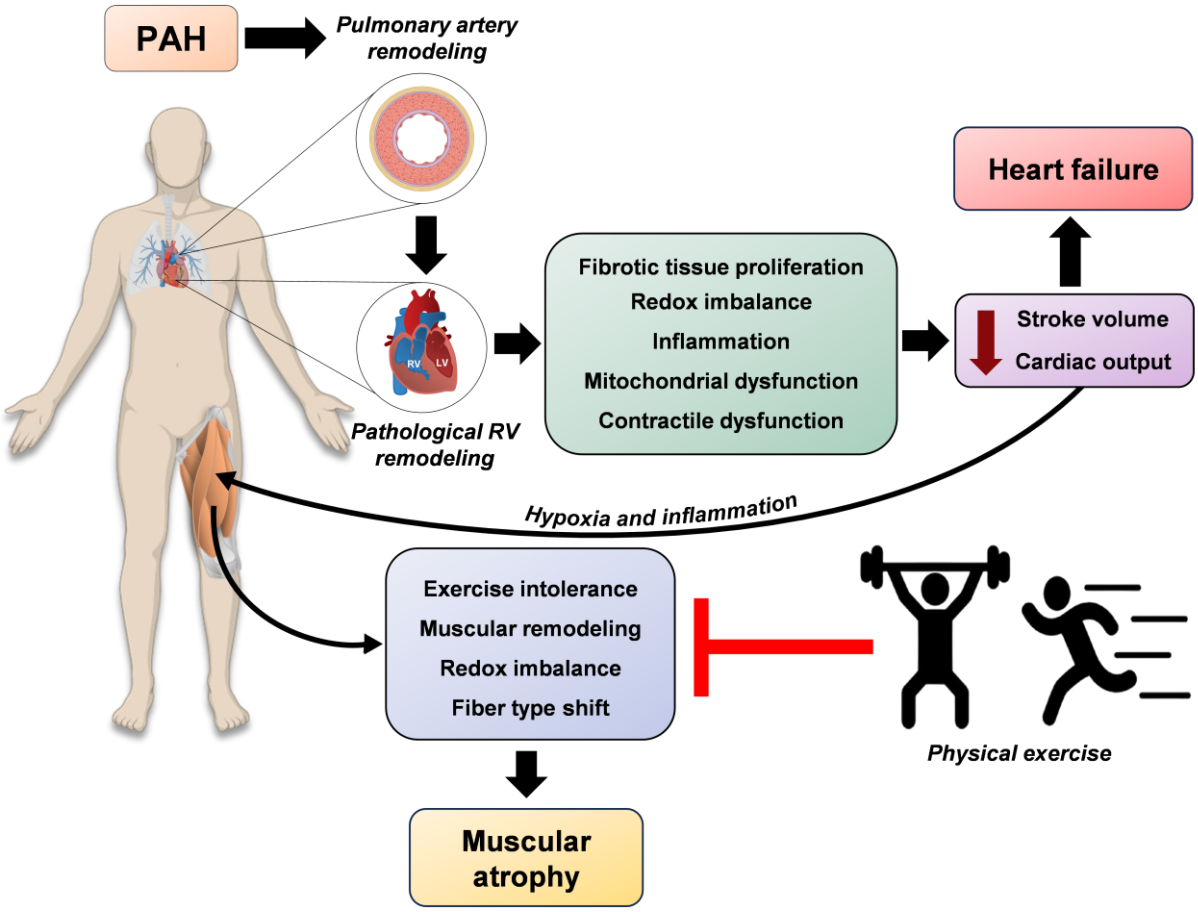

Figure 4. Summarizing diagram of the pathological mechanisms involved in skeletal muscle atrophy and dysfunctions induced by pulmonary arterial hypertension, and the potential effects of physical exercise in modulating these mechanisms. LV, left ventricle; PAH, pulmonary arterial hypertension. RV, right ventricle. Black arrows indicate the activation or progression of pathological processes, while the red arrow represents the attenuating effect of physical exercise as a therapeutic intervention.

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Author contributions

Conception and design of the research: SFF Costa; Conceptualization, data curation, writing, editing and visualization: SFF Costa, LL Soares, AMO Portes, LB Leite and AJ Natali. Critical review of the manuscript: LL Soares and AJ Natali.

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Competing interests

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Inoperable or Persistent Chronic Thromboembolic Pulmonary Hypertension. *Respiration* **100**, 369–378 (2021).

Capítulo 2: Resistance exercise training attenuates skeletal muscle atrophy in experimental pulmonary arterial hypertension

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Keywords: muscular atrophy; monocrotaline; histology; proteolysis; oxidative stress.

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ABSTRACT

Aim: To evaluate the effects of a RT program applied during the development of MCT-induced PAH on skeletal muscle atrophy in rats. **Methods:** Forty-two male Wistar rats were randomly distributed into three experimental groups (n = 14 per group): Sedentary Control (SC), Sedentary Hypertensive (SH), and Trained Hypertensive (TH). PAH was induced by a single intraperitoneal injection of monocrotaline (MCT; 60 mg/kg). Animals in the TH group underwent RT (vertical ladder; 15 climbs with 1-minute interval; 60% of the maximum load supported), 1 session/day, 5 days/week, for approximately 3 weeks. On the 24th day after injection, all animals were euthanized. Subsequently, the brachial biceps were removed, processed and destined for histological or biochemical analyses. **Results:** RT increased the tolerance to physical effort (i.e., maximum load supported) in rats with PAH. In addition, RT prevented adverse remodeling in skeletal muscle by preserving the number and cross-sectional area of myocytes and attenuated total collagen deposition. Furthermore, RT mitigated the gene expression of proteolytic agents (i.e., MuRF1, atrogin-1, and myostatin) and attenuated redox imbalance (i.e., CAT, NO, and CP). However, neither PAH nor RT influenced muscle hypertrophy pathways (i.e., Akt, phospo-Akt, eIF4E e phospo-eIF4E) in this model. **Conclusion:** The RT applied during the development of MCT-induced PAH protects against skeletal muscle atrophy, by mitigating adverse structural remodeling and atrophy through proteolysis modulation and attenuation of redox imbalance.

KEYWORDS: muscular atrophy; monocrotaline; histology; proteolysis; oxidative stress.

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by increased pulmonary vascular resistance, leading to elevated pulmonary artery pressure and right ventricular (RV) overload, which often progresses to heart failure and high mortality rates [1]. Although RV dysfunction is the main consequence, left ventricular impairment and systemic repercussions contribute to skeletal muscle atrophy and reduced exercise tolerance, significantly affecting the patients' quality of life [2–4]. Although advances in drug therapy have improved survival [5,6], current treatments are still insufficient to prevent or reverse cardiopulmonary and skeletal muscle remodeling, highlighting the need for complementary interventions.

Muscle fatigue is a prevalent and debilitating symptom in PAH, affecting approximately 93% of patients [7]. This manifestation is associated with the systemic consequences of RV dysfunction, which continuously induce structural and metabolic changes in skeletal muscle [8–12]. These modifications, such as activation of proteolytic pathways (i.e., ubiquitin proteasome, autophagy, and calpains), redox imbalance, and mitochondrial dysfunction, can result in progressive muscle atrophy and functional decline [13–15].

The monocrotaline (MCT)-induced PAH model is widely used to investigate critical aspects of the pathophysiology of the disease, including pulmonary vascular remodeling, ventricular dysfunction, metabolic alterations, and skeletal muscle impairment [16–18]. Regarding musculoskeletal dysfunctions, the disease significantly compromises muscle homeostasis, resulting in mitochondrial dysfunctions that lead to reduced oxidative capacity and increased oxidative stress [19–21]. These changes, associated with the activation of proteolytic pathways and the suppression of anabolic mechanisms, exacerbate skeletal muscle atrophy and exercise intolerance [22–24].

Although previous studies have investigated the mechanisms of muscle atrophy in PAH [13–15], the effects of resistance training (RT) on this process are still poorly understood. Emerging evidence suggests that RT protects against skeletal muscle loss in PAH [4,21,25], but the underlying molecular mechanisms have not yet been fully elucidated. Considering the potential of this intervention to preserve skeletal muscle mass and functionality, this study investigated the effects of RT applied during the progression of MCT-induced PAH on skeletal muscle atrophy.

2. METHODS

2.1. Experimental Design

Forty-two young Wistar rats (Body weight: ~ 200 g) were used to carry out this study. The animals were housed in polyethylene boxes (3-4 animals per box). The housing environment was maintained at a controlled temperature of 22 ± 1 °C, relative humidity of approximately 60%, and a 12-hour light/dark cycle. Throughout the experimental protocol, the rats had free access to water and food. The animals were randomly distributed into three experimental groups (n = 14 per group): Sedentary Control (SC), Sedentary Hypertensive (SH), and Trained Hypertensive (TH). The experimental protocol was approved by the Ethics Committee for the Use of Animals of the Federal University of Viçosa (no. 11/2022 – Anexo 2), following the guidelines established by the Guide for the Care and Use of Laboratory Animals.

2.2. Induction of experimental PAH

For the induction of experimental PAH, animals in the SH and TH groups received a single intraperitoneal injection (60 mg/kg body weight) of MCT (Sigma-Aldrich, St. Louis, MO, USA) dissolved in saline solution (140 mM NaCl; pH 7.4) [26]. Animals in the SC group received the same volume with just saline solution (140 mM NaCl; pH 7.4).

2.3. Resistance exercise training protocol

The RT protocol was adapted from Hornberger & Farrar (2004) [27], using a vertical ladder (1.1 m height, 0.18 m width, 2 cm between steps, 80º incline). Initially, the animals were familiarized with the protocol over the course of two weeks. In the first week, the rats performed 10 climbs per day, interspersed with one minute of rest at the top of the ladder in a cage (20 x 20 x 20 cm), for 5 days. The climbs were performed with a device attached to their tails, but without additional weight. On the second week, the same adaptation procedure was repeated, but the animals carried ~15 g in the device attached to their tails.

Following the adaptation, a maximum load test was conducted, starting with

75% of the animal's body weight. The load was augmented by 30 g after each successful climb, with a 2-minute rest between attempts. Such procedure occurred until the animal failed. The maximum weight carried was recorded as the maximum carrying load.

Training sessions were performed 5 times per week at 60% of the maximum carrying load test, with each session consisting of 15 climbs and 1-minute rest between climbs. The exercise intensity follows the recommendations for patients with cardiovascular diseases [28] and has been used in previous investigations conducted by our research group using the same experimental model of PAH [4,21,29,30]. The maximum load test was repeated on day 21 after MCT administration to assess the physical effort tolerance. The relative maximum load was determined by dividing the maximum carrying load by body weight.

2.4. Echocardiographic examination

Echocardiography was performed on day 23 after MCT administration to confirm the development of PAH in animals in the SH and TH groups, as previously described [3,21]. The presence of PAH in these groups was assessed using the tricuspid valve annular plane systolic excursion (TAPSE) and pulmonary artery resistance, determined by the ratio between acceleration time (AT) and ejection time (ET) of the right ventricle (RV).

2.5. Sample collection and processing

2.5.1. Euthanasia

The 24th day after MCT administration was established for euthanasia of animals in the experimental groups (SH and TH). This time point was chosen as it represents the midpoint of heart failure development, as described in previous studies from our laboratory [3,20,26]. Animals in the SC group were euthanized on the same day to ensure consistency in the analyses.

Euthanasia was performed by decapitation, without prior anesthesia, using a specific guillotine for rodents (Insight EB 271, Ribeirão Preto, SP-Brazil). Then, the left and right biceps brachii muscles were dissected, weighed, and stored for later

analyses. Additionally, the heart was also dissected, fragmented, and weighed to evaluate the development of experimentally induced PAH.

2.5.2. Histological and stereological analysis

Fragments from the upper medial third of the left biceps brachii were stored in 10% buffered formalin fixative solution for 24 hours. After the fixation period, the samples were dehydrated in an increasing series of ethanol (70-100%) and embedded in resin. Semi-serial histological sections, 3 µm thick, were obtained using a rotary microtome (RM 2255 Leica Biosystems, Nussloch, Germany). To avoid analysis of repeated areas, one in every 10 sections was used, respecting a minimum interval of 30 µm between sections. The histological preparations were then stained with hematoxylin and eosin (H&E) to measure cross-sectional area (CSA), proportion of muscle fibers and extracellular matrix (ECM) or with picrosirius red to quantify total collagen content.

Slides were viewed under a brightfield photomicroscope (Olympus BX-53, Tokyo, Japan) and the images captured using a digital camera (Olympus AX-70; Tokyo, Japan). For CSA analysis, 30 random cells were selected per animal, while the other analyses were performed using 10 random images (20x magnification) per animal/organ. All analyses were conducted with 7 animals per group.

The proportions of muscle fiber and ECM were determined using the software ImageJ® (National Institute of Health, USA). To evaluate the total collagen and myocyte CSA, the software Image ProPlus 4.5 (Media Cybernetics, Silver Spring, EUA) was used.

2.5.3. Gene expression analysis by Real-Time PCR

To extract total RNA, approximately 100 mg of right biceps brachii fragments were homogenized in Trizol Reagent (Invitrogen) and processed with the addition of chloroform. After centrifugation, the RNA was precipitated with isopropyl alcohol, washed with 75% ethanol, and resuspended in DNAse- and RNAse-free water.

The cDNA synthesis was performed from 2 µg of total RNA, using Oligo dT as a primer. The samples were incubated at 70°C for 5 minutes, followed by incubation on ice. Then, a mixture containing nucleotides (dNTPs), RNAse inhibitor, reverse

transcriptase, $MgCl_2$, and buffer was added, and the reaction was incubated at 44°C for 1 hour and 30 minutes.

Gene expression quantification was performed by Real-Time polymerase chain reaction (RT-PCR) in an Applied Biosystems 7300 system using SYBR Green. Reactions, with a final volume of 20 µL, included cDNA, specific primers and SYBR Green Master Mix. Cycling conditions followed the manufacturer's protocol and results were normalized using β-actin as an endogenous control. The specific primers (Thermo Fisher Scientific, EUA) used for real-time PCR analysis were: MuRF1 (forward 5'-CCC CTT ACA AAG CAT CTT CCA-3' and reverse 5'-TGT TTT CCT TGG TCA CTC GG-3', atrogin-1 (forward 5'-CAA CAG ACT GGA CTT CTC GAC-3' and reverse 5'-GAA GTT CTT TTG GGC GAT GC-3'), myostatin (5'-forward ACG ATT ATC ACG CTA CCA CG-3' and reverse 5'-CTT TCC ATC CGC TTG CAT TAG-3'), calpain-1 (foward 5'-ACC CAG GAA CTA GAT GAC CAG-3' and reverse 5'-CTC CTT GAC ACT GAT CTC CAT G-3') and β-actin (forward 5'-CAC TTT CTA CAA TGA GCT GCG-3' and reverse 5'-CTG GAT GGC TAC GTA CAT GG-3').

2.5.4. Westen blotting

The expression of Akt, phospho-Akt, eIF4E, and phospho-eIF4E proteins was determined by Western blotting as described previously [31]. Frozen fragments of the right biceps brachii (approximately 100g) were homogenized in RIPA buffer (150 mM NaCl, 50 mM Tris-HCl [pH 8.0], 0.1% sodium lauryl sulfate, 1% NP-40, and protease and phosphatase inhibitor cocktail [1:100; Sigma-Aldrich, St. Louis, MO; PhosSTOP 1:10; Roche, Basel, Switzerland]). Insoluble biceps tissues were removed by centrifugation at 3,000 g, 4°C, for 10 min. Samples were loaded and subjected to 8–10% polyacrylamide gel electrophoresis by SDS-PAGE. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ). Sample loading (40 µg) and transfer efficiency were verified by staining with 0.5% Ponceau S on the blot membrane. The membrane was incubated in blocking buffer (5% nonfat dry milk, 10 mM Tris HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and then incubated with specific antibodies overnight at 4°C. Detection of labeled proteins was performed using the Odyssey® Fc imaging system (LI-COR; Lincoln, NE, USA).

The following primary antibodies were used: Akt-1 (#9272), phospho-Akt-1

(Ser473) (#9271), eIF4E (#9742), phospho-eIF4E (Ser209) (#9741), and GAPDH (#97166), all from Cell Signaling Technology (Danvers, MA, USA). All primary antibodies were used at 1:1000 dilution, and the secondary antibodies used were IRDye® 680RD or 800CW (1:15,000; LI-COR, Lincoln, NE, USA). Bands were analyzed with ImageJ® software (National Institute of Health, USA).

2.5.5. Analysis of antioxidant enzymes activity and oxidative stress markers

To determine the activity of antioxidant enzymes, the left biceps brachii stored at -80°C (100 mg/fragment) was homogenized in PBS and centrifuged at 3,500 × g for 10 minutes at 4°C. The supernatant obtained was used to analyze the activities of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST). The activity of SOD was estimated by the pyrogallol method, based on the ability of this enzyme to catalyze the reactions of superoxide (O^{-2}) and hydrogen peroxide (H_2O_2) [32]. The activity of CAT was evaluated by measuring the decomposition rate of H_2O_2, according to Aebi (1984) [33]. The activity of GST was determined as previously described [34] based on formadinitrochlorobenzene (CDNB) conjugated with glutathione. Malondialdehyde (MDA) levels in biceps brachii homogenate were used to assess lipid peroxidation in this organ [35]. Nitric oxide (NO) production was indirectly measured by nitrite levels in the supernatant of tissue homogenates using the Griess reaction [36]. To measure carbonyls protein (CP), protein oxidation was assessed by the method of de Levine et al. (1990) [37], quantifying the carbonyl groups present in the protein structure. Total protein (TP) content was measured to normalize the results of SOD, CAT, GST and MDA assays, using the method described by Lowry et al. (1951) [38].

4.6. Statistical analysis

The data were subjected to the Shapiro-Wilk test to verify normality. One-way analysis of variance (ANOVA) was used for comparison between groups when data presented normal distribution, followed by Tukey's post hoc test for multiple comparisons. For non-parametric data, the Kruskal-Wallis test was used for between groups comparisons, followed by Dunn's post hoc test for multiple comparisons. A mixed design ANOVA (Split plot ANOVA), followed by Bonferroni’s post-hoc test was

used to compare maximum load. Pearson's correlation coefficient was used to evaluate the relationship between CSA and the percentage of total collagen, being the correlation (r) classified as insignificant (0.0–0.3), weak (0.3–0.5), moderate (0.5–0.7), strong (0.7–0.9) and very strong (> 0.9) [39]. The data are presented as means ± SD. An alpha error probability of up to 5% was considered. All analyses were performed using GraphPad Prism Software version 8.0.2.

3. RESULTS

3.1. General parameters and echocardiographic data

Rats induced to PAH (SH and TH groups) exhibited significantly lower mean values of final BW and Δ BW than those in the control group (SC) (Table 1). Regarding the absolute weight of the biceps brachii and its ratio to BW (Biceps/BW) were reduced in the groups with PAH, compared with SC group. However, RT was able to attenuate such reduction (i.e., SH < TH).

The animals in the SH groups showed an increase in the absolute weight of the heart, RV and their ratios to BW, when compared with rats in the SC group, indicating pathological cardiac hypertrophy induced by the disease. However, RT prevented the increase in the weight of these cardiac structures (i.e., SH > TH).

The SH group exhibited significantly lower AT/ET ratio and TAPSE values compared with the SC group, respectively. However, RT implemented in the TH group partially mitigated the cardiopulmonary impairments induced by the disease, as indicated by improved AT/ET ratio and TAPSE values, compared with those of SH group. Despite these improvements, the TH group values remained significantly lower than those observed in the SC group.

Table 1. Effects of resistance exercise training on body weight, tissue weight, ratios, and right ventricular function.

	SC	SH	TH
Organs parameters			
BW initial (g)	196.70 ± 5.85	199.30 ± 4.03	199.70 ± 4.65
BW final (g)	281.40 ± 13.64	255.60 ± 23.98*	261.10 ± 8.86
Δ BW (g)	84.71 ± 14.22	56.29 ± 24.03*	61.43 ± 7.53*
Biceps brachii (g)	0.17 ± 0.01	0.12 ± 0.01*#	0.15 ± 0.02*
Heart (g)	0.99 ± 0.07	1.30 ± 0.08*#	0.93 ± 0.06
RV (g)	0.19 ± 0.03	0.41 ± 0.05*#	0.21 ± 0.04
Biceps/BW (mg/g)	0.61 ± 0.03	0.45 ± 0.07*#	0.56 ± 0.09
Heart/BW (mg/g)	3.50 ± 0.31	5.5 ± 0.86*#	3.50 ± 0.17
RV/BW (mg/g)	0.67 ± 0.12	1.60 ± 0.23*#	0.83 ± 0.13
RV function			
AT/ET	0.60 ± 0.05	0.21 ± 0.01*#	0.46 ± 0.06*
TAPSE	2.14 ± 0.12	1.38 ± 0.06*#	1.81 ± 0.06*

Data are means ± SD of 7 rats in each group. SC, sedentary control; SH, sedentary hypertensive; TH, trained hypertensive. BW, body weight; RV, right ventricle; AT/ET, acceleration time/ejection time ratio; TAPSE, tricuspid annular plane systolic excursion. * $p < 0.05$ vs. SC; # $p < 0.05$ vs. TH. One-way ANOVA followed by Tukey's post hoc test.

3.2. Exercise tolerance

Exercise tolerance was not different between groups before MCT injection (Pre injection; Fig. 1). However, by the 21st day after MCT injection and RT, rats in the TH group showed higher exercise tolerance values than those in the SC and SH groups. It was also observed that rats in the SC group showed higher values compared with those in the SH group. Additionally, while the SH group showed a trend towards a reduction in relative maximum load over time, both SC and TH groups demonstrated improved performance, being the most pronounced increase exhibited by the TH group.

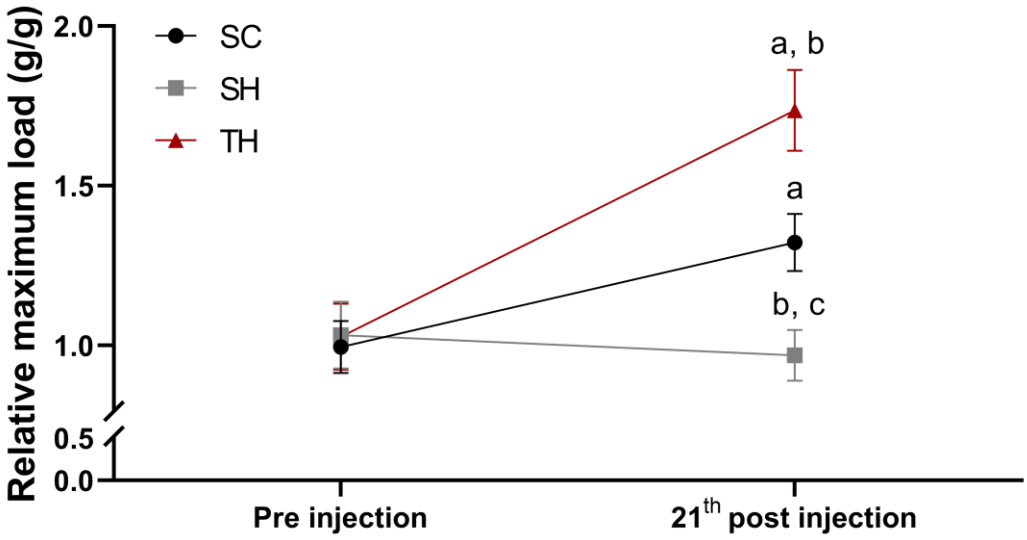

Figure 1. Effects of resistance exercise training on exercise tolerance. Relative maximum load tests. Values are means ± SD of 7 animals in each group. SC, sedentary control; SH, sedentary hypertensive; TH, trained hypertensive. [a] $p < 0.05$ vs. Pre injection; [b] $p < 0.05$ vs. SC; [c] $p < 0.05$ vs. TH. Mixed design ANOVA (Split plot ANOVA) followed by Bonferroni post-hoc.

3.3. Structural remodeling of skeletal muscle

Rats in the SH group showed lower CSA, compared with those in the other two experimental groups (Fig. 2C). Although CSA in the TH group was not at the level of that in SC group (i.e., TH < SC), RT was able to attenuate this structural loss (i.e., TH > SH).

Concerning the muscle volumetric proportion (Fig. 2D), SH rats exhibited a higher proportion of ECM and a lower proportion of muscle fiber as compared to SC and TH animals, which indicates a RT protection.

Regarding total collagen content (Fig. 2E), rats in the SH group had higher values than those in the other groups. RT, in turn, was able to avoid this adverse remodeling by mitigating the increase in total collagen (TH < SH). Additionally, a strong negative correlation (r = -0.89) was observed between CSA and the percentage of total collagen (Fig. 2F).

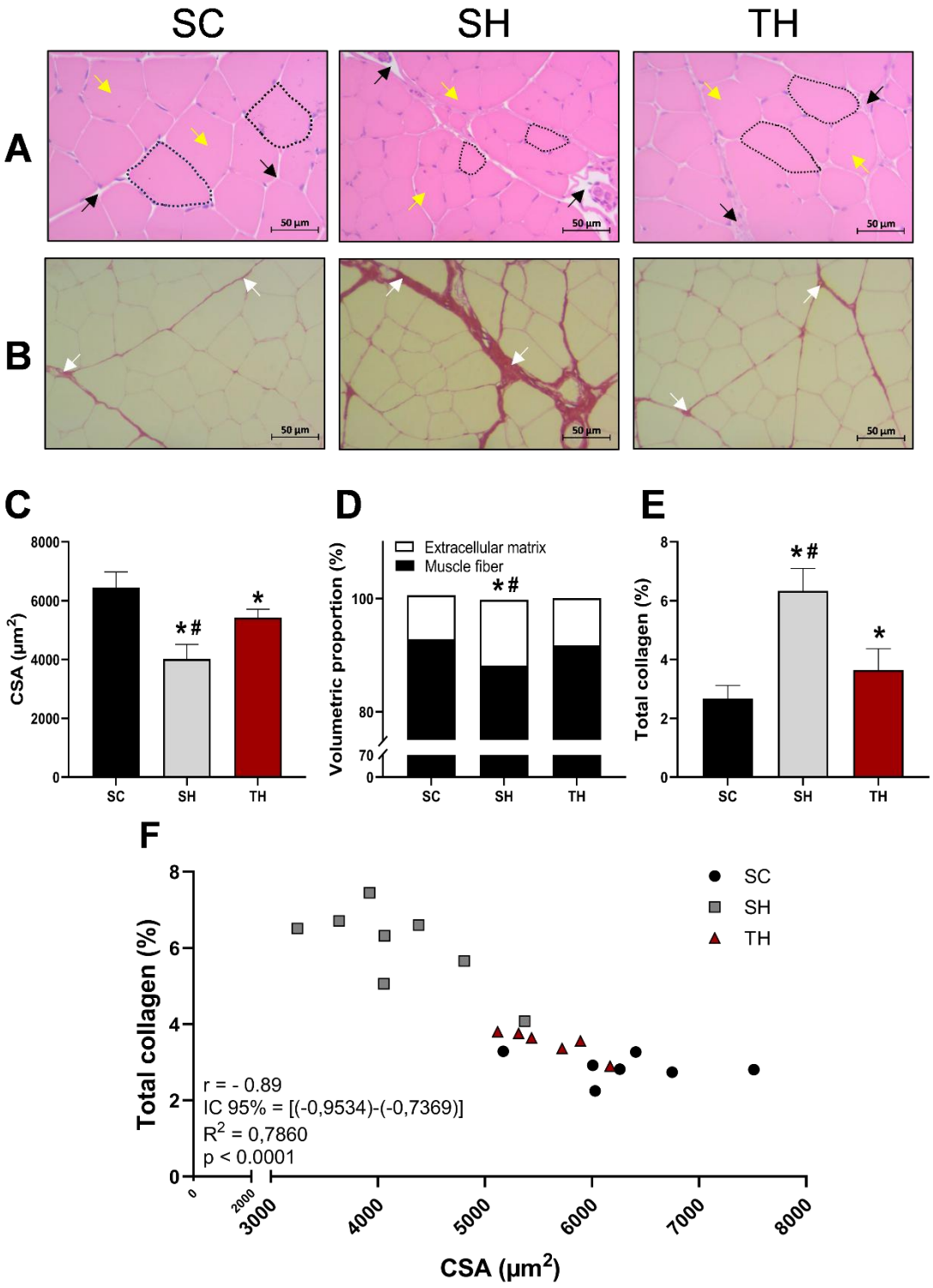

Figure 2. Effects of resistance exercise training on skeletal muscle structure. Representative photomicrograph of biceps brachii stained with H&E (A) and Picrosirius Red (B); (C) Cross-sectional area (CSA); (D) Volumetric proportion; (E) Percentage of total collagen (F) Correlation between CSA and percentage of total collagen. Data are means ± SD of 10 images per animal in each group (n = 7 animals per group). SC, sedentary control; SH, sedentary hypertensive; TH, trained hypertensive; CSA,

cross-sectional area. Black dotted line indicates CSA; Black arrow indicates extracellular matrix; Yellow arrow indicates muscle fiber; White arrow indicates collagen fiber. * $p < 0.05$ vs. SC; # $p < 0.05$ vs. TH. One-way ANOVA followed by Tukey's post hoc. The relationship between CSA and percentage of total collagen was tested by Pearson's correlation.

3.4. Skeletal muscle gene expression

Regarding gene expression of proteins associated with muscle atrophy, experimental PAH promoted an increase in MuRF1 and Atrogin-1 (Fig. 3A and 3B, respectively) in rats in the SH group, relative to those in SC and TH. However, RT was able to mitigate such augment (i.e. TH < SH), thus evidencing a protective effect against muscle atrophy.

As for calpain-1 (Fig. 3C), the disease augmented its expression in the SH and TH groups, compared with SC group. Therefore, RT was not able to impede such increase (i.e. TH = SH).

Finally, myostatin gene expression (Fig. 3D) was greater in the SH group, compared with the SC group. Nonetheless, RT downplayed such elevation (i.e. TH < SH).

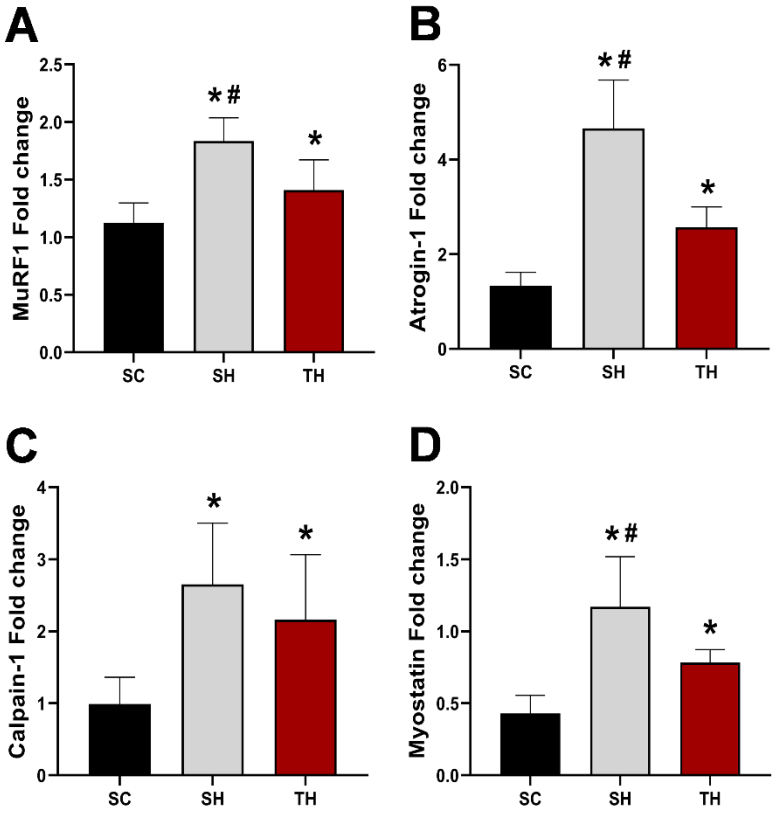

Figure 3. Effects of resistance exercise training on the gene expression of proteins associated with skeletal muscle atrophy. (A) Atrogin-1 (MAFbx; muscle atrophy F-box); (B) MuRF1 (muscle ring finger-1); (C) Myostatin; (D) Calpaína-1. Data are means ± SD of 6 animals in each group. SC, sedentary control; SH, sedentary hypertensive; TH, trained hypertensive. * $p < 0.05$ vs. SC; # $p < 0.05$ vs. TH. One-way ANOVA followed by Tukey's post hoc.

3.5. Skeletal muscle protein expression

Concerning the expression of proteins associated with the skeletal muscle hypertrophy, the levels of Akt-1 (Fig. 4A) and p-Akt-1$^{(Ser473)}$ (Fig. 4B) and the p-Akt-1$^{(Ser473)}$/Akt-1 ratio (Fig. 4C) did not show significant differences between groups. Statistical differences between groups were not observed for gene expression of eIF4E (Fig. 4D) and p-eIF4E$^{(Ser209)}$ (Fig. 4E) and the p-eIF4E$^{(Ser209)}$/eIF4E ratio (Fig. 4F) ratio.

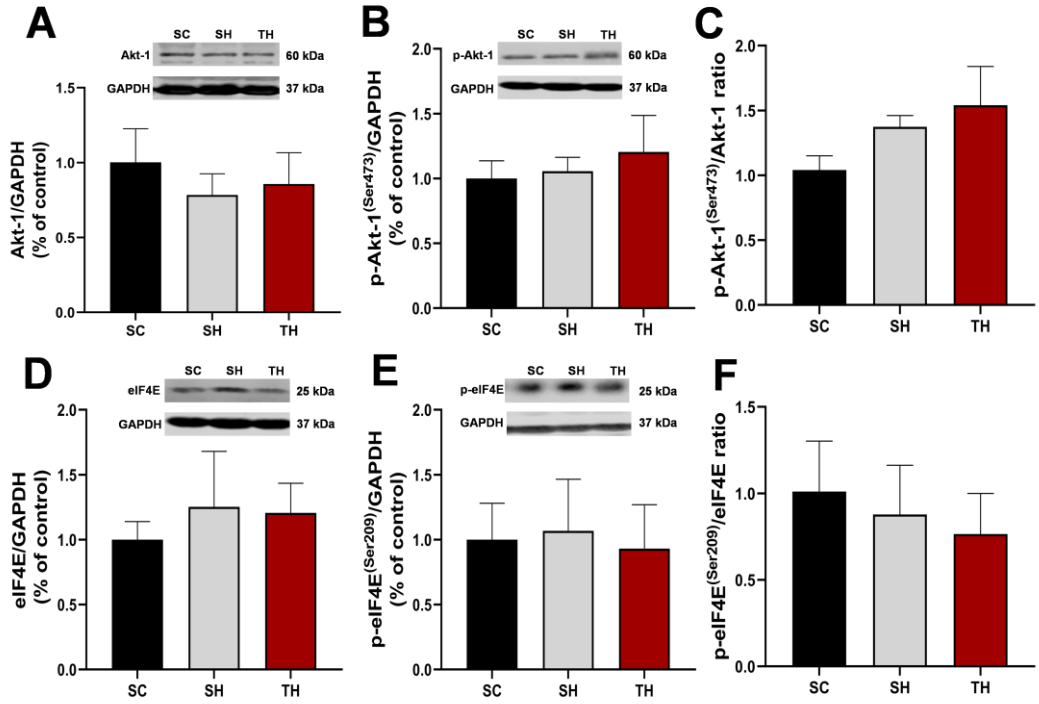

Figure 4. Effects of resistance exercise training on the expression of proteins related to skeletal muscle hypertrophy. (A) Akt-1, (protein kinase B); (B) p-Akt-1 (phospho-protein kinase B); (C) p-Akt-1/Akt-1 ratio; (D) eIF4E (eukaryotic translation initiation factor 4E); (E) p-eIF4E (phospho-eukaryotic translation initiation factor 4E); (F) p-eIF4E/eIF4E ratio. Data are means ± SD of 6 animals in each group. SC, sedentary control; SH, sedentary hypertensive; TH, trained hypertensive. $p > 0.05$. One-way ANOVA followed by Tukey's post hoc. Kruskal-Wallis followed by Dunn's post hoc for p-Akt-1/Akt-1 ratio.

3.6. Oxidative stress in skeletal muscle

The activities of antioxidant enzymes and markers of oxidative stress in skeletal muscle were measured. MCT-induced PAH reduced the activities of SOD (Fig. 5A) and CAT (Fig. 5 B) in rats in the SH group, related to those in the SC group. The employed RT did not avoid the diminution of SOD (i.e., TH = SH), nevertheless, the activity of CAT was maintained at the control rats (SC) levels (i.e., TH = SH), indicating an RT protection.

On the other hand, GST activity and MDA levels (Fig. 5C and 5D, respectively) were not statistically different between the experimental groups, indicating that neither the disease nor exercise influenced these parameters.

The concentration of NO (Fig. 5E) was lower in rats in the SH group than in those in the SC group. However, RT was able to downplayed against such reduction

(i.e., TH > SH). In addition, rats from SH group showed higher values of CP concentration, compared with those from SC and TH groups (Fig. 5F). Therefore, RT attenuated such increase in rats from TH group (i.e., TH < SH).

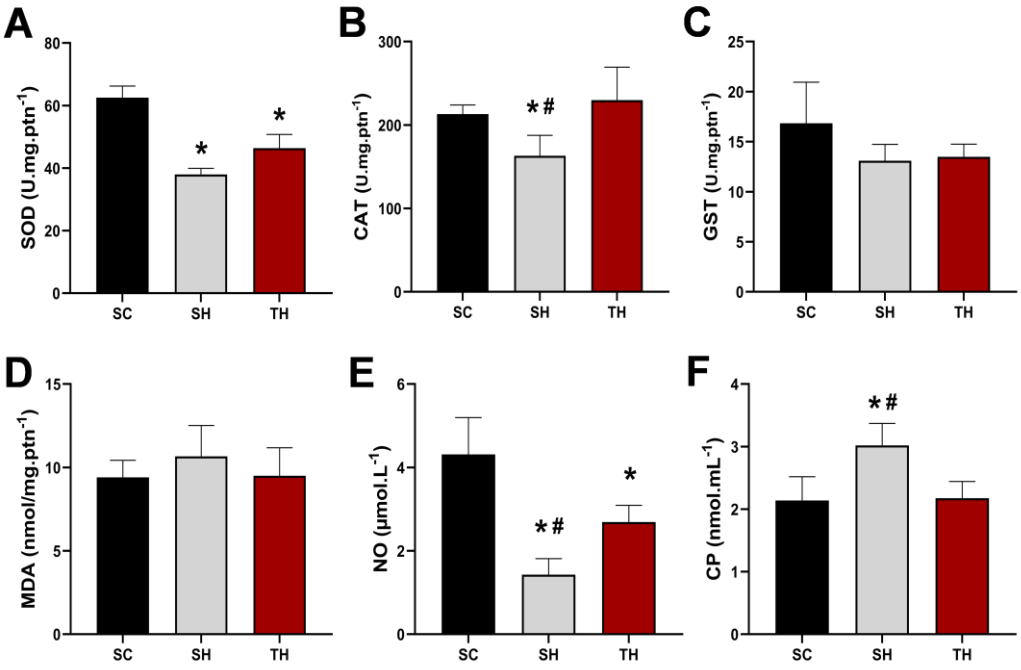

Figure 5. Effects of resistance exercise training on oxidative stress in skeletal muscle. (A) SOD (superoxide dismutase); (B) CAT (catalase); (C) GST (glutathione S-transferase); (D) MDA (malondealdehyde); (E) NO (nitric oxide); (F) CP (carbonyl protein). Data are the mean ± SD of 6 animals in each group. * $p < 0.05$ vs. SC; # $p < 0.05$ vs. TH. One-way ANOVA followed by Tukey's post hoc for SOD, CAT and NO. Kruskal-Wallis followed by Dunn's post hoc for GST, MDA and CP.

4. DISCUSSION

The present study aimed to investigate the effects of RT applied during the development of MCT-induced PAH on skeletal muscle atrophy in rats. The results demonstrate that RT improved hypertensive rats' tolerance to physical effort and protected them against disease's progression by preventing increases in pulmonary artery resistance and RV function. Hence, skeletal muscle mass loss was inhibited as its redox imbalance, structural adverse remodeling, and gene expression of proteins associated with muscle proteolysis pathways were mitigated.

The observed reduction in weight, CSA, and the percentage of myocytes in the brachial biceps of hypertensive rats indicates muscle atrophy. Indeed, we found that increased expression of MuRF1 and atrogin-1 indicated activation of the ubiquitin-proteasome system (UPS), the main protein degradation system in the body [40,41]. Furthermore, increased expression of myostatin suggests inhibition of cell growth and proliferation in muscle tissue [42], as well as activation of the UPS [14,43], reinforcing the contribution of these pathways to muscle mass loss in these rats. The activation of the UPS in skeletal muscle in PAH has been linked to central consequences of the disease, which trigger a range of systemic abnormalities (i.e., redox imbalance, mitochondrial dysfunction, increased inflammation, and tissue hypoxia) [43]. These alterations may converge to stimulate proteolytic pathways and contribute to the loss of muscle mass observed in PAH. More importantly, the employed RT protocol partially prevented muscle atrophy, as it mitigated reductions in weight, CSA, and the percentage of myocytes in the brachial biceps of hypertensive rats. Moreover, we observed reduced expression of MuRF1, atrogin-1, and myostatin in hypertensive trained rats, indicating an attenuation of the protein degradation process.

Reduced physical effort tolerance was observed in animals treated with MCT on the 21st day. In contrast, resistance training (RT) improved exercise tolerance, suggesting a protective effect against muscle atrophy and functional decline. Although this association was not directly evaluated in the present study, previous evidence from both MCT-induced PAH models [14,15] and human studies [13] suggests a link between decreased muscle CSA and impaired exercise capacity.

Additionally, PAH increased total collagen deposition and the proportion of ECM in brachial biceps, possibly mediated by the pro-inflammatory environment and the action of myostatin, which stimulate fibroblasts to promote ECM accumulation, including collagen [44,45]. These increases are associated with increased muscle stiffness and reduced functional capacity [46]. In this sense, the increased deposition of collagen and ECM may contribute to muscle atrophy and physical effort intolerance. In fact, we observed a strong negative correlation between CSA and total collagen content ($r = -0.89$) which suggests that muscle fibrosis may be directly associated with PAH-induced atrophy. However, it is noteworthy that the applied RT prevented such deleterious effects by reducing muscle adverse remodeling, which has been previously reported in the model by our group [21]. These results suggest mitigation of skeletal muscle morphological and structural changes associated with PAH by RT.

Regarding redox imbalance, sedentary hypertensive rats exhibited higher CP concentrations, reduced activity of antioxidant agents (i.e., SOD and CAT), and lower NO concentrations. The increased oxidative stress in PAH is related to mitochondrial dysfunctions induced by systemic alterations like reduced cardiac output and blood perfusion and increased inflammation [11,47,48], which results in excessive production of ROS and decreased cells' antioxidant capacity. Consequently, oxidative damage to molecules and cellular structures occurs, thus contributing to muscle degradation and atrophy. Our results reveal that RT protected hypertensive rats against these alterations, promoting therefore redox homeostasis and attenuating deleterious effects of PAH on skeletal muscle, which might be related to the UPS modulation [49–51]. The improvement in redox balance appears to be one of the mechanisms by which RT promotes improved muscle capacity and resistance to fatigue, as evidenced by increased tolerance to physical exertion. The present protection is supported by studies demonstrating that physical exercise can modulate oxidative stress and improve muscle function in experimentally induced PAH condition [21,52].

Interestingly, our study showed that the PI3K/Akt/mTOR/eIF4E signaling pathway was not inhibited in this model of PAH, in agreement with findings reported by Moreira et al. (2015)[14], though different from those by Batt et al. (2014)[13]. The activity of such pathway was preserved in sedentary hypertensive rats, despite the increased expression of myostatin, MuRF1, and atrogin-1, suggesting partial maintenance of muscle anabolism. Nevertheless, the conservation of such pathway did not prevent muscle atrophy together with increased ECM and collagen deposition and worse functional capacity, indicating that maintaining the anabolic pathway alone was not sufficient to counteract the deleterious effects of PAH.

On the other hand, the employed RT attenuated muscle mass loss and improved physical effort tolerance, possibly by modulating redox homeostasis and reducing adverse skeletal muscle remodeling. Moreover, the lower ECM deposition in trained hypertensive rats suggests that RT mitigated muscle stiffness and preserved contractile function. These effects may be related to the activation of the PI3K/Akt/mTOR/eIF4E pathway, which was preserved in rats with PAH, reinforcing the role of RT in sustaining skeletal muscle anabolism and lessening PAH-induced muscle loss.

These findings suggest that, although the PI3K/Akt/mTOR/eIF4E pathway was preserved in PAH, it was not sufficient to prevent the progression of muscle atrophy

and functional impairment in sedentary animals. Resistance training (RT), on the other hand, demonstrated a protective effect by attenuating muscle mass loss, improving exercise tolerance, and modulating muscle remodeling, involving the PI3K/Akt/mTOR/eIF4E pathway, thus reinforcing its therapeutic potential in PAH.

Finaly, the analyses presented here were restricted to a single skeletal muscle (i.e., brachial biceps), restraining the understanding of adaptive responses in muscles with different fiber compositions. We consider it as a study limitation, and thus future studies should include analyses of different types of skeletal muscle, as well as different components in the atrophy and hypertrophy pathways.

5. CONCLUSION

In conclusion, the RT applied during the development of MCT-induced PAH protects against skeletal muscle, by mitigating adverse structural remodeling and muscle atrophy through proteolysis modulation and attenuation of redox imbalance. These findings highlight the therapeutic potential of RT as a non-pharmacological strategy to preserve muscle functionality and physical effort tolerance in PAH conditions.

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Author contributions

Study design conceptualization, data collection, analysis, interpretation and drafted the manuscript: SFF Costa, LL Soares, LB Leite, AJ Natali; Data collection, analysis, and interpretation: SFF Costa, LL Soares, LB Leite, SCL Generoso, MAM Xavier, MS Faria, AE Quintão, LOG Ervilha, TI Lima, BRA Pelozin, TF, EM Oliveira, MM Neves, LL Oliveira, ECCR Reis, AJ Natali. Critical review of the manuscript: LL Soares and AJ Natali.

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Disclosures

None.

Competing interests

The authors declare that there is no conflict of interest with this article.

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CONSIDERAÇÕES FINAIS

Apresenta-se aqui as considerações finais sobre esta dissertação. No Capítulo 1, a revisão narrativa destaca a atrofia muscular esquelética na hipertensão arterial pulmonar (HAP) envolvendo a ativação de vias de proteólise, especialmente o sistema ubiquitina-proteassoma, a autofagia e as calpaínas. Além disso, foram apontadas alterações nos tipos de fibras musculares e a inibição de vias de sinalização relacionadas à hipertrofia como fatores que contribuem para a degradação muscular. Nesse sentido, o exercício físico apresenta-se como uma terapia não-farmacológica promissora para o tratamento da atrofia muscular no contexto da HAP. No Capítulo 2, o estudo experimental demonstra que o treinamento resistido (TR) realizado durante o desenvolvimento da HAP induzida por monocrotalina (MCT) atenua a atrofia muscular. Esse efeito protetor está associado à mitigação do remodelamento morfoestrutural e o desequilíbrio redox, bem como à modulação das vias de proteólise muscular minimizando, assim, a sua ativação. No entanto, estudos futuros são necessários para investigar diferentes tipos de músculos esqueléticos e explorar outros componentes envolvidos nas vias de sinalização relacionadas tanto à atrofia quanto à hipertrofia muscular.

Anexo 1 – Primeira página do artigo publicado (Capítulo 1)

Heart Failure Reviews
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REVIEW

Skeletal muscle atrophy in pulmonary arterial hypertension: potential mechanisms and effects of physical exercise

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Abstract
Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by pathological remodeling of the pulmonary arteries, resulting in increased pulmonary vascular resistance and right ventricular overload. This condition triggers common symptoms such as dyspnea and exercise intolerance, compromising thus the quality of life of individuals affected by this pathology. Skeletal muscle atrophy is one of the main determinants of these symptoms, which is mediated by an imbalance between protein synthesis and degradation, triggered by adverse systemic adaptations promoted by PAH, such as decreased blood perfusion and increased inflammation. This review addresses the main cellular and molecular mechanisms that potentially trigger or inhibit protein degradation pathways, and how they interact in the context of PAH. Furthermore, we focus on physical exercise as a non-pharmacological approach capable of modulating muscle atrophy induced by PAH.

Keywords Exercise intolerance · Inflammation · PAH · Pathological remodeling · Protein degradation

Introduction

Pulmonary arterial hypertension (PAH) is a rare disease characterized by a mean resting pulmonary arterial pressure above 20 mmHg [1, 2] and its worldwide prevalence is around 2.0 to 7.6 cases per million people. The pathophysiology of PAH involves adverse remodeling of the pulmonary artery and arterioles (vasculopathy), resulting in a progressive increase in pulmonary vascular resistance (PVR) [3].

Chronic increases in PVR lead to increased right ventricular (RV) afterload. This overload generates an adaptive response or adverse RV remodeling, resulting in pathological hypertrophy characterized by fibrotic tissue proliferation, redox imbalance, inflammation, mitochondrial dysfunction, and contractile dysfunction [4–6]. Such framework leads to RV failure characterized by reduced stroke volume (SV) and cardiac output (CO) and ultimately leads to patient's death [6, 7].

The clinical manifestations commonly observed in patients with PAH (i.e., dyspnea and fatigue) result from the adverse cardiovascular adaptations [8, 9]. These symptoms contribute to reduced tolerance to physical exertion, a striking feature in individuals with PAH [10].

Intolerance to physical effort in PAH is the result of a complex interaction between central factors (e.g. reduced CO and ventilatory inefficiency) and peripheral factors (e.g. rarefaction of skeletal muscle capillarization and reduced blood perfusion) that limit the adequate supply of oxygen and nutrients to skeletal muscles [11, 12] which contributes directly to muscle atrophy.

Muscle atrophy is a phenomenon characterized by the progressive loss of muscle mass and function, resulting from an imbalance between the synthesis and degradation of muscle proteins [13, 14]. Such process is driven by an increase in the activity of proteolytic pathways, as well as the inhibition of anabolic and myogenic pathways [15–17]. Hyperactivation of proteolytic pathways, associated with the suppression of protein synthesis mechanisms, results in progressive loss of muscle mass and strength [18].

While the molecular mechanisms underlying skeletal muscle atrophy and exercise intolerance have been

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Anexo 2 – Aprovação ética

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE VIÇOSA
PRÓ REITORIA DE PESQUISA E PÓS GRADUAÇÃO
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Viçosa, 08 de julho de 2022

Prof.
Antônio José Natali
Coordenador do projeto
DES/UFV

Sr. Coordenador,

Após avaliação da Metodologia utilizada no Projeto de Pesquisa intitulado **"Efeitos do exercício resistido ou terapia com extrato de mirtilo nas funções cardiopulmonares, marcadores inflamatórios e células do sistema imune em modelo experimental de hipertensão arterial pulmonar"**, aqui nomeado Processo 11/2022, a CEUA/UFV emite parecer favorável ao protocolo de utilização de animais proposto, tendo como base para análise a Legislação vigente (Lei Nº 11.794, de 08 de outubro de 2008), as Resoluções Normativas editadas pelo CONCEA/MCTIC, bem como a DBCA (Diretriz Brasileira de Prática para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos) e as Diretrizes da Prática de Eutanásia preconizadas pelo CONCEA/MCTIC.

Acresce a esse Parecer a exigência de Relatório Final de Atividades conforme itens a seguir:

RESUMO DOS RESULTADOS FINAIS OBTIDOS A PARTIR DOS EXPERIMENTOS ENVOLVENDO A ***UTILIZAÇÃO DE ANIMAIS*** *NO PROJETO DE PESQUISA*

1 Número do protocolo de submissão do projeto de pesquisa à CEUA/UFV:
2 Metodologia completa obrigatoriamente com:
- Local (is) Geral (is) e específico (s) oficial (is) onde ocorreu a experimentação;
- O nome científico do animal em questão;
- Número total de animais utilizados na pesquisa.
3 Resultados:
4 Nome do Coordenador do Projeto:
Assinatura:
5 Nome do Responsável Técnico:
Assinatura:
Inscrição em CRMV:

Prof. Fabrício Luciani Valente
Coordenador
Comissão de Ética no Uso de Animais – CEUA/UFV

CERTIFICADO

A Comissão de Ética no Uso de Animais - CEUA/UFV certifica que o processo nº 11/2022, intitulado **"Efeitos do exercício resistido ou terapia com extrato de mirtilo nas funções cardiopulmonares, marcadores inflamatórios e células do sistema imune em modelo experimental de hipertensão arterial pulmonar",** coordenado pelo professor Antônio José Natali do Departamento de Educação Física, está de acordo com a Legislação vigente (Lei Nº 11.794, de 08 de outubro de 2008), as Resoluções Normativas editadas pelo CONCEA/MCTIC, a DBCA (Diretriz Brasileira de Prática para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos) e as Diretrizes da Prática de Eutanásia preconizadas pelo CONCEA/MCTIC, portanto sendo aprovado por esta Comissão em 08/07/2022, com validade de 2 anos

CERTIFICATE

The Ethic Committee in Animal Use/UFV certify that the process number 11/2022, named **"Effects of resistance exercise or blueberry extract therapy on cardiopulmonary functions, inflammatory markers and immune system cells in an experimental model of pulmonary arterial hypertension"**, is in agreement with the a actual Brazilian legislation (Lei Nº 11.794, 2008, Normative Resolutions edited by CONCEA/MCTIC, the DBCA (Brazilian Practice Guideline for the Care and Use of Animals for Scientific and the Guidelines of Practice the Euthanasia recommended by CONCEA/MCTIC therefore being approved by the Committee on July 08, 2022 valid for 2 years.

Prof. Fabrício Luciani Valente
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