UNIVERSIDADE FEDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA INTERINSTITUCIONAL DE PÓS-GRADUAÇÃO EM CIÊNCIAS FISIOLÓGICAS – ASSOCIAÇÃO AMPLA UFSCar/UNESP

MAÍRA CAMARGO SCARPELLI

MECANISMOS DE HIPERTROFIA MUSCULAR INDUZIDA PELO TREINAMENTO DE FORÇA: ANÁLISE DE MARCADORES DE PROTEÓLISE, REMODELAMENTO DE MATRIZ EXTRACELULAR E ADIÇÃO DE MIONÚCLEOS MEDIADA POR CÉLULAS SATÉLITE

SÃO CARLOS – SP
2024

MAÍRA CAMARGO SCARPELLI

MECANISMOS DE HIPERTROFIA MUSCULAR INDUZIDA PELO TREINAMENTO DE FORÇA: ANÁLISE DE MARCADORES DE PROTEÓLISE, REMODELAMENTO DE MATRIZ EXTRACELULAR E ADIÇÃO DE MIONÚCLEOS MEDIADA POR CÉLULAS SATÉLITE

Tese apresentada ao Programa Interinstitucional de Pós-graduação em Ciências Fisiológicas – Associação Ampla UFSCar/UNESP, da Universidade Federal de São Carlos, como parte dos requisitos para a obtenção do título de Doutora em Ciências Fisiológicas.

Orientador: Prof. Dr. Cleiton Augusto Libardi

SÃO CARLOS – SP

2024

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas

Folha de Aprovação

Defesa de Tese de Doutorado da candidata Maíra Camargo Scarpelli, realizada em 21/03/2024.

Comissão Julgadora:

Prof. Dr. Cleiton Augusto Libardi (UFSCar)

Profa. Dra. Anabelle Silva Cornachione (UFSCar)

Prof. Dr. Felipe Cassaro Vechin (USP)

Prof. Dr. Paulo Henrique Caldeira Mesquita (OMRF)

Prof. Dr. Manoel Emilio Lixandrão (Colorado)

O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas.

Dedicatória

Aos meus pais, Heloisa e Moacir, pelo amor e apoio incondicionais.

AGRADECIMENTOS

Concluir um curso de pós-graduação não é fácil. Alguns diriam que “não é para ser fácil”. Mas o que não contam é que frente a todo o contexto, o “estudar e escrever” acaba por ser a parte mais fácil. A parte difícil é o esforço para trabalhar sem o mínimo de estrutura e investimento; é a obrigação de atender às demandas e cobranças que não são parte da nossa função, mas que cumprimos por receio de que possam nos prejudicar num futuro próximo; é a insegurança em relação ao futuro, totalmente incompatível com a dedicação que investimos para seguir na carreira acadêmica. Concluí esse processo tão cansada que pensei várias vezes em não escrever estes agradecimentos para poder finalizar o documento o quanto antes. Mas quando relembrei de todas essas dificuldades, foi impossível não pensar nas pessoas que me ajudaram a superá-las dia após dia.

Agradeço ao meu orientador pela atenção e zelo com o meu trabalho e minha formação e pelas oportunidades que me foram oferecidas ao longo de todo o meu período no laboratório. Agradeço à UFSCar, que apesar de todas as dificuldades, segue sendo uma das melhores Universidades do país. A UFSCar faz parte da minha história e de toda a minha família e sempre me sinto em casa quando estou ali. Agradeço também à *Auburn University School of Kinesiology*, Auburn – AL, EUA pelo período de doutorado sanduíche e agradeço aos colegas que ali me receberam, me ensinaram e se tornaram grandes amigos e parceiros de trabalho.

Agradeço aos meus colegas de laboratório. Ninguém conhece melhor que eles as dificuldades que passamos. Se hoje finalizo esse processo com sucesso é, em grande parte, porque eles estavam ao meu lado, trabalhando junto, ensinando e aprendendo, fazendo sempre mais que o esperado. De colegas de trabalho, se tornaram amigos, os quais levo orgulhosa comigo para toda a vida. Agradeço também aos amigos que não estão na mesma carreira, e que me lembravam que existe vida para além do laboratório. Que entendem pouco de como funciona a pesquisa e a Universidade pública, mas ainda assim me ouviam, me incentivavam e

diziam “você é nosso orgulho, acreditamos em você”. Em seguida, mudávamos de assunto e eu me conectava novamente com um mundo exterior de milhares de possibilidades.

Agradeço ao meu irmão. Ao longo dos anos ele se tornou meu melhor amigo, meu confidente, aquele para quem ligo quando quero só conversar, quando quero ouvir alguém que me pergunte com sinceridade “e você, como está?” e ouça atentamente a resposta. Além disso, me presenteou com um sobrinho. O amor por ele transbordou e foi essencial para tornar mais leves esses últimos meses do meu doutorado.

Agradeço aos meus pais. Quando eu era mais nova dizia que queria fazer qualquer coisa exceto ser como minha mãe porque ela trabalhava demais. Sigo achando que ela trabalha demais, a única diferença é que agora eu a faço companhia. Mas não tinha como ser diferente. Aprendi desde nova que estudar era legal, que tinha mundo demais e coisas demais nesse mundo para a gente aprender. Concluí que questionar é fundamental e responder aos questionamentos é viciante. Obrigada pai e mãe por me mostrarem isso. Obrigada por terem me presenteado com todas as ferramentas e oportunidades para que eu pudesse seguir por esse caminho. O mundo é realmente grande demais, mas eu não vou sair daqui sem ver e explorar o máximo que eu puder.

Por fim, agradeço ao apoio financeiro fornecido pela CAPES (Processos n. 88887.634296/2021-00 e 88887.717127/2022-00) que viabilizou a realização deste projeto de pesquisa.

RESUMO

O aumento da massa muscular (i.e., hipertrofia muscular) promovido pelo treinamento de força (TF) resulta da ativação e coordenação de vários mecanismos moleculares. Alguns deles têm sido amplamente investigados, como as células satélites e o acréscimo de mionúcleos às fibras musculares. Por outro lado, outros mecanismos, como a atuação das enzimas que regulam a proteólise e o remodelamento da matriz extracelular (MEC), foram pouco investigados. Considerando que o aumento da massa muscular impacta positivamente a saúde e o bem-estar, parece imperativo investigar o efeito do TF sobre estes mecanismos, bem como explorar estratégias para otimizar as respostas individuais de hipertrofia, por exemplo, por meio da progressão da sobrecarga de treinamento. Assim, este estudo tem como objetivo investigar se: (1) as respostas individuais de hipertrofia muscular a diferentes protocolos de progressão da sobrecarga podem ser explicadas por alterações no conteúdo total de mionúcleos e células satélites ou no conteúdo proteico e níveis de atividade enzimática de biomarcadores proteolíticos e fatores de remodelamento da MEC; (2) as respostas agudas do conteúdo proteico e dos níveis de atividade enzimática de biomarcadores proteolíticos e dos fatores de remodelamento da MEC a uma sessão de TF realizada em estado treinado são atenuadas em relação às respostas a uma sessão realizada em estado destreinado. Os resultados indicam que: (1) a prescrição individual da progressão da sobrecarga pode melhorar a responsividade intra-sujeitos ao TF; porém, a maior capacidade de resposta a um protocolo ou outro não é explicada pelos mecanismos moleculares investigados; (2) as respostas dos marcadores não são atenuadas no estado treinado em relação às respostas observadas no estado destreinado, sugerindo que as respostas de proteólise e remodelamento da MEC são provavelmente sustentadas para promover a hipertrofia e remodelamento muscular.

Palavras-chave: Estado de treinamento; Modelos de progressão da sobrecarga; Respostas individuais.

ABSTRACT

The increase in muscle mass (i.e., muscle hypertrophy) promoted by resistance training (RT) results from the activation and coordination of various molecular mechanisms. Some of them have been widely examined, such as satellite cells and the addition of myonuclei to muscle fibers. On the other hand, other mechanisms, such as the activity of enzymes that regulate proteolysis and extracellular matrix (ECM) remodeling, have been poorly investigated. Considering that increased muscle mass positively impacts health and well-being, it seems imperative to investigate the effect of RT on these mechanisms, as well as to explore strategies to optimize individual hypertrophic responses, for example, through RT overload progression. Thus, this study aims to investigate whether: (1) individual muscle hypertrophy responses to different overload progression protocols can be explained by changes in total myonuclei and satellite cells content or changes in the protein content and enzymatic activity levels of proteolytic biomarkers and ECM remodeling factors; (2) acute responses of protein content and enzymatic activity levels of proteolytic biomarkers and ECM remodeling factors to an RT bout performed in the trained state are attenuated compared to responses to a naïve bout (i.e., performed in the untrained state). The results indicate that: (1) individual prescription of overload progression can improve intra-subject responsiveness to RT; however, the greater responsiveness to one protocol or another is not explained by the investigated molecular mechanisms; (2) biomarker responses are not attenuated in the trained state compared to responses observed in the untrained state, suggesting that proteolysis and ECM remodeling responses are likely sustained to promote muscle hypertrophy and remodeling.

Keywords: Training status; Overload progression models; Individual responses.

LISTA DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

LISTA DE TABELAS

CAPÍTULO 1

CAPÍTULO 2

SUMÁRIO

CONTEXTUALIZAÇÃO

Minha trajetória como aluna de doutorado no Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas UFSCar-Unesp (PIPGCF) teve início em março de 2019. Ao longo do primeiro ano, concluí os créditos em disciplinas necessários para o cumprimento dos requisitos do Programa e, sob orientação do Prof. Cleiton Libardi, foi dado início à elaboração do projeto de pesquisa de Doutorado. No ano seguinte, 2020, seria realizada a coleta de dados do projeto. Entretanto, em março de 2020, teve início a pandemia de COVID-19, causada pelo vírus SARS-CoV-2. A constatação dos riscos que a COVID-19 apresentava à saúde da população, levou à interrupção de praticamente todas as atividades presenciais, em especial as atividades de pesquisa com seres humanos realizadas na Universidade Federal de São Carlos. Logo, o cronograma de execução do projeto foi adiado até que pudessem ser retomadas as atividades presenciais. No entanto, a duração da pandemia e das limitações por ela impostas se estenderam para muito além do que imaginávamos a princípio. O retorno às atividades presenciais em nosso laboratório foi autorizado somente em maio de 2022, após aprovação da nossa solicitação apresentada ao Núcleo Executivo de Vigilância em Saúde (NEVS), vinculado ao Comitê Gestor da Pandemia da UFSCar.

O período de interrupção de atividades imposto pela pandemia impactou não somente o meu cronograma, como também de outros colegas de doutorado cujos prazos para qualificação e defesa eram similares aos meus. A proximidade de nossos prazos tornava claramente inviável a condução paralela de três projetos de pesquisa distintos, uma vez que projetos que envolvem a aplicação de períodos de treinamento fazem uso de um espaço físico limitado e requerem a colaboração de todos os membros do laboratório. Optamos, portanto, em elaborar um design experimental que permitisse a investigação de diferentes perguntas de pesquisa a partir de dados coletados em um mesmo conjunto de participantes submetidos a um mesmo período

experimental. Dessa maneira, pudemos otimizar a demanda de trabalho e a aplicação de nossos recursos para recrutar um número recorde de voluntários

O estudo foi desenvolvido em duas etapas. A primeira fase foi realizada entre os meses de junho e outubro de 2022 na Universidade Federal de São Carlos. Nesta fase, os participantes foram recrutados e submetidos a um período de treinamento e a diversas avaliações, incluindo 8 biópsias musculares. Neste período, me inscrevi e fui aprovada no processo de seleção do edital Capes-PrInt. Isso permitiu que a segunda fase do estudo fosse realizada em um período de doutorado sanduíche (fevereiro a agosto de 2023) na *Auburn University*, em Auburn, Alabama, EUA, sob supervisão do Prof. Dr. Michael D. Roberts, diretor do laboratório *Nutrabolt Applied and Molecular Sciences Laboratory.* As 690 amostras de tecido muscular coletadas na Fase 1 foram enviadas para o laboratório do Dr. Roberts, aonde chegaram em 13 de março de 2023. Após receber o adequado treinamento, realizei as análises biomoleculares que compõem minha tese: imuno-histoquímica, *Western Blotting* e ensaios enzimáticos de luminescência. Ao final do período de doutorado sanduíche, em agosto de 2023, retornei ao Brasil para concluir a interpretação e análise dos dados, bem como a redação desta tese.

Por fim, cabe ressaltar que, conforme alternativa proporcionada pelas normas do Programa Interinstitucional de Pós-Graduação em Ciências Fisiológicas UFSCar-Unesp, a presente tese é composta por dois capítulos apresentados em língua inglesa, acrescentados de uma síntese geral em língua portuguesa. Um relatório das atividades extracurriculares desenvolvidas ao longo do período de doutorado é apresentado ao final do documento.

SÍNTESE GERAL

Apresentação do problema de pesquisa

O aumento da massa muscular (i.e., hipertrofia muscular) promovido pelo treinamento de força (TF) resulta da ativação e coordenação de vários mecanismos moleculares. Alguns deles têm sido amplamente investigados, como por exemplo, a diferenciação e fusão das células satélites e o acréscimo de mionúcleos às fibras musculares. Considerando-se que os núcleos das fibras musculares têm uma capacidade transcricional limitada, acredita-se que caso a magnitude da hipertrofia muscular induzida pelo TF dependa de atividade transcricional que supere esse limite, é necessária a adição de novos mionúcleos, os quais seriam fornecidos pela fusão de células satélites às fibras musculares em crescimento (BAMMAN *et al.*, 2018). Além disso, evidências sugerem que há uma associação entre os ganhos de hipertrofia em resposta ao TF e o conteúdo de células satélites ou o acréscimo de mionúcleos mediado por células satélites (PETRELLA *et al.*, 2008; BELLAMY *et al.*, 2014; ANGLERI *et al.*, 2022a; SMITH *et al.*, 2023).

Por outro lado, há poucos estudos que examinam outros mecanismos, como a atuação das enzimas que regulam a proteólise e o remodelamento da matriz extracelular (MEC) (ROBERTS *et al.*, 2023). A MEC é composta por colágenos, proteoglicanos e outras glicoproteínas que passam por remodelamento constante promovido pela ação de uma família de enzimas chamadas metaloproteinases (MMPs) (GILLIES *et al.*, 2011; DAVIS *et al.*, 2013). A MEC desempenha um papel vital em uma multiplicidade de processos celulares (NABA *et al.*, 2016) (KJAER, 2004) (MACKEY *et al.*, 2017) e tem sido sugerido que o seu remodelamento pode contribuir para um maior crescimento das fibras musculares (MILLWARD, 1995). Evidências indicam que os fatores de remodelamento da MEC (por exemplo, MMPs e inibidores teciduais das metaloproteinases [TIMPs]) são aumentados ou

inalterados após uma sessão de exercícios (RULLMAN *et al.*, 2007; RULLMAN *et al.*, 2009; HOIER *et al.*, 2012). No entanto, a literatura atual ainda não delineou como essas respostas agudas mudam após várias sessões ao longo de um período de TF.

No que diz respeito à proteólise muscular, a atuação coordenada da via da Ubiquitina-proteassoma e da via da Calpaína é crucial para o remodelamento muscular (HYATT *et al.*, 2020), uma vez que promovem a reciclagem de componentes celulares danificados ou ineficientes (PASIAKOS *et al.*, 2014). Evidências sugerem que há um aumento na atividade proteolítica após uma sessão de exercícios (FÉASSON *et al.*, 2002; NEDERGAARD *et al.*, 2007; GODWIN *et al.*, 2023b), que pode ser atenuada após sessões subsequentes (STUPKA *et al.*, 2001; WILLOUGHBY *et al.*, 2003; NEDERGAARD *et al.*, 2007; MASCHER *et al.*, 2008) devido ao "efeito da carga repetida" (atenuação do dano muscular induzido pelo TF) (DAMAS *et al.*, 2016a). No entanto, novas perspectivas têm surgido no que diz respeito à importância dos processos de degradação e remodelamento no músculo esquelético (HÖHFELD *et al.*, 2021; ROBERTS *et al.*, 2023), sugerindo que, se o estímulo oferecido pelo TF for mantido, essas atividades podem permanecer elevadas, mesmo com uma atenuação do dano muscular.

O aumento da massa muscular impacta positivamente a saúde e o bem-estar e pode contribuir, por exemplo, na prevenção ou atenuação da obesidade, diabetes tipo 2 e outras condições crônicas comuns (WOLFE, 2006; DANKEL *et al.*, 2015, 2016b, a). Assim, parece imperativo investigar, além dos mecanismos moleculares envolvidos na hipertrofia, estratégias para otimizar o aumento da massa muscular. Dentre estas estratégias, sugere-se que a progressão gradual da sobrecarga imposta pelo TF é essencial para garantir que a hipertrofia ocorra de forma contínua (KRAEMER *et al.*, 2004). Tradicionalmente, a progressão da sobrecarga é realizada estabelecendo-se uma zona de repetições máximas e ajustando a carga (peso em kg) série a série, de modo a garantir que a falha muscular concêntrica ocorra dentro da zona de repetições alvo (LOADprog). Alternativamente, é possível realizar a progressão por

meio do simples aumento do número de repetições (REPSProg). Nesse modelo, a carga não é alterada e não há limite para o número de repetições, que pode ser aumentado sempre que o indivíduo conseguir adiar o momento da falha muscular concêntrica (MITCHELL *et al.*, 2012; LASEVICIUS *et al.*, 2018; NOBREGA *et al.*, 2018).

Neste contexto, duas questões merecem destaque. Em primeiro lugar, embora a maioria dos indivíduos possa se beneficiar de diferentes protocolos de TF, alguns podem apresentar maiores ganhos musculares em resposta a um protocolo do que a outro. De fato, por meio de um desenho experimental intra-sujeitos, estudos revelaram que alguns indivíduos têm maiores ganhos de massa muscular ao treinar com alta frequência semanal (DAMAS *et al.*, 2019) ou maior volume de TF (LIXANDRÃO *et al.*, 2024), enquanto outros se beneficiam mais de uma baixa frequência ou menor volume. Além disso, pode haver uma interação entre as características do indivíduo e o protocolo de treinamento que provoca maior ou menor ativação de processos moleculares que podem regular o nível de hipertrofia alcançado. Portanto, para otimizar os aumentos da massa muscular promovidos pelo TF é importante investigar como diferentes protocolos de treinamento podem modular os mecanismos moleculares associados à hipertrofia, bem como as respostas hipertróficas individuais. Em segundo lugar, como o crescimento muscular induzido pelo TF não ocorre de forma linear ao longo do tempo (DAMAS *et al.*, 2018a), os mecanismos moleculares relacionados à hipertrofia podem ser diferentemente modulados de acordo com o nível de experiência de treinamento. Logo, para melhor compreender o continuum de crescimento muscular ao longo de um período de treinamento, é primordial entender como as vias moleculares respondem ao TF em diferentes estágios de adaptação muscular.

Tomando em conjunto o conteúdo exposto até aqui, o presente estudo tem como objetivo responder duas questões principais de pesquisa:

- ***Capítulo 1:*** *As respostas individuais de hipertrofia muscular aos protocolos LOADProg e REPSProg podem ser explicadas por alterações induzidas pelo treinamento no conteúdo total de mionúcleos e células satélites ou no conteúdo proteico e níveis de atividade enzimática de biomarcadores proteolíticos e fatores de remodelamento da matriz extracelular?*

- ***Capítulo 2:*** *As respostas agudas do conteúdo proteico e níveis de atividade enzimática de biomarcadores proteolíticos e fatores de remodelamento da matriz extracelular a uma sessão de treinamento de força realizada em estado treinado são atenuadas em relação às respostas a uma sessão realizada em estado destreinado?*

Principais métodos utilizados

Este estudo foi realizado a partir da análise de dados e amostras musculares derivadas de um de um ensaio clínico controlado randomizado (Registro Brasileiro de Ensaios Clínicos - RBR-57v9mrb) com um delineamento experimental intra-sujeitos e medidas repetidas ao longo do tempo.

Neste delineamento experimental, os participantes realizaram uma primeira visita ao laboratório para avaliação por ultrassonografia da área de secção transversa do músculo vasto lateral de ambas as pernas. Após 72 horas, repetiu-se a avaliação por ultrassonografia. Em seguida ,foi realizado o teste de força máxima dinâmica, com um reteste após 72 horas, seguindo os procedimentos recomendados por Brown e Weir (2001). Os participantes tiveram então seus membros inferiores alocados aleatoriamente aos protocolos experimentais empregando o modelo de randomização em blocos e balanceamento por dominância de membros e força muscular. Os treinamentos foram administrados em 24 sessões realizadas ao longo de 10 semanas. Biópsias musculares foram realizadas imediatamente antes e 24h após ambas a primeira e a última sessão de treinamento. A avaliação da área de secção transversa muscular e o teste de força máxima dinâmica foram repetidos ao final do período de treinamento.

Ambos os protocolos de treinamento foram compostos por 4 séries de extensão unilateral do joelho em um equipamento do tipo cadeira extensora (Effort NKR; Nakagym, SP, Brasil). No protocolo LOADProg, estabeleceu-se uma zona-alvo de 9 a 12 repetições máximas e a carga foi ajustada série a série de forma que a falha muscular concêntrica acontecesse sempre dentro deste intervalo de repetições. No protocolo REPSProg, a cada série o participante realizou o maior número de repetições possível até o ponto de falha muscular concêntrica utilizando uma carga pré-estabelecida em 80% da carga estipulada no teste inicial de 1-RM.

A avaliação da área de secção transversa do músculo vasto lateral foi realizada por meio de ultrassonografia modo-B, utilizando-se um probe linear de 7,5 MHz (MySono U6, Samsung Industria e Comércio Ltda., São Paulo, Brasil). As imagens foram obtidas na região do ponto médio do fêmur, calculado como 50% da distância entre o trocanter maior e o epicôndilo lateral, e posteriormente analisadas utilizando-se o software ImageJ, seguindo o protocolo previamente validado por Lixandrão *et al*. (2014).

Amostras do músculo vasto lateral foram obtidas por meio de biópsia muscular com sucção manual, realizada por profissional médico habilitado e com vasta experiência neste procedimento. O tecido extraído foi limpo de todo o sangue e tecido conectivo e separado em alíquotas para as análises. Uma porção (20-30 mg) foi colocada em meio de incorporação para temperatura de corte ideal (OCT), com as fibras perpendiculares à superfície horizontal e rapidamente congelada em isopentano resfriado por nitrogênio líquido para realizar as análises imuno-histoquímicas. Foram realizadas análises imuno-histoquímicas para mensuração da área de secção transversa das fibras musculares (fCSA) do tipo I e II e quantificação de mionúcleos e células satélites associados a estas fibras. A porção restante do tecido foi colocada em criotubos livres de RNA e destinada à análise de: (i) conteúdo proteico – por meio do método de Western Blotting – de calpaína-1, calpaína-2, 20S proteassoma, proteínas poliubiquitinadas, P62, MMP-9, MMP-14, TIMP-1 e TIMP-2; (ii) atividade enzimática – utilizando-se kits de luminescência comercialmente disponíveis – de calpaína, proteassoma e MMPs.

Principais resultados encontrados

Capítulo 1

Sete participantes apresentaram hipertrofia muscular superior em resposta do protocolo LOADProg em comparação a REPSProg. Um total de 12 participantes apresentaram hipertrofia muscular superior em resposta a REPSProg em comparação a LOADProg. Para estes participantes, foi observada uma associação entre o aumento da massa muscular e o aumento no número de células satélite. Para 13 participantes não houve diferenças na hipertrofia promovida por REPSProg e LOADProg. Cinco participantes foram considerados não respondedores, uma vez que nenhum dos protocolos promoveu hipertrofia acima do critério de dois erros típicos (5,7%). De forma geral, a análise de covariância indicou que os marcadores moleculares não foram diferentemente modulados pelos protocolos de treinamento em nenhum dos subgrupos de responsividade.

Capítulo 2

O protocolo de treinamento promoveu um aumento na área de secção transversa do músculo vasto lateral. Em relação às fibras musculares, somente as fibras do Tipo II sofreram aumento significante em sua área de secção transversa, enquanto não houve diferenças na área das fibras do Tipo I. Observou-se um aumento agudo na atividade de Calpaína e uma redução aguda no conteúdo de calpaína-1 e -2. Os valores de calpaína-2 foram maiores no estado treinado que no estado não treinado. A atividade do proteassoma aumentou agudamente, com valores no estado treinado inferiores aos do estado não treinado. A expressão proteica de 20S proteassoma aumentou agudamente em ambos os estados e as proteínas poliubiquitinadas não foram alteradas. Houve uma redução aguda na atividade global das MMPs em ambos os estados de treinamento. Em ambas as condições, o conteúdo proteico de MMP-9 aumentou agudamente, enquanto o conteúdo de TIMP-1 e TIMP-2 diminuiu. O conteúdo de MMP-14 não

sofreu alterações agudas, porém os valores no estado não treinado foram superiores aos do estado treinado.

Considerações gerais

A presente tese explora os resultados da análise secundária de dados coletados ao longo de um ensaio clínico randomizado. O objetivo desta tese foi investigar as respostas hipertróficas individuais aos protocolos aplicados, bem como analisar alguns mecanismos moleculares candidatos a explicar as diferenças na responsividade. Além disso, investigou-se se as respostas destes mecanismos a uma sessão realizada em estado não treinado diferem das respostas a uma sessão em estado treinado, i.e., após um período crônico de 10 semanas de treinamento.

Os resultados do primeiro estudo sugerem que os modelos de progressão de sobrecarga podem melhorar a responsividade intra-sujeitos ao treinamento de força. Curiosamente, um terço dos participantes apresentou maior hipertrofia muscular quando a sobrecarga foi progredida pelo aumento das repetições. Assim, ao contrário do que habitualmente se recomenda, este modelo de progressão parece vantajoso, pois produz hipertrofia muscular maior ou igual para uma parcela significativa de indivíduos em comparação ao protocolo realizado com aumento da carga. Além disso, parece haver uma associação entre a hipertrofia muscular e o aumento do número de células satélite. No entanto, as diferenças observadas na hipertrofia parecem não ser explicadas pelas mudanças crônicas observadas na contagem de mionúcleos, nos marcadores de atividade de proteólise e nos fatores de remodelamento da MEC.

Os resultados do segundo estudo indicam que as respostas de grande parte dos biomarcadores relacionados à proteólise e ao remodelamento da MEC a uma sessão de TF não são atenuadas no estado treinado. Isso sugere que as respostas de proteólise e remodelamento da matriz extracelular podem ser sustentadas para promover ainda mais a hipertrofia do músculo esquelético e o remodelamento tecidual. Além disso, não foram observadas diferenças entre homens e mulheres para nenhum dos marcadores investigados.

CAPÍTULO 1

Manuscript: Individual muscle hypertrophy is affected by the overload progression model and is associated with changes in satellite cell content

ABSTRACT

We aimed to compare individual hypertrophic responses to resistance training in which overload progressed either by adjusting the load (LOADProg) or by increasing the number of repetitions (REPSProg). Furthermore, we investigated whether greater responsiveness to one protocol was associated with chronic changes in myonuclei and satellite cells, proteolysis and extracellular matrix (ECM) remodeling biomarkers. Thirty-seven untrained participants had their legs randomized into LOADProg and REPSProg and underwent 10 weeks of training. Muscle cross-sectional area (mCSA) ultrasound and muscle biopsies were performed pre- and post-training. Based on mCSA changes between protocols, we applied a criterion of 2 typical errors (5.7%) to create 4 clusters. Twelve participants (~34%) showed greater mCSA increases after REPSProg (14.2 ± 7.6%) than LOADProg (3.4 ± 8.7%, $p = 0.004$). Seven participants (~19%) responded better to LOADProg (21.5 ± 7.5% vs. 12 ± 7.5%, $p = 0.041$). Thirteen participants (~35%) showed no differences between protocols ($p = 0.852$). Five participants were nonresponders (mCSA changes smaller than the 5.7% threshold) for both protocols. There were no differences ($p > 0.05$) in most of the assayed markers within any of the clusters. Notably, for those who responded better to REPSProg, this protocol promoted greater satellite cell changes (108.6 ± 77.0%) than LOADProg (48.9 ± 63.1%, $p = 0.015$). In conclusion, overload progression by increasing repetitions could produce greater or equal muscle hypertrophy for most individuals, which seems to be associated with greater satellite cell addition. However, responsiveness was not explained by changes in myonuclei, proteolysis or ECM remodeling biomarkers.

Keywords: Muscle cross-sectional area; Resistance training; Responsiveness; Satellite cells; Proteolysis.

INTRODUCTION

Resistance training (RT) has been well established as an effective non-pharmacological intervention to promote muscle hypertrophy (ROBERTS *et al.*, 2023). To ensure that hypertrophy occurs continuously, it is recommended to gradually progress the overload imposed by RT (KRAEMER *et al.*, 2004). Conventionally, overload the load within a repetition maximum zone (i.e., the load is adjusted set by set to ensure that concentric muscle failure occurs within the targeted repetition zone (LOADprog)). Nonetheless, we recently reported that LOADprog promotes muscle gains similar to those observed when increasing the number of repetitions, (i.e., individuals perform as many repetitions as possible until muscle failure with a fixed load (REPSprog)) (CHAVES *et al.*, 2024a). However, some individuals may present greater muscle gains in response to one RT protocol than to another (e.g., REPSProg vs. LOADProg). Through a within-subject design in which each leg of the same participant is allocated to a different protocol, studies revealed that some individuals have greater muscle mass gains when training at high weekly frequency (DAMAS *et al.*, 2019) or higher RT volume (LIXANDRÃO *et al.*, 2024), while others benefit more from a low frequency or lower volume. Hence, it is reasonable to suggest that different overload progression protocols could result in distinct individual responses, with some participants benefiting more from one protocol than from the other.

The difference in muscle mass gains between protocols performed by the same subject suggests the existence of a participant-by-training interaction which would distinctly modulate molecular processes regulating muscle hypertrophic response. Among those processes satellite cell activation has received considerable attention from the scientific community (ROBERTS *et al.*, 2018; ROBERTS *et al.*, 2023). It is believed that myocyte resident myonuclei have a limited transcriptional capacity, eventually reach a ceiling effect. Therefore, to maintain hypertrophy capacity, it would be required the addition of new myonuclei, which are provided

by the fusion of differentiated satellite cells into growing muscle fibers (BAMMAN *et al.*, 2018). Additionally, several associations have been observed between RT responsiveness and satellite cell content or satellite cell-mediated myonuclear accretion (PETRELLA *et al.*, 2008; BELLAMY *et al.*, 2014; ANGLERI *et al.*, 2022a; SMITH *et al.*, 2023). Thus, it is plausible that if overload progression protocols promote distinct individual responses, the abundance of satellite cells and myonuclei may also be differentially modulated.

Other mechanisms have been indicated to be involved in muscle hypertrophy, but little is known about their contributions to individual hypertrophic responses (ROBERTS *et al.*, 2018). For instance, the structural remodeling of skeletal muscles involves the activity of proteolytic systems that promote the recycling of damaged or inefficient cellular components (PASIAKOS *et al.*, 2014). Muscle remodeling involves changes not only in myocytes but also in the extracellular matrix (ECM). The ECM participates in several molecular processes, particularly as a repository for growth factors (NABA *et al.*, 2016), facilitator in mechanical sensing and force transmission (KJAER, 2004), and is fundamentally involved in skeletal muscle regeneration (MACKEY *et al.*, 2017). Evidence suggests that remodeling factors (e.g., matrix metalloproteinases [MMPs] and tissue inhibitors of metalloproteinases [TIMPs]) act to balance the ECM content, which can either be reduced to allow for myofiber hypertrophy or increased to strengthen the cellular scaffold (ROBERTS *et al.*, 2023). However, none of these mechanisms have been explored regarding individual responsiveness to different RT protocols.

Therefore, the present study aimed to compare individual muscle hypertrophy in response to REPSProg and LOADProg. For this, we used data from our previous group analysis study that assessed young untrained participants in a within-subject design (CHAVES *et al.*, 2024a). Additionally, we sought to explore whether protocol responsiveness could be explained by training-induced changes in satellite cell and myonuclei counts per muscle fiber, as well as

in the protein content and enzymatic activity levels of proteolytic and ECM remodeling markers following 10 weeks of RT (i.e., chronic changes).

METHODS

Participants

This is an ancillary analysis of data and muscle samples derived from a randomized controlled trial (Brazilian Registry of Clinical Trials – RBR-57v9mrb). The study was approved by the local ethics committee and conducted in accordance with the most recent version of the Declaration of Helsinki. Following the interviews and screening, 53 recreationally active young women and men were selected from the community to participate. The inclusion criteria were as follows: 18 to 35 years of age and not engaged in any exercise training program for at least 6 months prior to the study. Exclusion criteria involved the use of any drugs that could affect skeletal muscle mass or strength and any musculoskeletal disorders or injuries that could contraindicate RT practice. After being informed about the experimental procedures and potential associated risks and benefits, all participants signed a written consent form agreeing to enroll in the study. Eleven participants reported great discomfort at the first muscle biopsy procedure and opted to discontinue participation in the study. Four participants withdrew during the experimental period for personal reasons unrelated to the intervention. One participant did not complete post-intervention muscle biopsies and was therefore excluded from this analysis. Thus, the final sample of this study included 37 participants: eighteen women (age: 23.8 ± 4.6 years; body mass: 61.7 ± 7.7 kg; height: 163 ± 4.8 cm; BMI: 23.3 ± 3.3 kg/m2) and nineteen men (age: 24.5 ± 3.5 years; body mass: 73.0 ± 13.9 kg; height: 176 ± 6.6 cm; BMI: 23.4 ± 4.2 kg/m2).

Experimental design

This was an intra-subject study with repeated measures over time. Initially, participants had the muscle cross-sectional area of their vasti lateralis acquired via ultrasound at two different visits, with a 72-hour interval in between, to calculate the reproducibility of measurements. Next, a one repetition maximum (1RM) test was performed as described previously by our group (SCARPELLI *et al.*, 2022). For each participant, the right and left legs were randomly allocated to one of two different RT protocols. The randomization and allocation processes were based on leg dominance, leg strength (stronger/weaker) and the degree of strength difference between legs (high/moderate/low). Over the following 10 weeks, participants engaged in supervised RT sessions, 2-3 times a week, totaling 23 training sessions. Both RT protocols were performed in the same session in a randomized and counterbalanced order. Further details on the randomization and allocation processes are described in Chaves et al. (CHAVES *et al.*, 2024a). Muscle samples were collected via muscle biopsies from each leg immediately before the first RT session. The ultrasound assessments and muscle biopsies were repeated 96 hours after the last (23rd) RT session.

Training protocols

LOADprog consisted of 4 sets of 9-12 repetitions maximum of the unilateral leg extension exercise. The initial load of 80% of the 1RM was subsequently adjusted set by set when concentric muscle failure did not occur within the repetition maximum zone. Therefore, overload progressed through increases in load (LOADprog). The REPSprog was performed on the contralateral leg and consisted of 4 sets of unilateral leg extension exercises performed until concentric muscle failure with a fixed load equivalent to 80% of the initial 1RM. The load was not adjusted during the experimental period; therefore, overload progression was achieved by increments in the number of repetitions (REPSprog) whenever the participant was able to

postpone muscle failure. Participants were verbally encouraged throughout both protocols, and a 90-second rest interval was allowed between sets. For each protocol, the load (kg) and the number of repetitions were recorded for each set at each training session. The sum of repetitions performed throughout the intervention period was used to obtain the average number of repetitions per set per session. The sum of loads utilized throughout the intervention period was calculated to obtain the average load per set per session. The accumulated volume load was calculated as sets × repetitions × load (kg) considering the entire training period.

Muscle cross-sectional area (mCSA)

Participants were instructed to abstain from vigorous physical activity for at least 72 h before each assessment in order to minimize post-exercise edema (DAMAS *et al.*, 2016b). After arriving at the laboratory, the distance between the greater trochanter and the lateral epicondyle of the participants was measured, and the point corresponding to 50% of the femur length was marked as a reference for image acquisition. From this point, successive markings were made transversally, every 2 cm, in both the medial and lateral directions. The participant then laid still in a supine position for 15 minutes to allow tissue fluid stabilization. Sequential B-mode US images were acquired using a 7.5 MHz linear probe (MySono U6, Samsung, Sao Paulo, Brazil) and a water-soluble transmission gel to ensure acoustic coupling with minimal skin compression. Skin markings were used as a guide for the displacement of the probe in the sagittal plane, starting at the most medial mark (over the rectus femoris muscle). Using PowerPoint software (Microsoft, USA), the captured images were rotated and arranged to obtain a reconstructed panoramic image of the CSA of the entire muscle. The image was then opened on ImageJ (RASBAND, 2011), and the “polygon” tool was used to trace and calculate vastus lateralis CSA, excluding connective and bone tissue from the delimited area. The typical

error between two image acquisitions and quantifications separated by 72 h was 0.60 cm^2 (2.85%).

Muscle biopsies

Samples of the vastus lateralis muscle were obtained through a percutaneous muscle biopsy performed by a qualified medical professional with extensive experience in the procedure. Before tissue extraction, the region was cleaned with an antiseptic wash, and local subcutaneous anesthesia was applied [2-3 ml of 1% xylocaine (lidocaine)]. A small incision was made with a surgical scalpel, and a biopsy needle (Bergström model with manual suction) was introduced to a depth of approximately 4 cm to remove ~100 mg of muscle tissue, which was cleaned of all blood and connective tissue and separated into aliquots for analysis. The incision was closed and covered with bandages. A portion of tissue (20-30 mg) was positioned in liquid embedding medium for optimal cutting temperature (OCT), with the fibers perpendicular to the horizontal surface, and quickly frozen in nitrogen-cooled isopentane for immunohistochemical analyses. The remaining portion was placed in prelabeled RNA-free cryotubes for biochemical analyses. After separation, all the samples were immediately frozen in liquid nitrogen and stored at -80°C until processing.

Tissue homogenization

The neat muscle tissue was weighed using an analytical scale (Mettler Toledo ME303, Greifensee, Switzerland), and approximately 20 mg were placed in 1.7 ml microcentrifuge tubes. The samples were then homogenized in 500 µl of cell lysis buffer (cat. no. 9803; Cell Signaling Technology, Danvers, MA, USA) using hard plastic pestles and subsequently centrifuged at 500 × g for 5 minutes at 4°C. The resulting supernatants were transferred and stored in a new set of microtubes. Total protein concentrations were then determined using a

bicinchoninic acid (BCA) protein assay kit (cat. no. 23227; Thermo Fisher Scientific) and a spectrophotometer (BioTek Synergy H1, Winooski, VT, USA) following the manufacturer's instructions.

Western blotting

The cell lysates were added to 4× Laemmli buffer and distilled water (diH2O) to reach a 1 µg/µl protein concentration. Samples were then denatured for 5 min at 100°C prior to being stored at -80°C until analysis. Thereafter, the prepared samples (15 µl) were loaded onto the wells of 4−15% SDS–polyacrylamide gels (cat. no. 5671085, Criterion TGX; Bio-Rad Laboratories, Hercules, CA, USA) and subjected to electrophoresis at 180 V for 50 minutes with premade 1× SDS–PAGE running buffer (cat. no. RLMB-017, VWR-Avantor, Radnor, PA, USA). Proteins were then transferred to preactivated polyvinylidene difluoride membranes (cat. no. 1620177; Bio-Rad Laboratories) at 200 mA for two hours. Next, the membranes were stained with Ponceau for 10 minutes and digitally imaged with a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories). Next, the membranes were reactivated in methanol and blocked for one hour in a solution of 5% nonfat milk powder in Tris-buffered saline and 0.1% Tween-20 (TBST). The membranes were then incubated overnight at 4°C in an antibody solution containing a 1:1000 dilution in TBST supplemented with 5% bovine serum albumin (BSA) and one of the following: (i) rabbit anti-calpain-1 (cat. no.: 2556; Cell Signaling Technology); (ii) rabbit anti-calpain-2 (cat. no.: 70655; Cell Signaling Technology); (iii) rabbit anti-ubiquitin (cat. no.: 3933; Cell Signaling Technology), (iv) rabbit anti-20S core subunit antibody cocktail (cat. no. BML-PW8155; Enzo Life Sciences; Farmingdale, NY, USA); (v) rabbit anti-p62/SQSTM1 (cat. no. 5114; Cell Signaling Technology); (vi) rabbit anti-human MMP-9 (cat. no. ab76003; Abcam, Cambridge, MA, USA); (vii) rabbit anti-human MT1-MMP/MMP-14 (cat. no. 13130; Cell Signaling Technology); (viii) rabbit anti-human TIMP-1

(cat. no. 8946; Cell Signaling Technology); (ix) rabbit anti-human TIMP-2 (cat. no. 5738; Cell Signaling Technology). The next day, the membranes were incubated for one hour with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (cat. nos. 7076 and 7074; Cell Signaling Technology). The membranes were then developed using chemiluminescent substrate (Immobilon Forte, cat. no. WBLUF0500; MilliporeSigma, Burlington, MA, USA) and digitally imaged using a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories) and densitometry associated software (Image Lab v6.0.1; Bio-Rad Laboratories). Densitometry values were normalized to Ponceau densities.

Enzymatic activity assays

Calpain and proteasome activities on lysates were assayed using commercially available luminescence kits (cat. no.: G8502 and cat. no.: G8622; Promega Corporation, Madison, WI, USA). Briefly, 25 µl of lysates were loaded onto white 96-well plates in duplicate and incubated at room temperature for 10 minutes with the reagent mix provided by each kit. For global MMP activity, a commercially available fluorometric assay kit was used (cat. no.: AS-72202; AnaSpec, Freemont, CA, USA). Muscle tissue lysates (50 µl) were loaded in duplicate into black 96-well microplates with the substrate solution provided by the kit and incubated at room temperature for 30 minutes. Luminescence and fluorescence readings were performed with a microplate luminometer (BioTek Synergy H1, Winooski, VT, USA), and the values were divided by the total protein loaded per well and are expressed as relative luminescence/fluorescence units (RLU; RFU) per µg of muscle soluble protein. The fluorescence intensity was read using 330 nm excitation and 390 nm emission settings. The average coefficients of variation for duplicate values of calpain, proteasome and MMP activity were 5.2%, 5.4% and 6.5%, respectively.

Immunohistochemical analyses

OCT-preserved samples were transferred to a cryostat (Leica Biosystems, Buffalo Grove, IL, USA) set at -23°C, sectioned at a thickness of 14 μm, adhered to positively charged histology slides and stored at -80°C until batch-processed for immunohistochemical analyses. Samples from the same participant were placed on the same slide and analyzed concomitantly.

After being air-dried at room temperature for two hours, the slides were fixed with acetone at -20°C for 5 minutes. Slides were incubated with 3% H2O2 for 10 minutes at room temperature, followed by a 1-minute incubation with autofluorescence quenching reagent (TrueBlack, cat. no. 23007; Biotium, Fremont, CA, USA). Next, the slides were blocked for 60 minutes with 5% goat serum, 2.5% horse serum, and 0.1% Triton-X solution at room temperature. Subsequently, the slides were blocked with streptavidin and biotin solutions (cat. no. SP2002; Vector Laboratories, Newark, CA, USA) at room temperature for 15 minutes each. After blocking, the slides were incubated overnight at 4°C with a primary antibody cocktail of 1:20 Pax-7 (cat. no. pax7; RRID: AB_528428; Developmental Studies Hybridoma Bank; Iowa City, IA, USA), 1:100 Dystrophin (cat. no. GTX57970; GeneTex, Irvine, CA, USA), 1:100 BA-D5 (Myosin Heavy Chain I) (cat. no. BA-D5; RRID: AB_2235587; Developmental Studies Hybridoma Bank) and 2.5% horse serum in phosphate-buffered saline (PBS). The next day, the slides were incubated for 60 minutes with a 1:1000 solution of biotin-conjugated anti-mouse IgG1 (cat. no. 115-065-205; RRID: AB_2338571; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) in 2.5% horse serum, followed by a 60-minute incubation with a secondary antibody cocktail of 1:500 streptavidin (SA-HRP, cat. no. S911; Thermo Fisher Scientific, Waltham, MA, USA), 1:250 goat anti-mouse IgG2b Alexa Fluor 647 (cat. no. A-21242; Thermo Fisher Scientific), 1:250 goat anti-rabbit IgG DyLight488 (cat. no. DI-1488; Vector Laboratories) in PBS, and a 20-minute incubation with 1:200 TSA-555 (cat. no. B40955; Thermo Fisher Scientific). All steps mentioned above involved gentle washes in PBS. Slides

were then stained with DAPI (4=,6-diamidino-2-phenylindole; cat. no. D3571; Thermo Fisher Scientific) for 10 minutes at room temperature and mounted with glass coverslips using 1:1 PBS and glycerol as mounting medium. The sections were stored in the dark at 4°C until imaging. Digital images were captured using a fluorescence microscope set at ×20 magnification (Nikon Instruments; Melville, NY, USA). At least 50 fibers per sample were quantified (MACKEY *et al.*, 2009), and all areas selected for analyses were free of freeze-fracture artifacts. The staining method allowed the identification of cell membranes (detected by the TRITC filter), type I fiber cell bodies (detected by the FITC filter), type II fiber cell bodies (unlabeled), satellite cells (detected by the Texas Red filter), and myonuclei (detected by the DAPI filter).

Standardized counting of muscle fibers and myonuclei was performed via open-sourced software (MyoVision) (WEN *et al.*, 2018), applying a conversion ratio of 0.46 μm/pixel and a detection range from 500 to 15,000 μm2. Next, an investigator visually verified the software results, and empty spaces or incorrectly outlined fractured fibers were manually excluded from the analysis. The number of satellite cells on the laminin border was determined via visual inspection and verification of the colocalization of Pax7+ and DAPI. For each sample, the content of myonuclei and satellite cells was normalized to the respective number of muscle fibers analyzed. Notably, all procedures – sectioning, staining, imaging – and analyses were performed by investigators who were blinded to the interventions.

Statistical analyses

Participants were separated into four clusters based on their hypertrophic responses, and 2 typical errors (2×TE) of mCSA measurements were used as the cutoff criterion. A change in mCSA greater than this threshold would have a high probability of being a true different

adaptation beyond any technical and/or biological variability (HOPKINS, 2001). Specifically, the TE was calculated using the following equation:

$$TE = \frac{SD_{diff}}{\sqrt{2}}$$

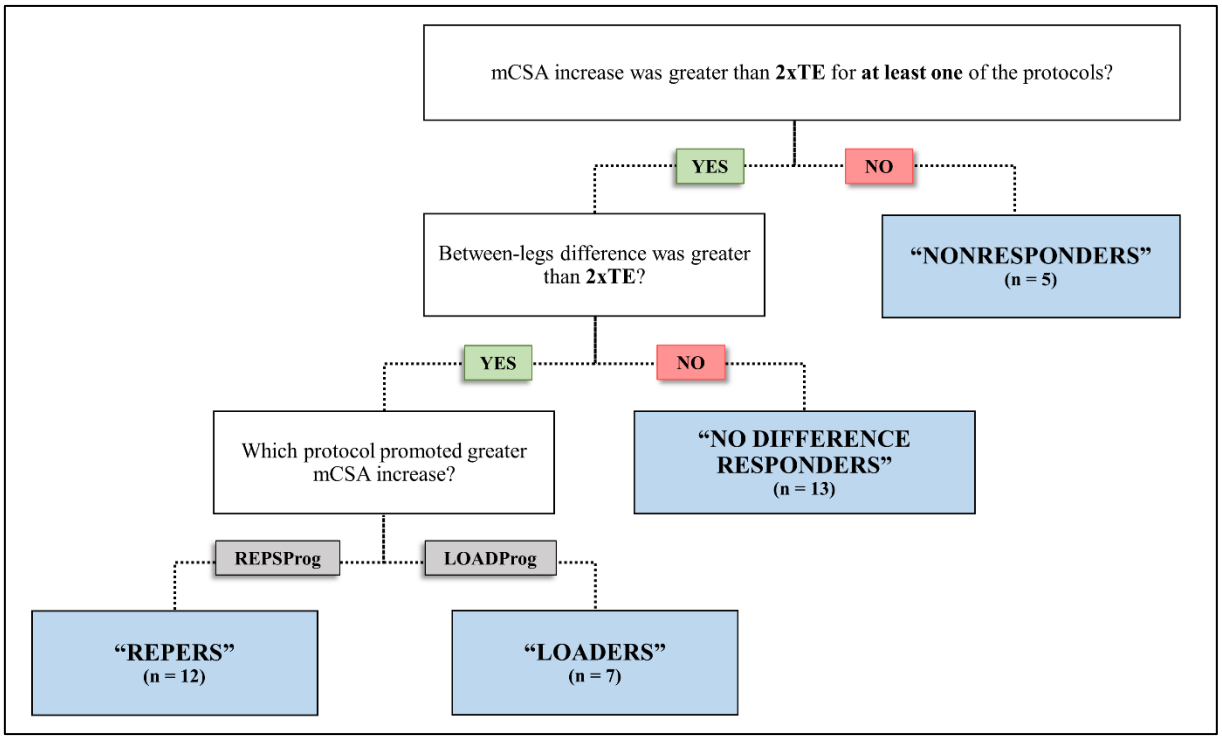
where SD_{diff} is the standard deviation (SD) of the difference between the two baseline mCSA measurements obtained through the US assessments. The TE of the present sample was 0.60 cm^2, yielding a 2×TE value of 1.2 cm^2. We used the 2×TE criterion, expressed as a percentage of the mean of the baseline mCSA measurements (HOPKINS, 2001), to compare it to the relative pre-to-post intervention changes in mCSA. Hence, our clustering criterion was 5.7%.

Figure 1 presents a flowchart summarizing the clustering procedure. First, a participant was defined as a "responder" to RT if she or he presented muscle gains greater than 2×TE for at least one of the RT protocols; otherwise, she or he was considered a "NONRESPONDER". Second, for "responders", we compared hypertrophy results between legs. If the difference between muscle gains was smaller than 2×TE, the participant was classified as a "NO DIFFERENCE RESPONDER". If the difference was greater than 2×TE, the participant was placed in either the "REPERS" or the "LOADERS" cluster, depending on whether greater gains were observed in response to REPSprog or LOADprog, respectively.

All statistical analyses were conducted for each cluster separately. Analyses of covariance (ANCOVA) were implemented for all outcomes to compare relative chronic changes (from Pre to Post) with training protocol as a fixed factor, Pre values as covariates, and participants as random factors. The normality of residuals and the effect of extreme observations were determined through residual analyses and influence diagnostics. Additionally, for each outcome, the effect size (ES) and the respective 95% confidence interval (CI) were calculated (HEDGES *et al.*, 1985) between relative changes observed in the REPSprog and LOADprog protocols. Positive and negative CIs not crossing zero (0) were considered significant (NAKAGAWA *et al.*, 2007). Repeated measures correlations (r_{rm}) were

calculated (BAKDASH *et al.*, 2017) to determine the within-cluster associations between relative chronic changes in mCSA and relative chronic changes in the assayed markers. Data from the same participant following the two protocols were considered as paired measures. Correlations were classified as very weak ($|r| < 0.20$), weak ($0.20 < |r| < 0.39$), moderate ($0.40 < |r| < 0.59$), strong ($0.60 < |r| < 0.79$) or very strong ($|r| > 0.80$) (EVANS, 1996). Paired t tests were used to compare the accumulated volume load, the average number of repetitions, and the average load between protocols. A chi-square test of goodness-of-fit was performed to determine whether the observed frequency of REPERS and LOADERS was significantly different. All data are presented as mean ± standard deviation (SD), unless otherwise stated. Significance was set at $p < 0.05$. Analyses were performed using SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA), the rmcorrShiny application (MARUSICH *et al.*, 2021) and GraphPad Prism 9.5 software (GraphPad Software, San Diego, CA, USA). Graphs were constructed using GraphPad Prism 9.5.

Figure 1 – Flowchart of the clustering process



RESULTS

Loaders

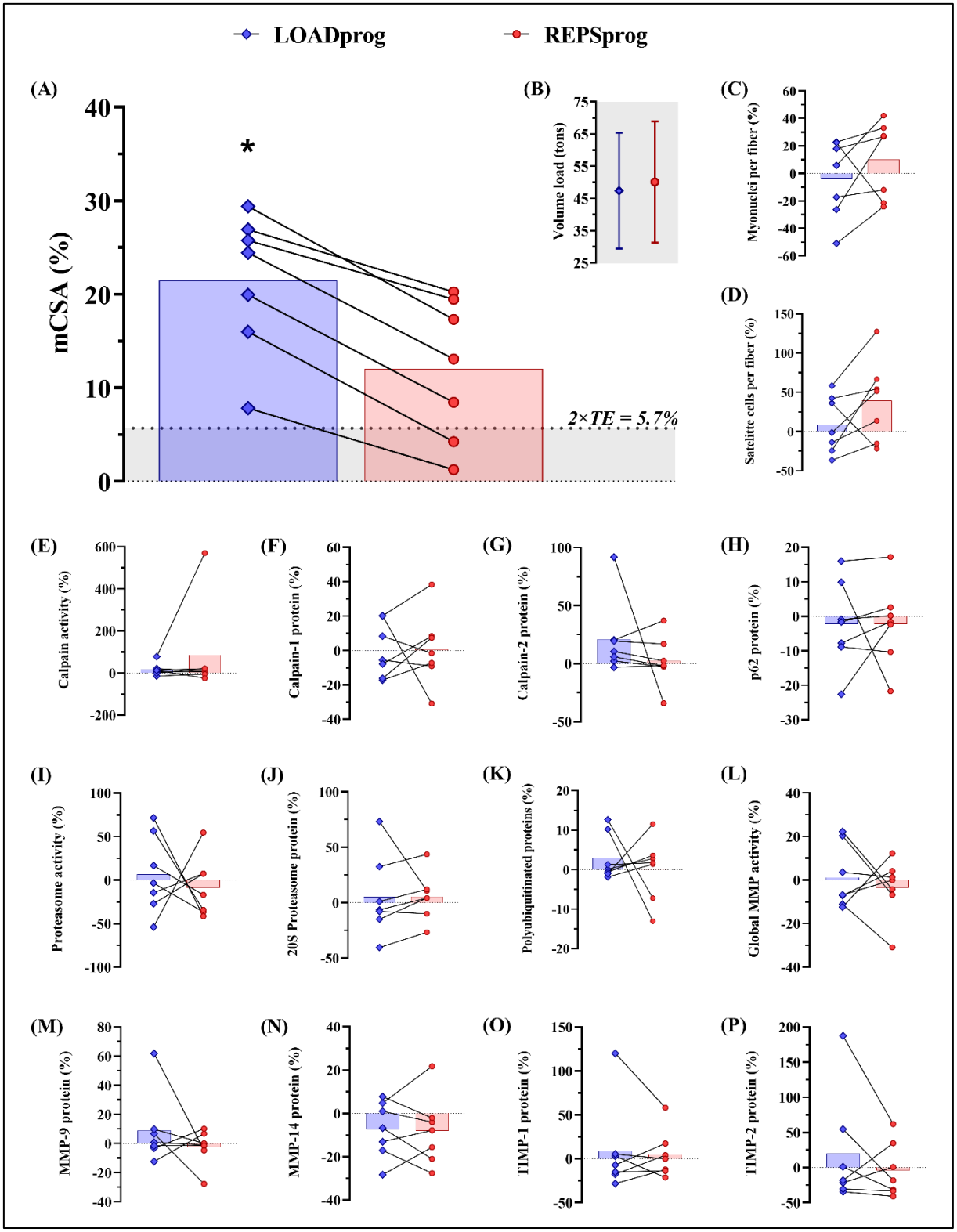
Seven participants (3 women and 4 men) were deemed "LOADERS"; i.e., their response to RT met the 2×TE criterion and muscle gains favored LOADProg in over 5.7% (2×TE) compared to REPSProg. As per design, ANCOVA results showed that mCSA relative changes from pre- to post-intervention were lower for REPSProg (12 ± 7.5%) than for LOADProg (21.5 ± 7.5%; $F = 70.5$, $p = 0.0004$). The CI of the ES (ES: -1.18; 95% CI: -2.31 to -0.04) confirmed the inferential analysis.

A t-test revealed no significant differences in volume load between LOADProg (47.4 ± 17.9 tons) and REPSProg (50.1 ± 18.8 tons; $p = 0.616$). The average number of repetitions performed per set per training session was greater for REPSProg (13.2 ± 3.1) than for LOADProg (10.4 ± 1.0; $p = 0.021$), with no significant differences in the average load utilized per set per training session (LOADProg: 48.6 ± 15.1 kg; REPSProg: 41.6 ± 14.8 kg; $p = 0.194$).

ANCOVA revealed no significant differences between protocols on any of the biomarkers investigated: (i) myonuclei ($F = 0.52$, $p = 0.505$), (ii) satellite cell counts ($F = 2.26$, $p = 0.193$), (iii) global calpain activity ($F = 2.20$, $p = 0.198$), (iv) global proteasome activity ($F = 0.26$, $p = 0.631$), (v) global MMP activity ($F = 0.10$, $p = 0.769$), (vi) calpain-1 protein content ($F = 0.00$, $p = 0.956$), (vii) calpain-2 protein content ($F = 0.00$, $p = 0.967$), (viii) 20S proteasome protein content ($F = 0.51$, $p = 0.506$), (ix) polyubiquitinated proteins ($F = 0.32$, $p = 0.598$), (x) p62 ($F = 0.00$, $p = 0.990$), (xi) MMP-9 protein content ($F = -0.38$, $p = 0.717$), (xii) MMP-14 protein content ($F = 0.11$, $p = 0.756$), (xiii) TIMP-1 protein content ($F = 0.01$, $p = 0.937$), or (xiv) TIMP-2 protein content ($F = 0.27$, $p = 0.628$). The inferential analysis was confirmed by the CIs of the respective ESs. Repeated measures correlation analysis revealed no correlation between chronic changes in mCSA and chronic changes in the assayed markers.

A table with the relative changes (post vs. pre), the correlation coefficients and the ES and CI between protocols for all outcomes is presented in the supplemental material.

Figure 2 – Relative changes in muscle cross-sectional area and selected biomarkers for "LOADERS"



Legend: Relative changes observed within the "LOADERS" (n = 7) cluster following 10 weeks of resistance training using LOADProg and REPSProg in (A) muscle cross-sectional area (mCSA); (C) myonuclei and (D) satellite cell count per muscle fiber; enzymatic activity levels of (E) calpain, (I)

proteasome and (L) MMPs; and relative protein expression of (F) calpain-1, (G) calpain-2, (H) p62, (J) 20S proteasome, (K) poly-ubiquitinated proteins, (M) MMP-9, (N) MMP-14, (O) TIMP-1 and (P) TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel (B) shows the accumulated volume load, with diamond and circle representing the mean values. LOADERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion, and muscle gains favored the LOADProg protocol in over 5.7% (2×TE) compared to REPSProg; TE = typical error; *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

Repers

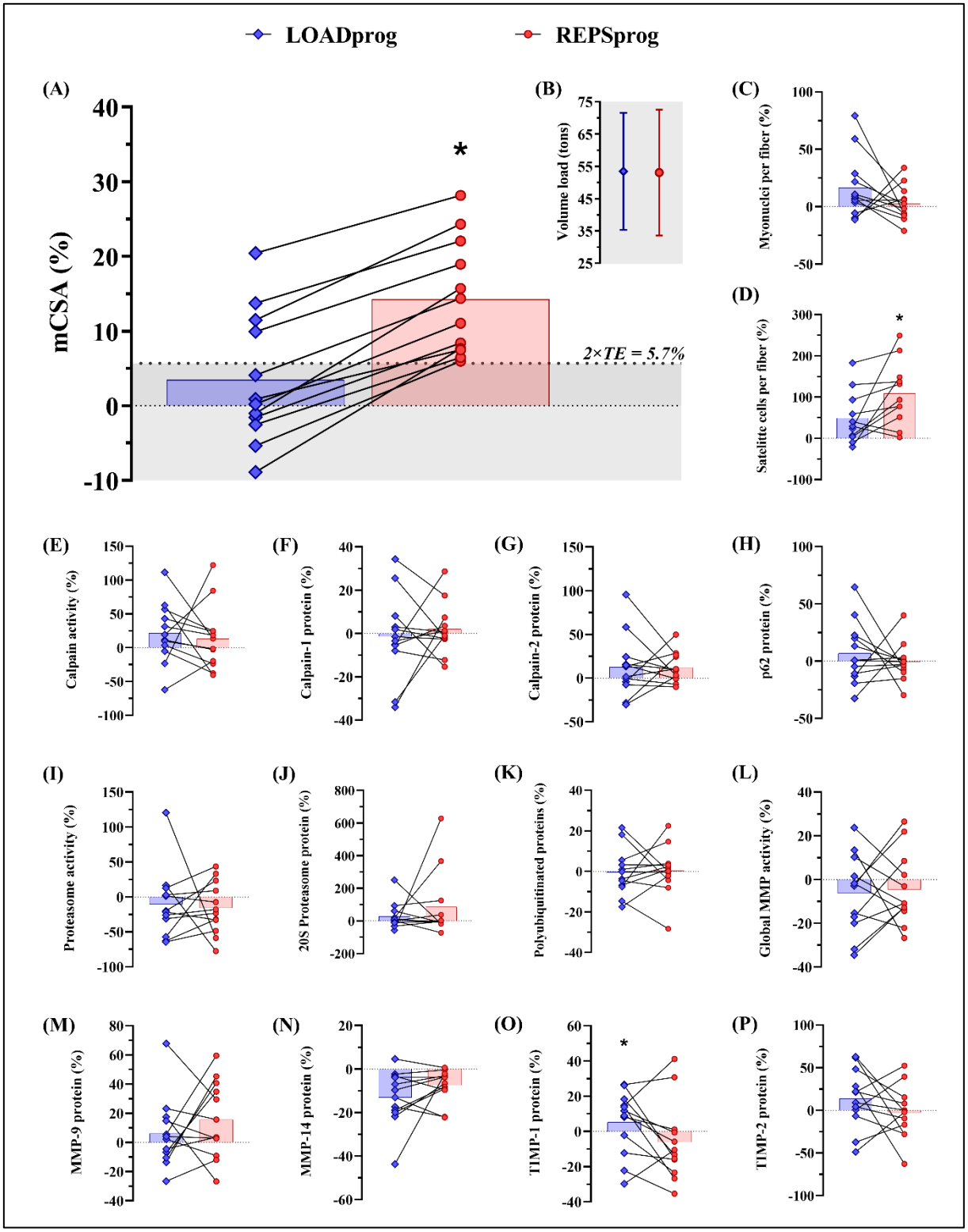
A total of 12 participants (6 women and 6 men) were classified as "REPERS", i.e., the response to RT met the 2×TE criterion, and muscle gains favored the REPSProg protocol, with a difference greater than 2×TE compared to the LOADProg protocol. In accordance with our design, the ANCOVA results indicated that mCSA relative changes were greater for REPSProg (14.2 ± 7.6%) than for LOADProg (3.4 ± 8.7%; $F = 145.94$, $p < 0.0001$). The CI of the ES (ES: 1.27; 95% CI: 0.39 to 2.15) confirmed the inferential analysis.

A t-test showed no significant differences in the volume load between LOADProg (53.4 ±18.1 tons) and REPSProg (53.1 ± 19.5 tons; $p = 0.829$). The average number of repetitions performed per set per training session was greater for REPSProg (14.0 ± 3.1) compared to LOADProg (10.7 ± 0.8; $p < 0.001$), while the average load utilized per set per training session was greater for LOADProg (55.3 ± 17.4 kg) than for REPSProg (41.8 ± 14.2 kg; $p < 0.001$).

For the biomarkers assessed, results revealed that the relative chronic changes in satellite cell count per fiber were greater for REPSProg (108.6 ± 77.0%) than for LOADProg (48.9 ± 63.1%; $F = 9.04$, $p = 0.015$). As for the protein content of TIMP-1, chronic changes were lower for REPSProg (-6.1 ± 22.4) than for LOADProg (5.1 ± 18.2%; $F = 7.75$; $p = 0.019$). There were no significant differences in the other variables including: (i) the number of myonuclei ($F = 0.84$; $p = 0.381$), (ii) global calpain activity ($F = 0.49$; $p = 0.500$), (iii) global proteasome activity ($F = 0.13$, $p = 0.724$), (iv) global MMP activity ($F = 0.14$, $p = 0.719$), (v) calpain-1 protein content ($F = 0.04$, $p = 0.829$), (vi) calpain-2 protein content ($F = 0.19$, $p = 0.674$), (vii) 20S proteasome protein content ($F = 0.55$, $p = 0.474$), (viii) polyubiquitinated proteins ($F =$

0.03, p = 0.861), (ix) p62 protein content (F = 1.08, p = 0.323), (x) MMP-9 protein content (F = 0.31, p = 0.589), (xi) MMP-14 protein content (F = 0.00, p = 0.970), or (xii) TIMP-2 protein content (F = 4.62, p = 0.057). The CIs of the respective ESs (Table 2) did not confirm the difference observed in the relative changes in satellite cell count (ES: 0.82; 95% CI: -0.01 to 1.65) or in TIMP-1 (ES: -0.54; 95% CI: -1.35 to 0.28) but confirmed inferential results for all other outcomes. There was a strong and positive correlation between relative changes in mCSA and satellite cell count per fiber (r_{rm} = 0.68; 95% CI: 0.17 to 0.90; p = 0.016), and a moderate and negative correlation between relative changes in mCSA and TIMP-1 protein content (r_{rm} = -0.58; 95% CI: -0.86 to -0.05; p = 0.035). There were no significant correlations with the other assayed markers (supplemental material).

Figure 3 – Relative changes in muscle cross-sectional area and selected biomarkers for "REPERS"



Legend: Relative changes observed within the "REPERS" (n = 12) cluster following 10 weeks of resistance training using LOADProg and REPSProg in (A) muscle cross-sectional area (mCSA); (C) myonuclei and (D) satellite cell count (n = 11) per muscle fiber; enzymatic activity levels of (E) calpain,

(I) proteasome and (L) MMPs; and relative protein expression of (F) calpain-1, (G) calpain-2, (H) p62, (J) 20S proteasome, (K) poly-ubiquitinated proteins, (M) MMP-9, (N) MMP-14, (O) TIMP-1 and (P) TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel (B) shows the accumulated volume load, with diamond and circle representing the mean values. REPERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion, and muscle gains favored the REPSProg protocol in over 5.7% (2×TE) compared to LOADProg; TE = typical error; *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

No difference responders

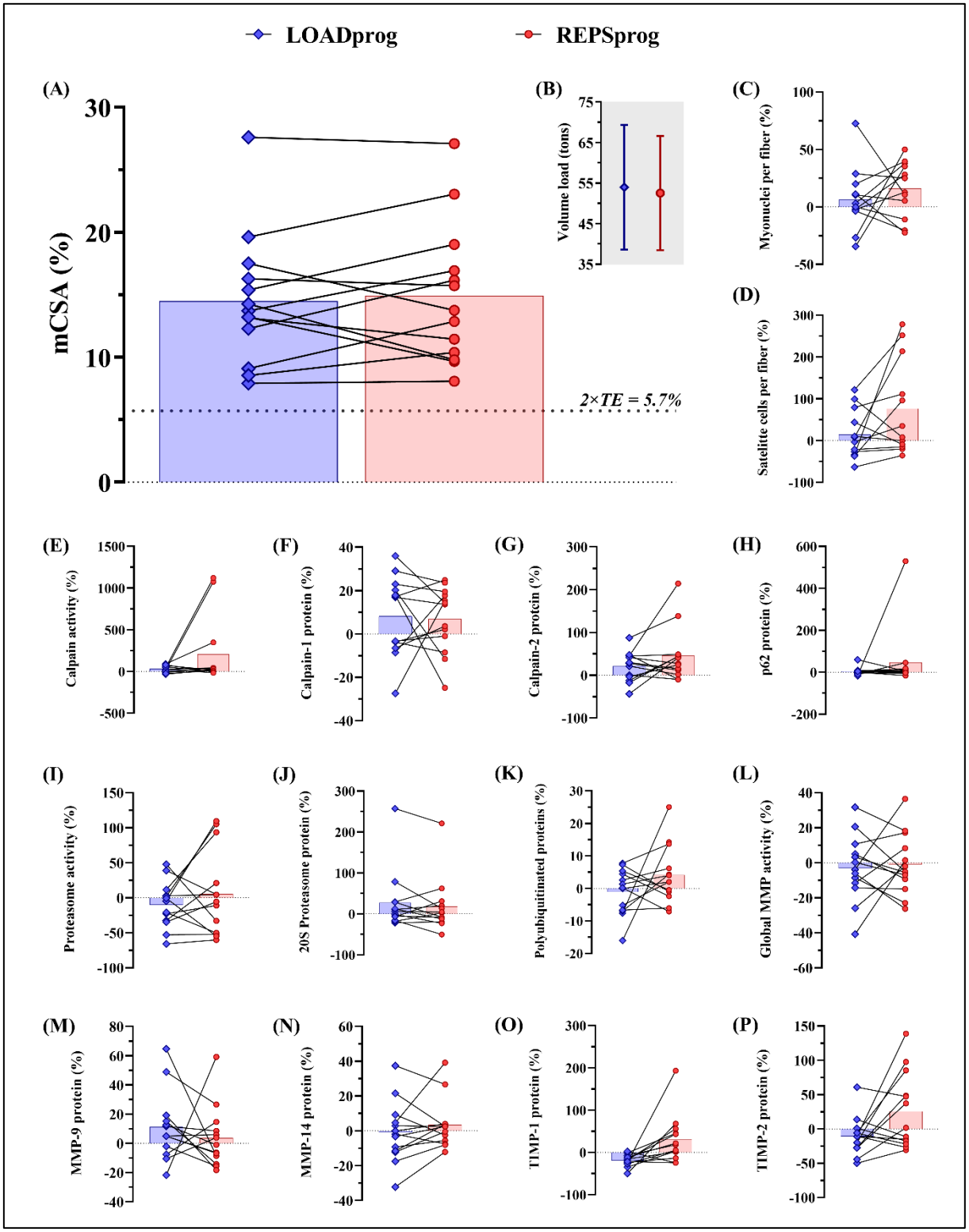
A total of 13 participants (5 women and 8 men) were classified as "NO DIFFERENCE RESPONDERS"; i.e., muscle gains in response to RT met the 2×TE criterion for at least one of the protocols, but the difference between protocols was smaller than the criterion (5.7%). According to the study design, the ANCOVA results revealed no significant differences when comparing the relative changes in mCSA between REPSProg (14.9 ± 5.6%) and LOADProg (14.5 ± 5.2%; $F = 0.22$, $p = 0.650$). The CI of the ES (ES: 0.08; 95% CI: -0.69 to 0.84) confirmed the inferential analysis.

A t-test revealed no significant differences between the volume load achieved in LOADProg (53.9 ± 15.3 tons) and that achieved in REPSProg (52.5 ± 14.1 tons; $p = 0.582$). The average number of repetitions performed per set per training session was greater for REPSProg (14.4 ± 2.9) than for LOADProg (10.5 ± 0.5; $p < 0.001$), while the average load used per set per session was greater for LOADProg (56.9 ± 14.5 kg) than for REPSProg (40.6 ± 11.0 kg; $p < 0.001$).

ANCOVA results revealed no significant differences in the assayed markers: (i) myonuclear number ($F = 0.51$, $p = 0.490$), (ii) satellite cell counts ($F = 2.92$, $p = 0.118$), (iii) global calpain activity ($F = 4.50$, $p = 0.057$), (iv) global proteasome activity ($F = 1.25$, $p = 0.287$), (v) global MMP activity ($F = 0.17$, $p = 0.692$), (vi) calpain-1 protein content ($F = 0.04$, $p = 0.846$), (vii) calpain-2 protein content ($F = 3.78$, $p = 0.078$), (viii) 20S proteasome protein content ($F = 0.01$, $p = 0.919$), (ix) polyubiquitinated proteins ($F = 2.91$, $p = 0.116$), (x) p62 protein content ($F = 1.26$; $p = 0.285$), (xi) MMP-9 protein content ($F = 0.49$, $p = 0.500$), (xii)

MMP-14 protein content (F = 0.54, p = 0.480), (xiii) TIMP-1 protein content (F = 3.75, p = 0.079), or TIMP-2 protein content (F = 4.29, p = 0.063). The CI of the respective ES (Table 3) confirmed the absence of difference observed in most of the markers but indicated significant effects in the protein content of TIMP-1 (ES: 1.14; 95% CI: 0.31 to 1.97) and TIMP-2 (ES: 0.81; 95% CI: 0.01 to 1.61), both favoring changes observed in REPSProg rather LOADProg. There were no significant correlations between changes in mCSA and in the assayed markers (supplemental material).

Figure 4 – Relative changes in muscle cross-sectional area and selected biomarkers for "NO DIFFERENCE RESPONDERS"



Legend: Relative changes observed within the "NO DIFFERENCE RESPONDERS" (n = 13) cluster following 10 weeks of resistance training using LOADProg and REPSProg in (A) muscle cross-sectional area (mCSA); (C) myonuclei (n = 12) and (D) satellite cell count (n = 12); enzymatic activity levels of

(E) calpain, (I) proteasome and (L) MMPs; and relative protein expression of (F) calpain-1, (G) calpain-2, (H) p62, (J) 20S proteasome, (K) poly-ubiquitinated proteins, (M) MMP-9, (N) MMP-14, (O) TIMP-1 and (P) TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel (B) shows the accumulated volume load, with diamond and circle representing the mean values. NO DIFFERENCE RESPONDERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion but the difference between protocols was smaller than 5.7% (2×TE); TE = typical error; *, indicates a significant difference between protocols. The significance level was set at $p < 0.05$.

Nonresponders

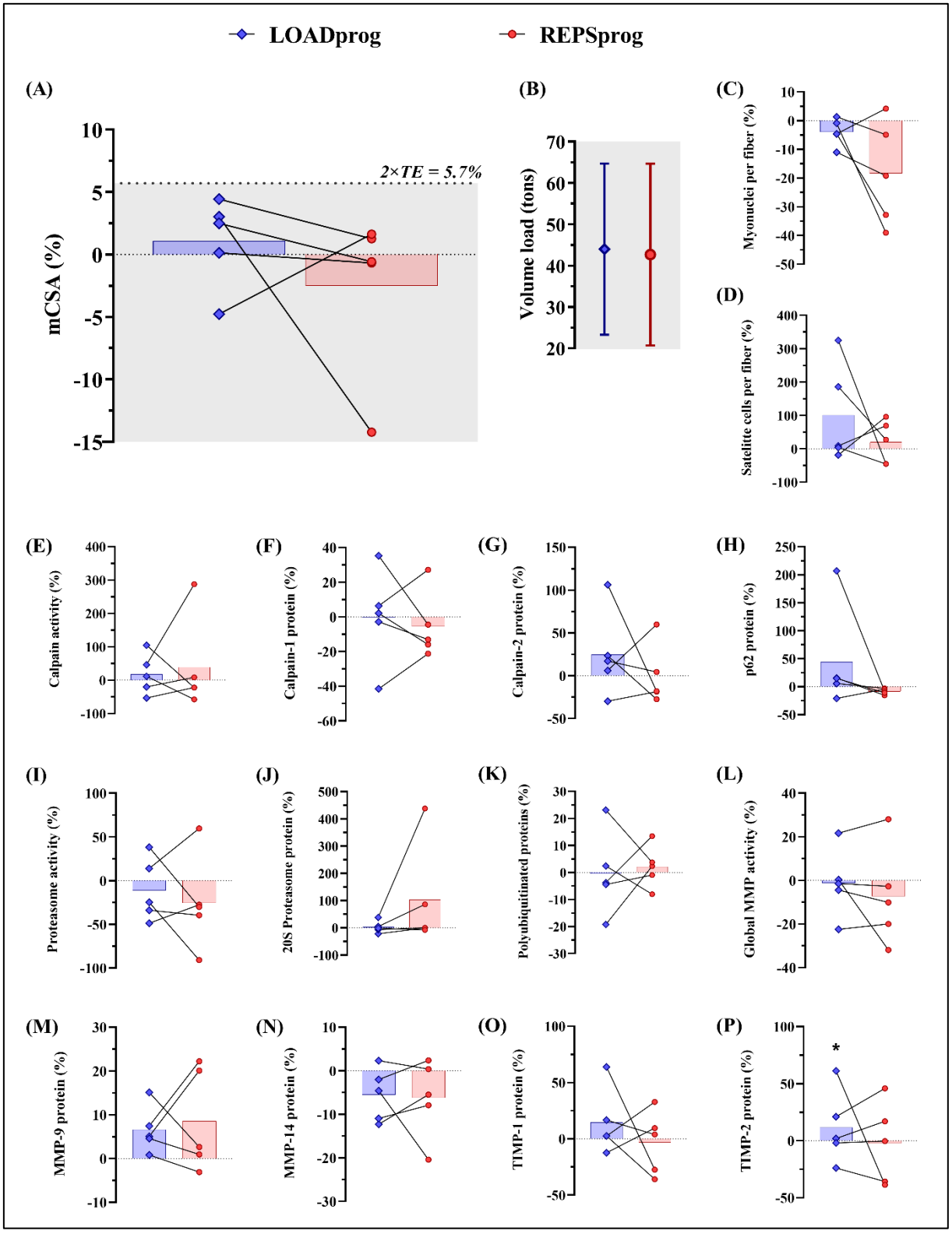
Five participants (4 women and 1 man) were considered "NONRESPONDERS"; i.e., muscle gains were smaller than 5.7% in response to both protocols. According to the study design, the ANCOVA results revealed no significant differences in the mCSA relative changes between LOADProg (1.1 ± 3.6%) and REPSProg (-2.5 ± 6.6%; $F = 0.92$, $p = 0.408$). The CI of the ES (ES: -0.61; 95% CI: -1.87 to 0.66) confirmed the inferential analysis.

There were no significant differences between the volume load observed in LOADProg (43.9 ± 20.7 tons) and REPSProg (42.6 ± 21.9 tons; $p = 0.241$). There were no differences in the average number of repetitions performed (LOADProg: 10.5 ± 0.6; REPSProg: 12.3 ± 1.9 kg; $p = 0.058$) or the average load utilized per set per session (LOADProg: 45.0 ± 20.1 kg; REPSProg: 36.4 ± 12.7 kg; $p = 0.069$).

ANCOVA revealed that changes in TIMP-2 protein content were greater for LOADProg (11.6 ± 32.0%) than for REPSProg (-2.4 ± 35.8%; $F = 20.45$, $p = 0.020$). There were no differences in the changes of the following biomarkers: (i) myonuclear number ($F = 2.61$, $p = 0.205$), (ii) satellite cell counts ($F = 3.48$, $p = 0.159$), (iii) global calpain activity ($F = 0.08$, $p = 0.795$), (iv) global proteasome activity ($F = 2.53$, $p = 0.209$), (v) global MMP activity ($F = 2.83$, $p = 0.191$), (vi) calpain-1 protein content ($F = 4.21$, $p = 0.132$), (vii) calpain-2 protein content ($F = 1.46$, $p = 0.314$), (viii) 20S proteasome protein content ($F = 1.01$, $p = 0.389$), (ix) polyubiquitinated proteins ($F = 0.00$, $p = 0.951$), (x) p62 protein content ($F = 2.32$, $p = 0.225$), (xi) MMP-9 protein content ($F = 0.13$, $p = 0.740$), (xii) MMP-14 protein content ($F = 0.05$, $p = 0.830$), or (xiii) TIMP-1 protein content ($F = 2.02$, $p = 0.250$). The CIs of the ESs did not

confirm the difference observed in the relative changes in TIMP-2 protein content (ES: -0.37; 95% CI: -1.62 to 0.88) but confirmed the remaining results of the inferential analysis. There were no significant correlations between changes in mCSA and in the assayed markers (supplemental material).

Figure 5 – Relative changes in muscle cross-sectional area and selected biomarkers for "NONRESPONDERS"



Legend: Relative changes observed within the "NONRESPONDERS" (n = 5) cluster following 10 weeks of resistance training using LOADProg and REPSProg in (A) muscle cross-sectional area (mCSA); (C) myonuclei and (D) satellite cell count per muscle fiber; enzymatic activity levels of (E)

calpain, (I) proteasome and (L) MMPs; and relative protein expression of (F) calpain-1, (G) calpain-2, (H) p62, (J) 20S proteasome, (K) poly-ubiquitinated proteins, (M) MMP-9, (N) MMP-14, (O) TIMP-1 and (P) TIMP-2. The diamonds and circles refer to individual values, and the bars refer to group means. Panel (B) shows the accumulated volume load, with diamond and circle representing the mean values. NONRESPONDERS = cluster of participants whose muscle gains were smaller than 5.7% (2×TE) in response to both protocols; TE = typical error; *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

Cluster distribution analysis

The result of the chi-square test of goodness-of-fit reveals that the distribution of participants between REPERS and LOADERS was not significantly different (χ^2 [3, n = 37] = 1.316, p = 0.725). Considering that the chi-square test is sensitive to the sample size (MCDONALD, 2014), we have performed simulations to verify the sample size that would be necessary to observe a significant difference. Simulation analyses indicate that in a sample size greater than 222 participants, the same frequency amongst clusters would result in a significantly different distribution of participants between LOADERS and REPERS (χ^2 [3, n = 222] = 7.895, p = 0.048).

DISCUSSION

The current study is the first to investigate individual RT-induced responses to different models of overload progression. We were able to explore the variability in responsiveness due to the use of an intra-subject design, with each participant performing both the LOADProg and REPSProg protocols. Notably, one-third of participants presented greater muscle hypertrophy when overload progressed by increasing repetitions compared to increasing load, which was associated with chronic changes in the number of satellite cells.

For a considerable portion of our sample (n = 22 [~60%]), both overload progression models were effective at promoting muscle hypertrophy greater than the 2×TE criterion, which warrants a high probability of true adaptation. These muscle gains will likely have several other positive health impacts (SARTORI *et al.*, 2021), as evidence indicates that greater muscle mass

is associated with a reduced risk of cardiovascular outcomes (KIM *et al.*, 2024), better insulin sensitivity and a lower risk of transitional/pre- or overt diabetes (SRIKANTHAN *et al.*, 2011), prevention or better resolution of nonalcoholic fatty liver disease (KIM *et al.*, 2018) and a lower risk of overall and cause-specific mortality (LIU *et al.*, 2022). Considering that attaining some gain in muscle mass, regardless of its magnitude, is relevant for health, practitioners could consider individual preferences when selecting the overload progression model, whether it is LOADprog or REPSprog. On the other hand, some participants (n = 5 [13.5%]) were resistant to the training stimuli, which has been reported to occur in numerous other studies (BAMMAN *et al.*, 2007; PETRELLA *et al.*, 2008; AHTIAINEN *et al.*, 2016; STEC *et al.*, 2016; DAMAS *et al.*, 2019; LIXANDRÃO *et al.*, 2024). While non-responsiveness can be broadly affected by genetic factors and nutritional status (MANN *et al.*, 2014), it is possible that the training stimuli provided by both overload progression models used in the present study were insufficient to activate muscle hypertrophy-related molecular mechanisms (ROBERTS *et al.*, 2023). Although it is unknown whether these individuals would benefit from different RT stimuli, Lixandrão *et al.* (2024) recently reported that increased volume mitigated non-responsiveness in older adults. Hence, it is possible that other RT protocols could trigger muscle growth in nonresponders.

When comparing muscle hypertrophy between protocols, our results indicate that 35.1% of the participants showed no differences between them ("NO DIFFERENCE RESPONDERS"). Those findings are consistent with our previous group analysis which showed no differences in muscle hypertrophy following LOADProg and REPSProg (CHAVES *et al.*, 2024a). Nonetheless, most of our participants (51.3%) showed muscle gains favoring one or the other protocol, with responses differing by more than 5.7% (2×TE). It is striking that REPSProg promoted greater mCSA increases to 12 (~32.4%) participants ("REPERS"), while the conventional overload progression model (i.e., LOADProg) favored just approximately half of that amount (n = 7 [~18.9%]; "LOADERS"). Furthermore, for some individuals, the overload

progression protocol was a key determinant for reaching the responsivity threshold since 8 of the 12 “REPERS” responded only to REPSProg (i.e., changes in the mCSA of the leg that performed LOADProg within the non-responder threshold), and 2 of the 7 “LOADERS” responded exclusively to LOADProg. These differences were observed despite the similar volume loads achieved. However, the design of the protocols led to differences in the volume load components, with a greater volume (number of repetitions) being performed during REPSProg and greater loads being performed in LOADProg. Although the chi-squared test suggests the number of participants classified as REPERS and LOADERS is not significantly different, this test is sensitive to the sample size (MCDONALD, 2014). In a larger sample ($n \geq 222$), the same distribution of participants amongst clusters would result in a significant difference between the number of LOADERS and REPERS. Thus, it would be plausible to suggest that REPSProg could be successful in promoting higher – or at least similar – muscle hypertrophy for a greater share of individuals, i.e., opting for higher volumes (i.e., more repetitions) instead of higher loads could be further beneficial.

Our study is the first to explore, via an intra-subject approach, whether biomarkers of molecular processes are differentially modulated by RT protocols and thus divergently drive the hypertrophic response. Studies that have compared individual responses to RT protocols have not investigated molecular markers (DAMAS *et al.*, 2019; ANGLERI *et al.*, 2022b; LIXANDRÃO *et al.*, 2024). Even so, individual responses and molecular mechanisms have been investigated in low and high responders to the same RT protocol (BAMMAN *et al.*, 2007; PETRELLA *et al.*, 2008; DAVIDSEN *et al.*, 2011; OGASAWARA *et al.*, 2016; STEC *et al.*, 2016; MOBLEY *et al.*, 2018; HAUN *et al.*, 2019; GODWIN *et al.*, 2023a; SMITH *et al.*, 2023). For example, myonuclear accretion mediated by satellite cells is a primary mechanism associated with the individual variability in hypertrophic responses (PETRELLA *et al.*, 2006; PETRELLA *et al.*, 2008; ROBERTS *et al.*, 2018; ANGLERI *et al.*, 2022a). Petrella *et al.* (2008)

classified young and older participants of both sexes without previous RT experience into three clusters based on myofiber hypertrophy. After 16 weeks of RT, extreme responders expanded their myonuclei and satellite cell pool, while nonresponders did not. Similarly, in a cohort of 19 young resistant trained men, Angleri *et al.* (2022a) reported that changes in muscle fiber cross-sectional area were aligned with changes in the number of myonuclei and in the satellite cell pool in the higher responders and lower responders. Our findings corroborate the results for satellite cells but not for myonuclei. The greater hypertrophy observed for REPSProg in the REPERS cluster was accompanied by a greater addition of satellite cells compared to LOADProg (Fig. 3, Panel D), and there was a positive correlation between these variables (r_{rm} = 0.68). In contrast, the small number of participants may have hindered this observation in LOADERS. Regarding the changes in myonuclei count, Petrella *et al.* (2008) and Angleri *et al.* (2022a) compared clusters of high and low responders with critically contrasting hypertrophic responses. Myofiber hypertrophy averaged 58 and 0% respectively for extreme responders and nonresponders in Petrella *et al.* (2008), and 39 and -9% respectively for the top 4 responders and bottom 4 lower responders in Angleri *et al.* (2022a). Meanwhile, our analyses compared RT protocols that promoted muscle hypertrophy, which differed by an average of ~10% between protocols (for "REPERS" and "LOADERS"). In fact, a more robust muscle hypertrophy seems to be associated with greater myonuclear accretion (CONCEIÇÃO *et al.*, 2018). Thus, we hypothesize that any differences in myonuclei count that could arise due to a participant-by-training interaction would depend on observing a greater contrast in muscle adaptations, especially in response clusters of small size.

Both proteolytic activity and ECM remodeling seem to be involved in the regeneration of skeletal muscle (PASIAKOS *et al.*, 2014; AHMAD *et al.*, 2023; ROBERTS *et al.*, 2023). Moreover, proteolysis may be critical for muscle growth, as the inhibition or downregulation of calpain or the proteasome seem to impair muscle or myotube hypertrophy (BAEHR *et al.*,

2014; KITAJIMA *et al.*, 2014; OSBURN *et al.*, 2021). Although proteolysis has been speculated to be a possible factor driving differential individual responses, the evidence is sparse (ROBERTS *et al.*, 2018). Some studies have made inferences about proteolytic activity derived from inflammation markers, considering that chronic elevations in skeletal muscle pro-inflammatory cytokines (e.g., IL-6, TNF-α, and IL-1β) seem to upregulate muscle proteolysis (ZAMIR *et al.*, 1992; HADDAD *et al.*, 2005; RAJ *et al.*, 2008). From an individual response perspective, one's inability to control inflammatory signaling post-exercise could lead to heightened muscle proteolysis, which by its turn would hinder muscle growth (ROBERTS *et al.*, 2018). Nevertheless, the evidence regarding the association between inflammation and individual responsiveness to RT is equivocal. For instance, Mobley *et al.* (2018) reported that while IL-1β mRNA was downregulated in high responders compared with low responders, other inflammatory markers (e.g., IL-6 and TNF-α mRNA) were not differentially expressed. The same study showed that a cluster×time interaction approached significance for 20S proteasome activity ($p = 0.058$), indicating that low hypertrophy responders could have heightened proteolytic activity. However, the authors acknowledge that the plausibility of this hypothesis is limited by the lack of association between changes in 20S proteasome levels and changes in hypertrophy. To date, no study has investigated proteolysis markers from an individual response perspective comparing RT protocols. Notably, we assessed markers of protein content and enzymatic activity related to three different proteolysis pathways (i.e., autophagy, the calpain pathway and the ubiquitin–proteasome pathway). Our findings revealed that none of the associated markers were differentially modulated between the RT overload progression models. Thus, our results support a lack of association between RT responsiveness and certain markers of proteolytic activity.

There is also a paucity of studies exploring individual RT-responsiveness and markers associated with the remodeling of muscle ECM. Our findings suggest that the protein content

of TIMP-1 may be differentially modulated between overload progression models. Yet, all other ECM remodeling markers were not differentially modulated, corroborating results from previous studies (ANGLERI *et al.*, 2022a; GODWIN *et al.*, 2023a). Godwin *et al.* (2023a), using a between-subject design, showed no differential training responses in most skeletal muscle ECM markers when comparing low and high responders to 10 weeks of RT. Similarly, Angleri *et al.* (2022a) revealed no association between individual increases in total fCSA and gene expression of ECM remodeling markers. In addition, the study presents a heatmap stratified by myofiber hypertrophy that allows a qualitative analysis of the individual responses to two different RT protocols (ANGLERI *et al.*, 2022a). From this heatmap, it is clear that the variance observed in myofiber hypertrophy is not reflected in the gene expression of ECM remodeling markers in response to those protocols.

Like others, this study is not without limitations. Our sample comprised exclusively young healthy untrained participants, thus precluding the extrapolation of the results to other populations. Additionally, RT protocols were performed over a limited 10-week period. It is uncertain whether responsiveness favoring one or the other protocol would be altered if RT practice continued for a longer period. Finally, it is worth noting that our clustering procedure was based on mCSA changes and a threshold (2×TE) dichotomous classification that yielded groups of relatively small sizes and low power for detecting significant effects.

In conclusion, our results suggest that the overload progression models can improve responsiveness to muscle hypertrophy induced by RT. Notably, it is intriguing that, to a significant share of individuals, progressing overload by increasing the number of repetitions resulted in greater or equal muscle hypertrophy, which seems to be associated with greater chronic addition of satellite cells. However, overload progression models did not differentially affect chronic changes in myonuclei, proteolysis activity markers, or markers associated with ECM remodeling. Future studies should investigate other molecular mechanisms that may

explain the variability in individual responsiveness to different models of RT progression, especially to understand the hypertrophic response resulting from the progression of overload due to the increase in the number of repetitions.

SUPPLEMENTAL MATERIAL

Table 1 – Comparisons between protocols and correlation analyses for "LOADERS"

Variables	LOADProg Δ%	REPSProg Δ%	ES (95% CI) REPSProg vs. LOADProg	Correlation with mCSA r_{rm} (95%CI; *p*-value)
mCSA*	21.47 ± 7.51	12.01 ± 7.54	-1.18 (-2.31 to -0.04)	-
Myonuclei count per fiber	-3.65 ± 28.49	10.17 ± 28.17	0.46 (-0.60 to 1.52)	-0.54 (-0.90 to 0.27; *p* = 0.168)
Satellite cells count per fiber	8.61 ± 36.73	39.40 ± 52.11	0.64 (-0.43 to 1.71)	-0.67 (-0.93 to 0.06; *p* = 0.069)
Enzymatic Activity				
Global calpain	16.62 ± 29.2	85.15 ± 214.36	0.42 (-0.64 to 1.48)	-0.24 (-0.81 to 0.55; *p* = 0.558)
Global proteasome	6.48 ± 44.97	-8.56 ± 34.36	-0.35 (-1.41 to 0.70)	0.36 (-0.46 to 0.85; *p* = 0.385)
Global MMP	1.20 ± 14.65	-3.52 ± 13.55	-0.31 (-1.37 to 0.74)	0.30 (-0.51 to 0.83; *p* = 0.473)
Protein content				
Calpain-1	0.29 ± 15.99	0.78 ± 21.12	0.02 (-1.02 to 1.07)	-0.03 (-0.72 to 0.69; *p* = 0.948)
Calpain-2	21.01 ± 32.38	2.27 ± 21.57	-0.64 (-1.71 to 0.44)	0.45 (-0.37 to 0.88; *p* = 0.262)
20S Proteasome	5.27 ± 36.93	5.38 ± 21.60	0.00 (-1.04 to 1.05)	-0.12 (-0.76 to 0.64; *p* = 0.78)
Polyubiquitinated proteins	2.96 ± 5.90	0.10 ± 7.97	-0.38 (-1.44 to 0.68)	0.16 (-0.61 to 0.78; *p* = 0.694)
p62	-2.28 ± 12.71	-2.31 ± 11.93	0.00 (-1.05 to 1.05)	-0.03 (-0.72 to 0.69; *p* = 0.948)
MMP-9	8.82 ± 24.42	-2.61 ± 12.22	-0.55 (-1.62 to 0.51)	0.31 (-0.50 to 0.83; *p* = 0.453)
MMP-14	-7.45 ± 13.00	-8.09 ± 16.06	-0.04 (-1.09 to 1.01)	0.17 (-0.60 to 0.78; *p* = 0.68)
TIMP-1	8.61 ± 50.54	4.56 ± 26.89	-0.09 (-1.14 to 0.95)	-0.06 (-0.73 to 0.67; *p* = 0.88)
TIMP-2	19.73 ± 79.90	-3.86 ± 38.88	-0.35 (-1.41 to 0.70)	0.25 (-0.55 to 0.81; *p* = 0.55)

Legend: Relative chronic changes in muscle cross-sectional area (mCSA), myonuclei and satellite cell number per fiber, enzyme activity levels and relative protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers following 10 weeks of resistance training. Values are

means ± SD. LOADERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion, and muscle gains favored the LOADProg protocol in over 5.7% (2×TE) compared to REPSProg; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = mean relative change from Pre to Post; ES = effect size; CI = confidence interval; r_{rm} = repeated measures correlation coefficient. *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

Table 2 – Comparisons between protocols and correlation analyses for "REPERS"

Variables	LOADProg Δ%	REPSProg Δ%	ES (95% CI) REPSProg vs. LOADProg	Correlation with mCSA r_{rm} (95%CI; *p*-value)
mCSA*	3.45 ± 8.68	14.21 ± 7.64	1.27 (0.39 to 2.15)	-
Myonuclei count per fiber	16.47 ± 27.57	2.64 ± 15.18	-0.60 (-1.42 to 0.22)	-0.31 (-0.74 to 0.29; $p = 0.298$)
Satellite cells count per fiber*	48.93 ± 63.07	108.58 ± 77.01	0.82 (-0.01 to 1.65)	0.68 (0.17 to 0.90; $p = 0.016$)
Enzymatic Activity				
Global calpain	21.45 ± 44.54	13.41 ± 48.16	-0.17 (-0.97 to 0.63)	-0.14 (-0.64 to 0.44; $p = 0.647$)
Global proteasome	-10.78 ± 49.96	-15.74 ± 37.47	-0.11 (-0.91 to 0.69)	-0.11 (-0.62 to 0.47; $p = 0.731$)
Global MMP	-6.35 ± 17.86	-4.50 ± 16.49	0.10 (-0.70 to 0.90)	0.001 (-0.55 to 0.55; $p = 0.998$)
Protein content				
Calpain-1	-1.38 ± 19.48	1.99 ± 11.88	0.20 (-0.60 to 1.00)	0.12 (-0.46 to 0.63; $p = 0.686$)
Calpain-2	12.76 ± 35.16	12.19 ± 17.25	-0.02 (-0.82 to 0.78)	-0.07 (-0.59 to 0.50; $p = 0.827$)
20S Proteasome	28.28 ± 80.25	86.31 ± 205.03	0.36 (-0.45 to 1.17)	0.29 (-0.31 to 0.73; $p = 0.329$)
Polyubiquitinated proteins	-0.49 ± 11.65	0.34 ± 12.21	0.07 (-0.73 to 0.87)	-0.05 (-0.59 to 0.51; $p = 0.859$)
p62	6.77 ± 27.11	-0.84 ± 16.78	-0.33 (-1.13 to 0.48)	-0.17 (-0.66 to 0.42; $p = 0.571$)
MMP-9	6.12 ± 23.98	15.58 ± 26.32	0.36 (-0.44 to 1.17)	0.33 (-0.27 to 0.74; $p = 0.268$)
MMP-14	-13.04 ± 12.69	-7.54 ± 7.54	0.51 (-0.30 to 1.32)	0.37 (-0.22 to 0.77; $p = 0.206$)
TIMP-1*	5.15 ± 18.19	-6.16 ± 22.38	-0.54 (-1.35 to 0.28)	-0.58 (-0.86 to -0.05; $p = 0.035$)
TIMP-2	13.74 ± 34.97	-2.71 ± 30.66	-0.48 (-1.29 to 0.33)	-0.38 (-0.77 to 0.21; $p = 0.196$)

Legend: Relative changes in muscle cross-sectional area (mCSA), myonuclei and satellite cells number per fiber, enzyme activity levels and relative protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers following 10 weeks of resistance training. Values are means ± SD. REPERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion, and muscle gains favored the REPSProg protocol in over 5.7% (2×TE) compared to LOADProg; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = relative change from Pre to Post;

ES = effect size; CI = confidence interval; r_{rm} = repeated measures correlation coefficient. *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

Table 3 – Comparisons between protocols and correlation analyses for "NO DIFFERENCE RESPONDERS"

Variables	LOADProg Δ%	REPSProg Δ%	ES (95% CI) REPSProg vs. LOADProg	Correlation with mCSA r_{rm} (95%CI; *p*-value)
mCSA	14.50 ± 5.23	14.92 ± 5.60	0.08 (-0.69 to 0.84)	-
Myonuclei count per fiber	6.48 ± 27.23	15.93 ± 24.22	0.36 (-0.42 to 1.13)	0.21 (-0.38 to 0.68; *p* = 0.477)
Satellite cells count per fiber	14.70 ± 58.82	76.20 ± 113.49	0.66 (-0.13 to 1.45)	0.45 (-0.13 to 0.80; *p* = 0.122)
Enzymatic Activity				
Global calpain	29.63 ± 38.60	205.21 ± 407.15	0.59 (-0.20 to 1.37)	0.22 (-0.35 to 0.67; *p* = 0.445)
Global proteasome	-10.15 ± 32.90	5.52 ± 61.19	0.31 (-0.46 to 1.08)	0.05 (-0.49 to 0.57; *p* = 0.857)
Global MMP	-3.07 ± 18.85	-0.88 ± 17.44	0.12 (-0.65 to 0.89)	0.11 (-0.44 to 0.60; *p* = 0.705)
Protein content				
Calpain-1	8.22 ± 18.25	6.84 ± 15.09	-0.08 (-0.85 to 0.69)	0.09 (-0.46 to 0.59; *p* = 0.756)
Calpain-2	20.60 ± 34.82	45.46 ± 63.23	0.47 (-0.31 to 1.25)	0.24 (-0.33 to 0.68; *p* = 0.405)
20S Proteasome	27.02 ± 74.11	17.77 ± 67.00	-0.13 (-0.90 to 0.64)	0.17 (-0.39 to 0.64; *p* = 0.548)
Polyubiquitinated proteins	-1.04 ± 7.01	4.18 ± 8.96	0.63 (-0.16 to 1.42)	0.30 (-0.27 to 0.71; *p* = 0.301)
p62	3.98 ± 18.22	46.59 ± 145.90	0.40 (-0.38 to 1.17)	0.36 (-0.20 to 0.75; *p* = 0.198)
MMP-9	11.18 ± 23.37	3.71 ± 21.03	-0.33 (-1.10 to 0.45)	-0.16 (-0.63 to 0.41; *p* = 0.590)
MMP-14	-0.95 ± 17.55	3.45 ± 14.28	0.27 (-0.51 to 1.04)	0.19 (-0.38 to 0.65; *p* = 0.514)
TIMP-1	-19.01 ± 13.90	30.17 ± 57.32	1.14 (0.31 to 1.97)	0.20 (-0.36 to 0.66; *p* = 0.480)
TIMP-2	-10.64 ± 27.70	25.60 ± 55.02	0.81 (0.01 to 1.61)	0.29 (-0.29 to 0.71; *p* = 0.321)

Legend: Relative changes in muscle cross-sectional area (mCSA), myonuclei and satellite cell count per fiber, enzyme activity levels and relative protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers following 10 weeks of resistance training. Values are means ± SD. NO DIFFERENCE RESPONDERS = cluster of participants whose hypertrophic response to RT met the 2×TE criterion but the difference between protocols was smaller than 5.7% (2×TE); MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = relative change from Pre to

Post; ES = effect size; CI = confidence interval; r_{rm} = repeated measures correlation coefficient. *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

Table 4 – Comparisons between protocols and correlation analyses for "NONRESPONDERS"

Variables	LOADProg Δ%	REPSProg Δ%	ES (95% CI) REPSProg vs. LOADProg	Correlation with mCSA r_{rm} (95%CI; *p*-value)
mCSA	1.06 ± 3.61	-2.52 ± 6.63	-0.61 (-1.87 to 0.66)	-
Myonuclei count per fiber	-3.95 ± 4.68	-18.37 ± 18.22	-0.98 (-2.29 to 0.33)	0.33 (-0.66 to 0.90; *p* = 0.523)
Satellite cells count per fiber	100.99 ± 149.71	20.15 ± 64.83	-0.63 (-1.90 to 0.64)	0.08 (-0.78 to 0.84; *p* = 0.879)
Enzymatic Activity				
Global calpain	17.61 ± 61.00	38.73 ± 140.94	0.18 (-1.07 to 1.42)	-0.67 (-0.96 to 0.30; *p* = 0.142)
Global proteasome	-11.13 ± 35.96	-25.72 ± 54.19	-0.29 (-1.53 to 0.96)	-0.18 (-0.87 to 0.74; *p* = 0.728)
Global MMP	-1.28 ± 15.70	-7.36 ± 22.61	-0.28 (-1.53 to 0.96)	-0.50 (-0.93 to 0.53; *p* = 0.315)
Protein content				
Calpain-1	-0.15 ± 27.49	-5.53 ± 19.26	-0.20 (-1.45 to 1.04)	-0.47 (-0.93 to 0.55; *p* = 0.346)
Calpain-2	24.63 ± 50.11	0.07 ± 35.44	-0.51 (-1.77 to 0.75)	-0.41 (-0.92 to 0.60; *p* = 0.413)
20S Proteasome	2.50 ± 22.10	103.05 ± 191.01	0.67 (-0.61 to 1.94)	-0.22 (-0.88 to 0.72; *p* = 0.668)
Polyubiquitinated proteins	-0.39 ± 15.35	2.10 ± 7.80	0.18 (-1.06 to 1.43)	-0.44 (-0.92 to 0.58; *p* = 0.384)
p62	44.36 ± 92.06	-8.35 ± 5.00	-0.73 (-2.01 to 0.55)	-0.05 (-0.83 to 0.80; *p* = 0.928)
MMP-9	6.61 ± 5.31	8.56 ± 11.71	0.19 (-1.05 to 1.44)	-0.51 (-0.93 to 0.51; *p* = 0.297)
MMP-14	-5.53 ± 6.12	-6.23 ± 8.98	-0.08 (-1.32 to 1.16)	-0.13 (-0.85 to 0.76; *p* = 0.803)
TIMP-1	14.58 ± 29.41	-3.49 ± 28.15	-0.57 (-1.83 to 0.70)	-0.23 (-0.88 to 0.71; *p* = 0.653)
TIMP-2*	11.63 ± 32.02	-2.38 ± 35.85	-0.37 (-1.62 to 0.88)	-0.11 (-0.84 to 0.77; *p* = 0.837)

Legend: Relative changes in muscle cross-sectional area (mCSA), myonuclei and satellite cell count per fiber, enzyme activity levels and relative protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers following 10 weeks of resistance training. Values are means ± SD. NONRESPONDERS = cluster of participants whose muscle gains were smaller than 5.7% (2×TE) in response to both protocols; MMP = matrix

metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = mean relative change from Pre to Post; ES = effect size; CI = confidence interval; r_{rm} = repeated measures correlation coefficient. *, indicates a significant difference between protocols. Significance was set at $p < 0.05$.

CAPÍTULO 2

Manuscript: Resistance training-induced changes in muscle proteolysis and extracellular matrix remodeling biomarkers in the untrained and trained states

ABSTRACT

Purpose: Resistance training (RT) induces muscle growth at varying rates across RT phases, and evidence suggests that the muscle-molecular responses to training bouts become refined or attenuated in the trained state. This study examined how proteolysis-related biomarkers and extracellular matrix (ECM) remodeling factors respond to a bout of RT in the untrained (UT) and trained (T) state.

Methods: Participants (19 women and 19 men) underwent 10 weeks of RT. Vastus lateralis muscle samples were collected before (Pre) and 24 h after the first and last (24th) sessions (UT and T, respectively). Vastus lateralis cross-sectional area (CSA) assessments were performed before and after the experimental period.

Results: There were increases in vastus lateralis and type II fiber CSAs. In both the UT and T states, calpain activity was upregulated and calpain-1/-2 protein expression was downregulated from Pre to 24 h. Calpain-2 was higher in the T state. Proteasome activity and 20S proteasome protein expression were upregulated from Pre to 24 h in both the UT and T. However, proteasome activity levels were lower in the T state. The expression of poly-ubiquitinated proteins was unchanged. MMP activity was downregulated, and MMP-9 protein expression was elevated from Pre to 24 h in UT and T. Although MMP-14 protein expression was acutely unchanged, this marker was lower in T state. TIMP-1 protein levels were reduced Pre to 24 h in UT and T, while TIMP-2 protein levels were unchanged.

Conclusion: Our results are the first to show that RT does not attenuate the acute-induced response of proteolysis and ECM remodeling-related biomarkers.

Keywords: Calpain; Exercise training; Metalloproteinase; Muscle hypertrophy; Ubiquitin–proteasome.

INTRODUCTION

Skeletal muscle plays a crucial role in human metabolism, and increases in muscle mass with resistance training (RT) positively influence health and well-being (WOLFE, 2006). Skeletal muscle hypertrophy during periods of RT occurs due to the coordination of several molecular mechanisms induced by acute bouts of training (ROBERTS *et al.*, 2023). Since RT-induced muscle growth is more modest in the initial phases (~3 weeks), but considerably greater after 10 weeks (DAMAS *et al.*, 2018a), these mechanisms may be differentially impacted according to the level of training experience. Thus, to improve comprehension of the continuum of muscle growth, understanding how molecular pathways respond to RT at different stages of muscle adaptation is paramount. The molecular mechanisms impacted by RT include (but are not limited to) the differentiation and fusion of satellite cells, myonuclear accretion, DNA methylation, protein synthesis, proteolytic pathways, and extracellular matrix (ECM) remodeling (ROBERTS *et al.*, 2023). While some of these mechanisms have received much research attention, studies extensively examining enzymes that regulate proteolysis and ECM remodeling are scarce (ROBERTS *et al.*, 2023).

The ECM plays a vital role in a multitude of cellular processes and has several functions ranging from acting as a reservoir of growth factors (NABA *et al.*, 2016), aiding in mechanical sensing and force relaying (KJAER, 2004), as well as being a fundamental component of skeletal muscle regeneration (MACKEY *et al.*, 2017). The protein composition of the ECM includes collagens, proteoglycans, and other glycoproteins that constantly undergo remodeling by a family of enzymes called matrix metalloproteases (MMPs) (GILLIES *et al.*, 2011; DAVIS *et al.*, 2013). It has been suggested that this ECM remodeling may allow for greater fiber growth. Millward (1995) proposed a "bag-theory" to account for the intracellular protein accumulation. According to this theory, muscle cells exhibit a bag-like behavior, and upon reaching capacity, the bag (i.e., the extracellular matrix) necessitates remodeling to facilitate

continued myofiber growth. Evidence indicates that the ECM remodeling factors (e.g., matrix metalloproteinases [MMPs] and tissue inhibitors of metalloproteinases [TIMPs]) are either upregulated or unchanged following a bout of exercise (RULLMAN *et al.*, 2007; RULLMAN *et al.*, 2009; HOIER *et al.*, 2012). However, the current literature has not delineated how these responses change following various bouts throughout a period of RT.

Concerning muscle proteolysis, the ubiquitin-proteasome system is the primary proteolysis pathway in muscle, targeting proteins like titin and troponin (MURTON *et al.*, 2008). However, before ubiquitination, contractile proteins require disassembly, a process facilitated by the calpain system. This orchestrated function of both proteolytic pathways is crucial for muscle remodeling (HYATT *et al.*, 2020). Evidence suggests that there is upregulation after a bout of exercise (FÉASSON *et al.*, 2002; NEDERGAARD *et al.*, 2007; GODWIN *et al.*, 2023b), which may be attenuated after subsequent bouts (STUPKA *et al.*, 2001; WILLOUGHBY *et al.*, 2003; NEDERGAARD *et al.*, 2007; MASCHER *et al.*, 2008) due to the "repetitive bout effect" phenomenon (attenuation of RT-induced muscle damage) (DAMAS *et al.*, 2016a). However, novel insights have emerged regarding the importance of the processes of degradation and remodeling in skeletal muscle, suggesting that if the RT stimulus is continued, these activities could remain upregulated, even with an attenuation of muscle damage. While structural disruptions and microlesions are gradually reduced during the initial weeks of RT (DAMAS *et al.*, 2016a), the need for proteolysis and remodeling is likely sustained due to studies showing that muscle hypertrophy ensues months into training (DEFREITAS *et al.*, 2011; OGASAWARA *et al.*, 2012). Moreover, it has been suggested that muscle growth increases the need to recycle cellular components that might have become inefficient and the need for an intracellular pool of free amino acids (HÖHFELD *et al.*, 2021; ROBERTS *et al.*, 2023). Calpains play an essential role in the disassembly of myofibril proteins and the incorporation of new myosin isoforms (HYATT *et al.*, 2020) in fiber-type shifts – an

RT-induced adaptation that occurs further along the training continuum. There is also evidence that calpains have nonproteolytic functions. For example, calpains are considered an essential structural component for the conformation of muscle triad junctions (OJIMA *et al.*, 2011). Other findings suggest that the enhancement of the UPP is important for satellite cell proliferation and muscle regeneration (KITAJIMA *et al.*, 2020). In fact, evidence indicates that the inhibition or downregulation of either calpain (OSBURN *et al.*, 2021) or the UPP (BAEHR *et al.*, 2014; KITAJIMA *et al.*, 2014) might be detrimental to muscle hypertrophy. Therefore, it is plausible to assume that some level of protein breakdown is important for muscle growth, and that these processes are not attenuated following training bouts in the trained state.

Hence, this study aimed to examine how proteolysis- and remodeling-related biomarkers respond to a bout of RT in the untrained and trained state, thus assessing both transient and chronic RT-induced changes. In order to provide a comprehensive evaluation, protein levels and the activities of the ubiquitin–proteasome and calpain pathway markers as well as MMPs and TIMPs were assessed. We hypothesized that all these markers would be changed in response to a resistance exercise bout in both the trained and untrained states given the evidence and arguments put forth. As a secondary aim, given the rarity of RT research including women, we explored whether biological sex influences how these biomarkers responded.

METHODS

Participants

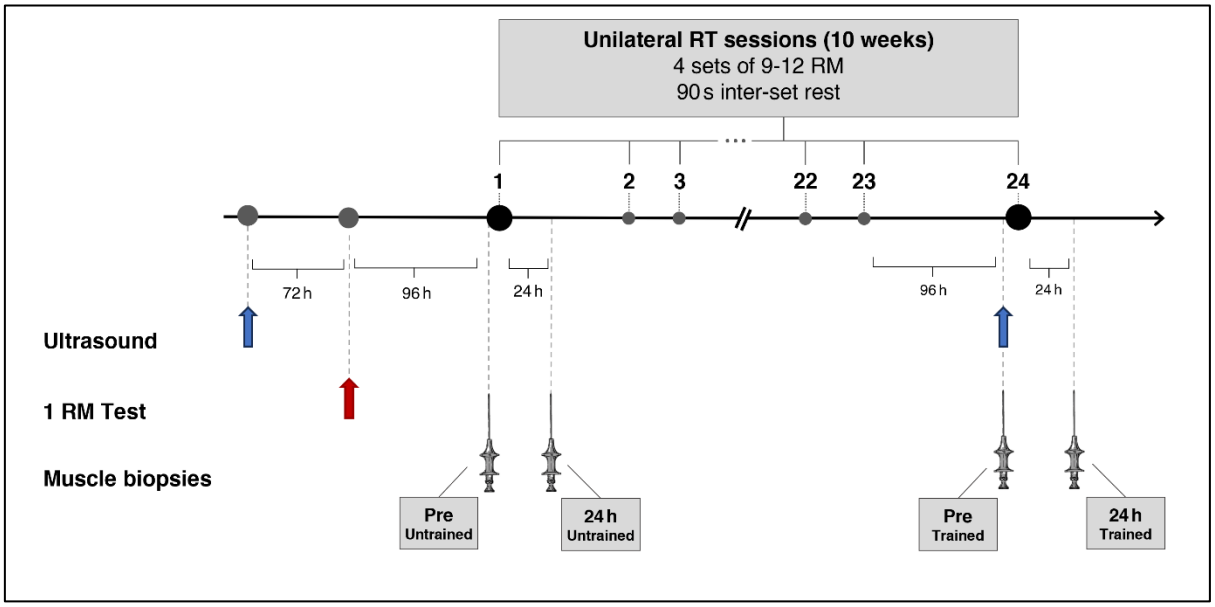
This study comprises an analysis of data obtained from participants of a randomized controlled trial (registered at the Brazilian Registry of Clinical Trials – RBR-57v9mrb), which was not previously proposed. The study was conducted in accordance with the most recent version of the *Declaration of Helsinki* and approved by the Ethics Committee of the Federal

University of São Carlos (no. 56259622.0.0000.5504). To be eligible, participants were needed to meet the following criteria: (a) not have engaged in RT, aerobic training, or any physical training program for at least six months prior to the beginning of the study; (b) not make use of any drugs that could affect skeletal muscle mass or strength; and (c) be free of any musculoskeletal injuries or neuromuscular disorders that could contraindicate or prevent the practice of RT. After screening, thirty-nine recreationally active young men and women were recruited. Participants were informed of all procedures and the benefits and risks of enrollment before signing an institutionally approved consent form. After the start of data collection, one participant withdrew consent to have his muscle biopsied; thus, thirty-eight participants were included in this analysis: 19 women (age: 24.2 ± 4.9; body mass: 62.7 ± 8.5 kg; height: 1.64 ± 0.1 m; BMI: 23.5 ± 3.3 kg/m^2) and 19 men (age: 24.5 ± 3.3; body mass: 73.6 ± 13.4 kg; height: 1.76 ± 0.1 m; BMI: 23.8 ± 4.2 kg/m^2).

Study design

The experimental design is illustrated in Figure 1. In short, an ultrasound assessment of the cross-sectional area of the vastus lateralis muscle was performed at baseline, followed by a one repetition maximum (1RM) test 72 hours later. Muscle samples were collected from the midpoint of the vastus lateralis via muscle biopsy before (Pre) and 24 h after the first and last (24th) training sessions (untrained and trained state, respectively). Participants underwent 10 weeks of RT, totaling 24 training sessions. A 96-h interval was allowed between the 23rd and 24th sessions. Another ultrasound assessment was performed at the end of the experimental period.

Figure 1 – Study design



Legend: Design of the 10-week resistance training (RT) study, displaying timepoints of the ultrasound muscle assessment, the 1RM test, muscle biopsies and training sessions. RM = repetitions maximum.

Resistance training protocol

The 1RM test was performed as described by SCARPELLI *et al.* (2022) to determine the load used in the first training session (80% of 1 RM). The RT protocol was composed of 4 sets of 9-12 maximum repetitions of unilateral leg extension exercise, with 90 seconds of rest between sets. The load was adjusted set by set to ensure that concentric muscle failure occurred within the targeted repetition zone. Training sessions were performed 2 to 3 times a week for 10 weeks, totaling 24 sessions. Verbal encouragement was provided to ensure maximum effort. Determining which leg of a participant would follow this protocol was performed by randomization and allocation strategies as described previously by our group (CHAVES *et al.*, 2024b).

Muscle cross-sectional area (mCSA)

Vastus lateralis mCSA was assessed by an experienced evaluator through B-mode ultrasound (US) imaging using a 7.5 MHz linear probe (MySono U6, Samsung, Sao Paulo, Brazil). The procedure has been described in further detail by Chaves *et al*. (2024b). Briefly, participants were instructed to refrain from vigorous physical activity for at least 72 h before each assessment (DAMAS *et al.*, 2016b). Upon arrival at the laboratory, participants laid in a supine position, and the point corresponding to 50% of femur length – measured as the distance between the greater trochanter and the lateral epicondyle – was marked as a reference for image acquisition. From this point, successive markings were made every 2 cm in both the medial and lateral directions at intervals of 2 cm to guide the displacement of the ultrasound probe in the sagittal plane. The participant laid still for 15 minutes to allow tissue fluid stabilization. Next, US images were captured sequentially, using the markings as a guide for the displacement of the probe in the sagittal plane, starting at the most medial mark (over the rectus femoris muscle). Water-soluble transmission gel was applied to ensure acoustic coupling of the probe without skin compression. Using PowerPoint software (Microsoft, EUA), captured images were manually rotated and sequentially overlaid to obtain a panoramic image of the CSA of the entire muscle. The ImageJ (SCHNEIDER *et al.*, 2012) "polygonal" tool was used to circle and calculate the vastus lateralis CSA, excluding connective and bone tissue from the delimited area. The typical error between two image acquisitions and quantifications separated by 72h was 0.52 cm^2 (2.47%).

Muscle biopsies

Muscle tissue samples were obtained from the vastus lateralis via percutaneous muscle biopsies performed by a qualified medical professional. The area around the extraction point was cleaned with an antiseptic wash and injected with local subcutaneous anesthesia [2-3 ml of

1% xylocaine (lidocaine)]. The professional then made a small incision with a surgical scalpel and introduced the biopsy needle (Bergström model with manual suction) to a depth of approximately 4 cm to remove ~100 mg of muscle tissue. The incision was closed and covered with bandages. The tissue was cleaned of blood and connective tissue and portioned for analysis. A fraction of the sample (40-50 mg) was allocated to cryotubes for protein content and enzymatic activity analyses. Another portion (20-30 mg) was positioned in a liquid embedding medium for optimal cutting temperature (OCT), with the fibers perpendicular to the horizontal surface, and frozen in nitrogen-cooled isopentane for immunohistochemical analyses. The remainder of the sample was stored in prelabeled cryotubes. After separation, all samples were immediately frozen in liquid nitrogen and stored at -80°C until processing.

Immunohistochemical analyses

The OCT-preserved samples were sectioned at a thickness of 14 μm using a cryostat (Leica Biosystems, Buffalo Grove, IL, USA) and adhered to positively charged histology slides. These were then stored at -80°C until batch-processed for immunohistochemical analyses to measure type I and type II fiber CSA (fCSA). All samples from the same participant were placed on the same slide and analyzed concomitantly.

Batch processing began with slides being air-dried at room temperature for two hours and fixed with acetone at -20°C for 5 minutes. Slides were incubated with 3% H_2O_2 for 10 minutes at room temperature, followed by a 1-minute incubation with autofluorescence quenching reagent (TrueBlack, cat. no. 23007; Biotium, Fremont, CA, USA). Slides were then blocked for one hour with a 5% goat serum, 2.5% horse serum, and 0.1% Triton-X solution at room temperature. After blocking, slides were incubated overnight at 4°C with a primary antibody cocktail: 1:100 Dystrophin (cat. no. GTX57970; GeneTex, Irvine, CA, USA) + 1:100 BA-D5 (Myosin Heavy Chain I) (cat. no. BA-D5; RRID: AB_2235587; Developmental Studies

Hybridoma Bank, Iowa City, IA, USA) + 2.5% horse serum in phosphate-buffered saline (PBS). The following day, the sections were incubated for 60 minutes in a secondary antibody cocktail: 1:250 goat anti-mouse IgG2b Alexa Fluor 647 (cat. no. A-21242; Thermo Fisher Scientific, Waltham, MA, USA) + 1:250 goat anti-rabbit IgG DyLight488 (cat. no. DI-1488; Vector Laboratories, Newark, CA, USA) in PBS. Slides were then stained with DAPI (4=,6-diamidino-2-phenylindole; cat. no. D3571; Thermo Fisher Scientific) for 10 minutes at room temperature and mounted with glass coverslips using 1:1 PBS and glycerol as mounting medium. Sections were stored in the dark at 4°C until imaging was conducted. Digital images were captured with a fluorescence microscope at ×20 magnification (Nikon Instruments; Melville, NY, USA). All areas selected for analyses were free of freeze-fracture artifacts, and at least 50 fibers per sample were quantified (MACKEY *et al.*, 2009).

Standardized measurements of type I and type II fiber counts and fCSAs were performed via open-sourced software (MyoVision) (WEN *et al.*, 2018), applying a conversion ratio value of 0.46 μm/pixel and a detection range from 500 to 15,000 μm^2. Visual verification of appropriate fiber detection was then carried out, and all empty spaces or fractured fibers wrongly outlined by the software were excluded from the analysis. Notably, all procedures – sectioning, staining, imaging – and analyses were accomplished by investigators blinded to the interventions.

Tissue homogenization

The fraction of muscle tissue stored in cryotubes was crushed using a set of ceramic mortar and pestle cooled in liquid nitrogen. The tissue was weighed, and approximately 20 mg was placed in 1.7 ml microcentrifuge tubes with 500 μl of a general cell lysis buffer (cat. no. 9803; Cell Signaling Technology, Danvers, MA, USA) according to Godwin *et al*. (2023a). Samples were then homogenized using hard plastic pestles and centrifuged at 500 × g for 5

minutes at 4°C. Next, supernatants were transferred to a new set of microtubes. Approximately 20 µL of the resulting cell lysates were used to determine the total protein concentrations using a commercially available bicinchoninic acid (BCA) protein assay kit (cat. no. 23227; Thermo Fisher Scientific) and a spectrophotometer (BioTek Synergy H1, Winooski, VT, USA), following the manufacturer's instructions.

Enzymatic activity assays

Calpain and proteasome activity assays were performed using commercially available luminescence kits (cat. no.: G8502 and cat. no.: G8622; Promega Corporation, Madison, WI, USA). Muscle tissue lysates (25 µl) were loaded in duplicate onto white 96-well plates with the reagent mix provided by the kits and incubated at room temperature for 10 min, separately for each assay. Luminescence was then read using a microplate luminometer (BioTek Synergy H1, Winooski, VT, USA). Luminometric readings were divided by total protein loaded per well and expressed as relative luminescence units (RLU) per µg muscle soluble protein. The average coefficients of variation for duplicate values of calpain and proteasome activity were 5.2% and 5.4%, respectively.

Global MMP activity was measured using commercially available fluorometric assay kits (cat. no.: AS-72202; AnaSpec, Freemont, CA, USA). Muscle tissue lysates (50 µl) were loaded in duplicate into black 96-well microplates with the substrate solution provided by the kit and incubated at room temperature for 30 minutes. The fluorescence intensity was then read using a microplate fluorometer (BioTek Synergy H1, Winooski, VT, USA) using 330 nm excitation and 390 nm emission settings. Fluorometric readings were divided by total protein loaded per well (i.e., RFU per µg muscle soluble protein). The average coefficient of variation for duplicate values of MMP activity data was 6.5%.

Western blotting

Part of the supernatants resulting from the homogenization process were prepared for Western blotting. Lysates were added to 4× Laemmli buffer and distilled water (diH_2O) to a 1 µg/µl concentration. The solutions were then denatured for 5 min at 100°C prior to being stored at -80°C until analysis. Next, prepared samples (15 µl) were loaded onto 4−15% SDS-polyacrylamide gels (cat. no. 5671085, Criterion TGX; Bio-Rad Laboratories, Hercules, CA, USA) and subjected to electrophoresis at 180 V for 50 minutes with premade 1× SDS–PAGE running buffer (VWR-Avantor, Radnor, PA, USA). Then, the proteins were transferred to preactivated polyvinylidene difluoride membranes (cat. no. 1620177; Bio-Rad Laboratories) at 200 mA for two hours. Membranes were then Ponceau stained for ten minutes and digitally imaged with a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories). Next, membranes were reactivated in methanol and blocked with 5% nonfat milk powder in Tris-buffered saline with 0.1% Tween-20 (TBST) at room temperature for one hour. Membranes were then incubated overnight with the following antibodies at a 1:1000 dilution in TBST with 5% bovine serum albumin (BSA): (i) rabbit anti-calpain-1 (cat. no.: 2556; Cell Signaling Technology); (ii) rabbit anti-calpain-2 (cat. no.: 70655; Cell Signaling Technology); (iii) rabbit anti-ubiquitin (cat. no.: 3933; Cell Signaling Technology), (iv) rabbit anti-20S core subunit antibody cocktail (cat. no. BML-PW8155; Enzo Life Sciences; Farmingdale, NY, USA); (v) rabbit anti-human MMP-9 (cat. no. ab76003; Abcam, Cambridge, MA, USA); (vi) rabbit anti-human MT1-MMP/MMP-14 (cat. no. 13130; Cell Signaling Technology); (vii) rabbit anti-human TIMP-1 (cat. no. 8946; Cell Signaling Technology); (viii) rabbit anti-human TIMP-2 (cat. no. 5738; Cell Signaling Technology). The next day, the membranes were incubated for one hour with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (cat. nos.: 7076 and 7074; Cell Signaling Technology). Membranes were then developed using chemiluminescent substrate (Immobilon Forte, cat. no. WBLUF0500; MilliporeSigma,

Burlington, MA, USA) and digitally imaged using a gel documentation system (ChemiDoc Touch; Bio-Rad Laboratories) and densitometry associated software (Image Lab v6.0.1; Bio-Rad Laboratories). Densitometry values were normalized to Ponceau densities.

Statistical analyses

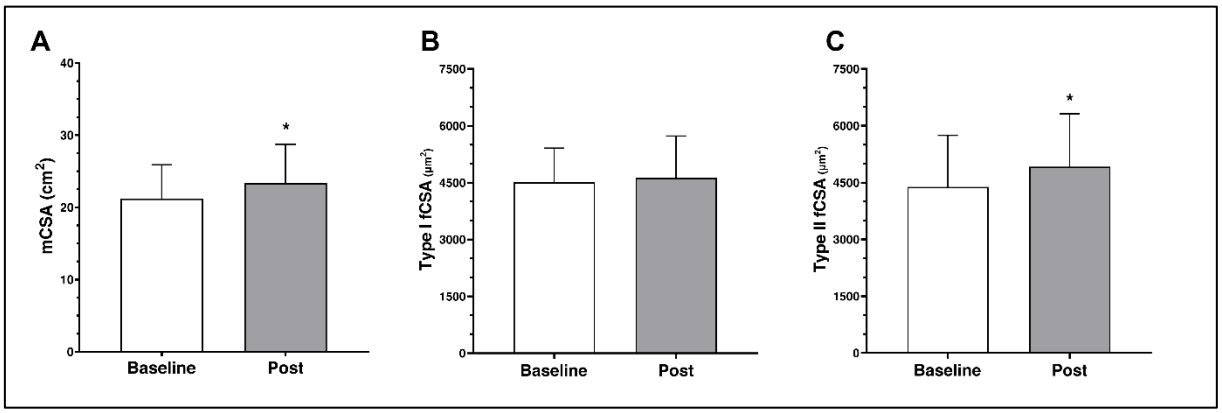
After visual inspection, the assumptions of homogeneity of variance and normality of data were verified. The *t test* for dependent samples was used to compare values of mCSA and type I and type II fCSA before and after RT. Mixed model analyses were employed for proteolysis and remodeling-related markers, with time (Pre and 24 h) and training status (untrained and trained) as fixed factors and participants as random factors. Tukey's adjustment was used for multiple comparisons in the case of significant *F* values. Effect sizes (ESs) and the respective 95% confidence intervals (CI) were calculated according to Hedges *et al.* (1985). The ES was calculated between from untrained to trained state for mCSA and type I and type II fCSA. For proteolysis and ECM-related biomarkers, the ES was calculated between acute absolute changes observed in the untrained and trained conditions. Positive and negative CIs not crossing zero (0) were considered significant (NAKAGAWA *et al.*, 2007). Additionally, the ESs were classified as "small" if lower than 0.2, "medium" if between 0.2 and 0.5, and "large" if higher than 0.8 (COHEN, 1988). An analysis of covariance (ANCOVA) was employed to compare the absolute changes in all outcomes (from Pre to 24 h), with biological sex as a fixed factor, Pre values as covariates, and participants as random factors. ANCOVA was implemented separately for the untrained and trained conditions. Values are presented as the mean ± standard deviation (SD). The significance level was set at $p \leq 0.05$. Analyses were performed using SAS 9.2 and GraphPad Prism 9 software.

RESULTS

Muscle and fiber cross-sectional area

The *t test* (Fig. 2) indicated an increase in mCSA from untrained (21.21 ± 4.7 cm^2) to trained state (23.36 ± 5.4 cm^2; $p < 0.0001$). The CI of the ES (ES = 0.42; 95% CI: 0.26 to 0.59) confirmed the inferential analysis. Regarding mean Type II fCSA, there was an increase from untrained (4475 ± 1108 μm^2) to trained state(4916 ± 1400 μm^2; $p = 0.026$). The CI of the ES (ES = 0.35; 95% CI: 0.06 to 0.63) confirmed the inferential analysis. There were no differences in mean Type I fCSA from untrained (4539 ± 891 μm^2) to trained state (4630 ± 1103 μm^2; $p = 0.49$). The CI of the ES (ES = 0.09; 95% CI: -0.22 to 0.40) confirmed the inferential analysis.

Figure 2 – Muscle and fiber cross-sectional area



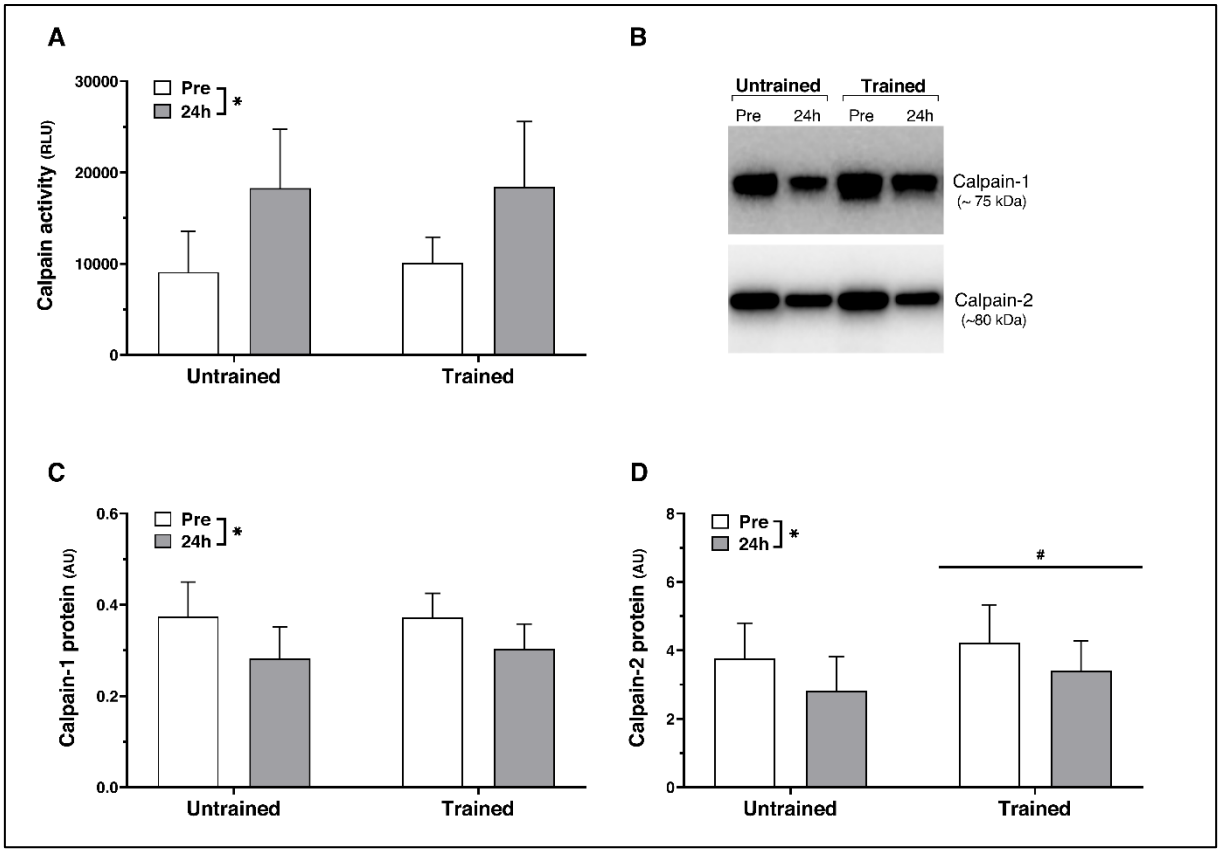
Legend: Values of the (s) vastus lateralis muscle cross-sectional area (mCSA), and (b) type I and (c) type II fiber cross-sectional areas (fCSA) before (Baseline) and after (Post) 10 weeks of resistance training. Values are mean ± SD. *, indicates an increase from baseline to post values. Significance was set at $p \leq 0.05$.

Calpain pathway markers

The mixed model revealed a main effect of time (Pre vs. 24 h) ($F_{[1,36.4]} = 70.84$, $p < 0.0001$) for global calpain activity (Fig. 3a). There was no effect of condition (trained vs. untrained) ($F_{[1,36.8]} = 0.50$, $p = 0.486$) or condition vs. time interaction ($F_{[1,36.4]} = 0.36$, $p = 0.551$). Calpain activity increased acutely from Pre to 24 h. Regarding the protein content of calpain-1

(Fig. 3c), there was a main effect of time ($F_{[1,66.3]} = 72.98, p < 0.0001$), with no effect of condition ($F_{[1,131]} = 0.85, p = 0.357$) or condition vs. time interaction ($F_{[1,113]} = 1.38, p = 0.244$). Calpain-1 content decreased acutely from Pre to 24 h. For the content of calpain-2 (Fig. 3d), there was a main effect of time ($F_{[1,107]} = 40.58, p < 0.0001$) and a main effect of condition ($F_{[1,108]} = 15.25, p = 0.0002$) but no condition vs. time interaction ($F_{[1,107]} = 0.17, p = 0.683$). Calpain-2 content decreased from Pre to 24 h, with values in the trained state being higher than those in the untrained state. The 95% CI of ESs of the differences between delta changes for calpain activity, calpain-1 and calpain-2 content indicated no significant differences between UT and T state (Table 1).

Figure 3 – Calpain pathway markers



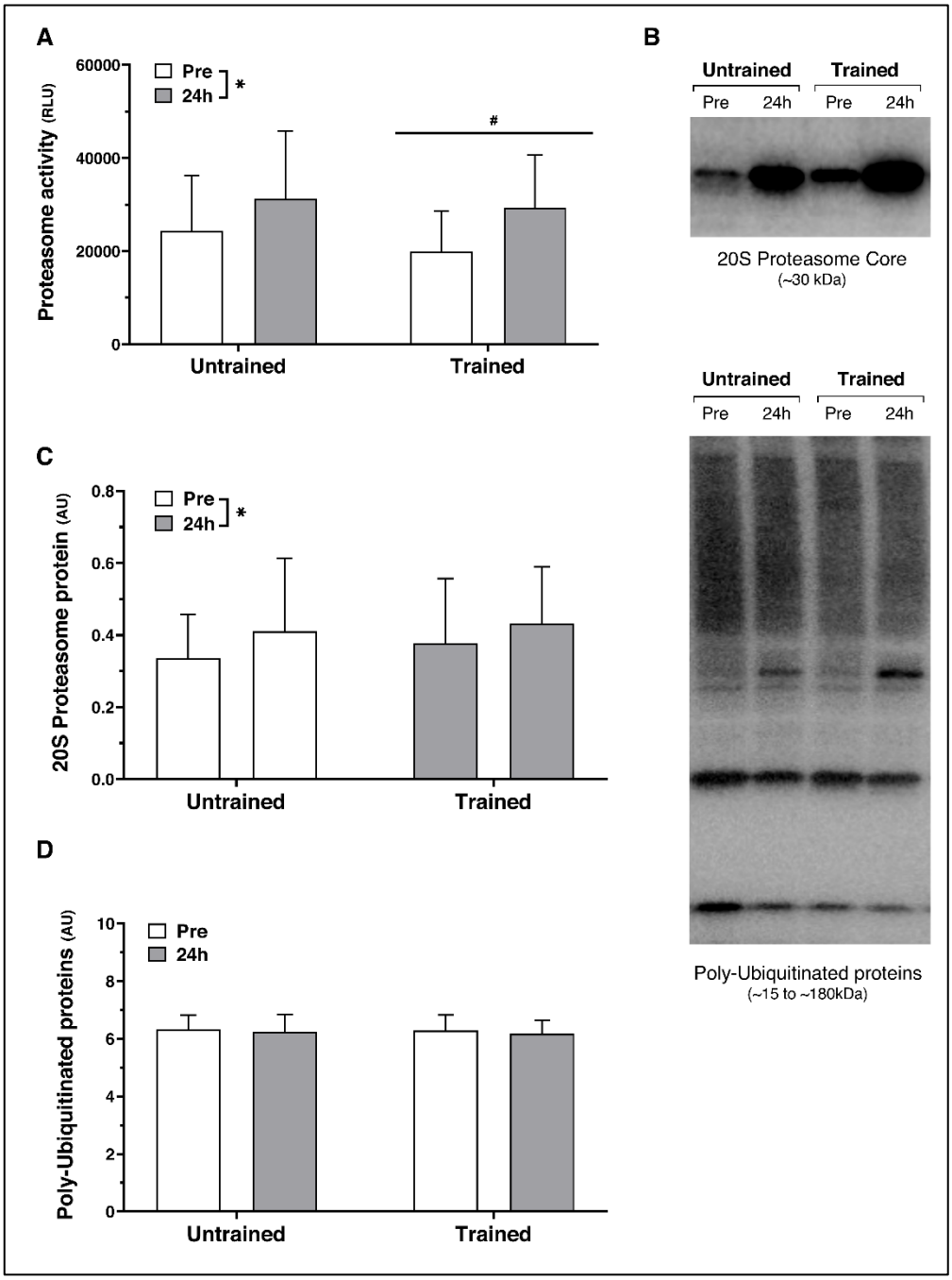
Legend: (a) Calpain activity levels and relative protein expressions of (c) calpain-1 and (d) calpain-2 measured Pre and 24 h after a resistance training session before (Untrained) and after (Trained) 10 weeks of resistance training. Panel (b) shows representative Western blots. Values are mean ± SD. *, indicates

a main effect of time (Pre vs. 24 h); #, indicates a main effect of condition (Trained vs. Untrained). Significance was set at $p \leq 0.05$. RLU = relative luminescence units; AU = arbitrary units.

Proteasome-ubiquitin pathway markers

Regarding proteasome activity (Fig. 4a), time ($F_{[1,108]} = 28.54$; $p < 0.0001$) and condition ($F_{[1,109]} = 4.79$, $p = 0.031$) effects were observed, with no interaction ($F_{[1,108]} = 0.62$, $p = 0.432$). Proteasome activity increased from Pre to 24 h, with values in the trained state being lower than those in the untrained state. For the content of the 20S proteasome proteins (Fig. 4c), there was a main time effect ($F_{[1,108]} = 5.83$, $p = 0.017$) with no condition ($F_{[1,109]} = 1.34$, $p = 0.250$) or interaction ($F_{[1,108]} = 0.13$, $p = 0.717$) effects. Protein expression of the 20S proteasome increased from Pre to 24 h. Regarding the content of polyubiquitinated proteins (Fig. 4d), there was no effect of time ($F_{[1,107]} = 3.01$, $p = 0.086$), condition ($F_{[1,108]} = 0.68$, $p = 0.412$) or interaction ($F_{[1,107]} = 0.11$, $p = 0.746$). The 95% CI of ESs of the differences between delta changes for proteasome activity, 20S proteasome and polyubiquitinated proteins indicated no significant differences between UT and T state (Table 1).

Figure 4 – Proteasome-ubiquitin pathway markers

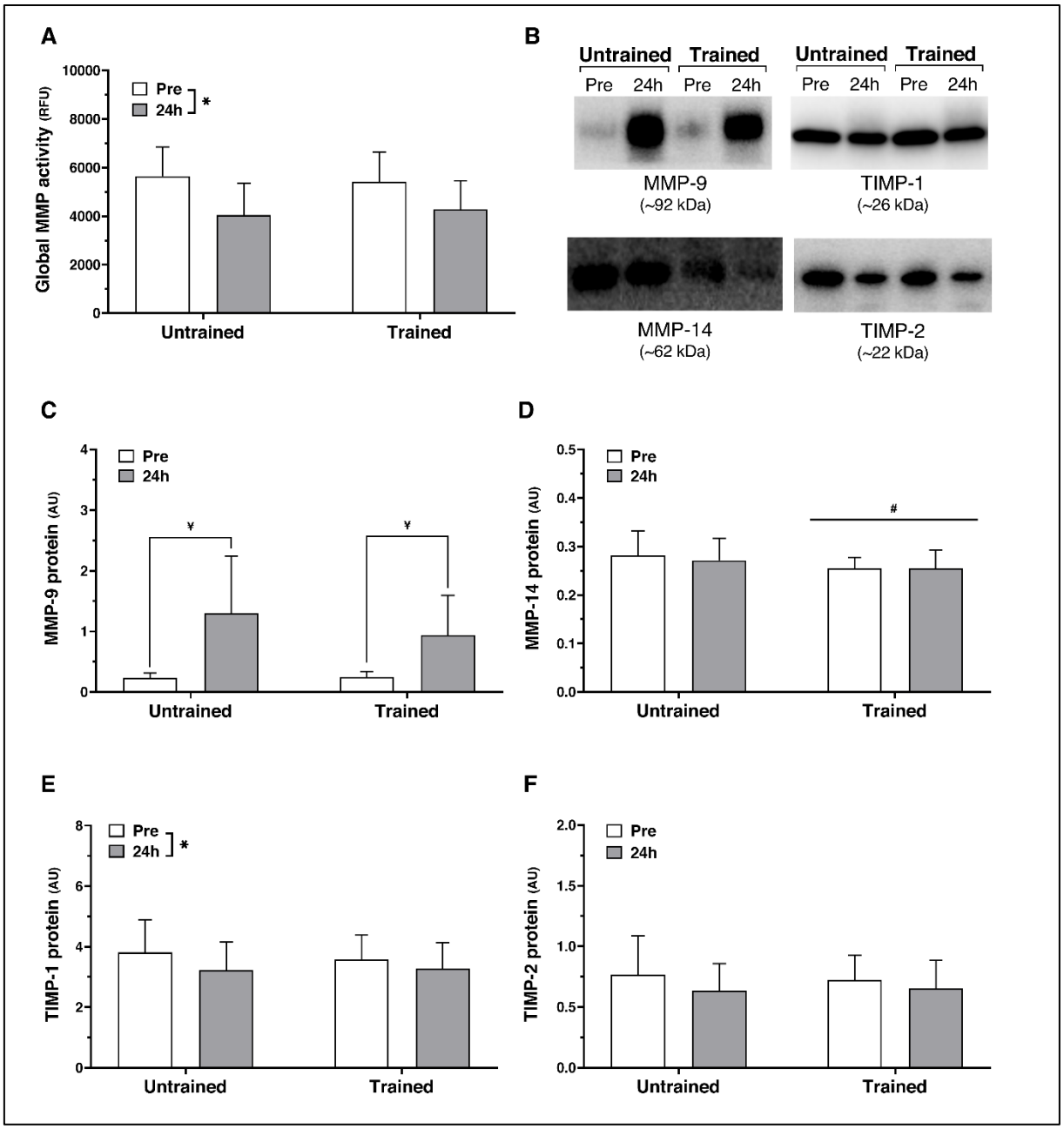


Legend: (a) Proteasome activity levels and the relative protein expressions of (c) 20S proteasome and (d) poly-ubiquitinated proteins measured Pre and 24 h after a resistance training session before (Untrained) and after (Trained) 10 weeks of resistance training. Panel (b) shows representative Western blots. Values are mean ± SD. *, indicates a main effect of time (Pre vs. 24 h); #, indicates a main effect of condition (Trained vs. Untrained). Significance was set at $p \leq 0.05$. RLU = relative luminescence units; AU = arbitrary units.

Extracellular matrix remodeling markers

The mixed model revealed a main effect of time ($F_{[1,107]}$ = 86.02, p < 0.0001) on global MMP activity (Fig. 5a). There was no effect of condition ($F_{[1,108]}$ = 0.04, p = 0.841) or interaction ($F_{[1,107]}$ = 1.81, p = 0.181). The activity of global MMPs decreased from Pre to 24 h. For MMP-9 protein (Fig. 5c), the mixed model revealed a condition vs. time interaction ($F_{[1,35.9]}$ = 4.76, p = 0.036). The post hoc analysis revealed that the content of MMP-9 increased from Pre to 24 h under both conditions, yet there were no differences between conditions at either time point. The CI of ES (Table 1) indicates that there might be a difference between the absolute changes observed in MMP-9 in the untrained and trained states. For the content of MMP-14 protein (Fig. 4d), the mixed model showed only a condition effect ($F_{[1,35.6]}$ = 14.03; p = 0.0006) but no time effect ($F_{[1,36.9]}$ = 0.93; p = 0.341) or interaction ($F_{[1,36.5]}$ = 1.31; p = 0.260), with values in the untrained state being higher than those in the trained state. Finally, there was a main effect of time on the abundance of TIMP-1 ($F_{[1,112]}$ = 31.41, p < 0.0001; Fig. 5e) and TIMP-2 ($F_{[1,106]}$ = 11.52, p = 0.001; Fig. 5f). There was no effect of condition ($F_{[1,139]}$ = 0.17, p = 0.680; and $F_{[1,106]}$ = 0.0, p = 0.982, respectively) or a condition vs. time interaction ($F_{[1,93.3]}$ = 2.8, p = 0.098; and $F_{[1,106]}$ = 1.34, p = 0.250, respectively). However, the CI of ES (Table 1) indicates that there might be a difference between absolute changes observed in TIMP-1 in the UT and T states. Protein expression of TIMP-1 and TIMP-2 decreased from Pre to 24 h. The 95% CI of ESs of the differences between delta changes for MMP-9, MMP-14 and TIMP-2 indicated no significant differences between UT and T state (Table 1).

Figure 5 – Extracellular matrix remodeling markers



Legend: (a) Global MMP activity levels and the relative protein expressions of (c) MMP-9, (d) MMP-14, (e) TIMP-1, and (f) TIMP-2 measured Pre and 24 h after a resistance training session before (Untrained) and after (Trained) 10 weeks of resistance training. Panel (b) shows representative Western blots. Values are mean ± SD. *, indicates a main effect of time (Pre vs. 24 h); #, indicates a main effect of condition (Trained vs. Untrained); ¥, significantly different from Pre in the same condition (time vs. condition interaction). Significance was set at $p \leq 0.05$. RFU = relative fluorescence units; AU = arbitrary units; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase.

Sex-based differences

The ANCOVA results indicate no differences ($p > 0.05$) between women and men in mCSA, type I, and type II fCSA changes. The results also show no differences between women and men acute responses (changes from Pre to 24 h) of any of the proteolysis and ECM remodeling biomarkers in both the untrained and trained states. The values observed separately for women and men, ANCOVA results, and effect sizes are presented in the supplemental material.

Table 1 – Muscle proteolysis and ECM remodeling biomarkers

	Untrained (UT)			Trained (T)			ES (95% CI)	Magnitude of ES
	Pre	24h	ΔUT	Pre	24h	ΔT	ΔT vs. ΔUT	
Enzymatic Activity								
Calpain (RLU)	9088 ± 4483	18289 ± 6432	9282 ± 8547	10091 ± 2819	18406 ± 7170	8367 ± 6820	-0.12 (-0.57 to 0.33)	Small
Proteasome (RLU)	24354 ± 11890	31234 ± 14565	7303 ± 15011	19895 ± 8701	29340 ± 11346	9596 ± 11958	0.17 (-0.28 to 0.62)	Small
Global MMP (RFU)	5651 ± 1197	4047 ± 1314	-1544 ± 1167	5417 ± 1227	4275 ± 1189	-1162 ± 1358	0.30 (-0.15 to 0.75)	Medium
Protein Content (AU)								
Calpain-1	0.37 ± 0.08	0.28 ± 0.07	-0.09 ± 0.09	0.37 ± 0.05	0.30 ± 0.05	-0.07 ± 0.07	0.32 (-0.13 to 0.77)	Medium
Calpain-2	3.76 ± 1.04	2.82 ± 0.99	-0.94 ± 1.17	4.22 ± 1.11	3.40 ± 0.88	-0.83 ± 1.04	0.10 (-0.35 to 0.55)	Small
20S Proteasome	0.34 ± 0.12	0.41 ± 0.20	0.08 ± 0.22	0.38 ± 0.18	0.43 ± 0.16	0.05 ± 0.23	-0.10 (-0.55 to 0.35)	Small
Polyubiquitinated proteins	6.33 ± 0.50	6.24 ± 0.59	-0.10 ± 0.63	6.29 ± 0.54	6.17 ± 0.46	-0.14 ± 0.47	-0.08 (-0.53 to 0.37)	Small
MMP-9	0.23 ± 0.09	1.30 ± 0.94	1.07 ± 0.93	0.25 ± 0.09	0.94 ± 0.65	0.69 ± 0.64	-0.48 (-0.93 to -0.02)	Medium
MMP-14	0.28 ± 0.05	0.27 ± 0.05	-0.01 ± 0.06	0.26 ± 0.02	0.25 ± 0.04	0.00 ± 0.03	0.23 (-0.22 to 0.68)	Medium
TIMP-1	3.81 ± 1.08	3.23 ± 0.92	-0.63 ± 0.66	3.57 ± 0.82	3.28 ± 0.86	-0.29 ± 0.75	0.49 (0.03 to 0.94)	Medium
TIMP-2	0.77 ± 0.32	0.63 ± 0.22	-0.14 ± 0.26	0.72 ± 0.21	0.66 ± 0.23	-0.07 ± 0.22	0.31 (-0.14 to 0.76)	Medium

Legend: Enzyme activity levels and the relative protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers measured Pre and 24 h after a resistance training session before (UT = Untrained) and after (T = Trained) 10 weeks of resistance training. Values are means ± SD. RLU = relative luminescence units; RFU = relative fluorescence units; AU = arbitrary units; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = absolute change from Pre to 24h; ES = effect size; CI = confidence interval.

DISCUSSION

In accordance with our hypothesis, the present study is the first to show that 10 weeks of RT does not attenuate the acute RT-induced response of proteolysis and remodeling-related biomarkers. Furthermore, no sex differences were evident for any of the assayed markers.

Calpain activity was upregulated from Pre to 24 h after an acute bout of RT in both the untrained and trained states (Fig. 3a). Our findings corroborate previous findings showing an acute upregulation in calpain activity 24 h after exercise (RAASTAD *et al.*, 2010). In contrast, Godwin *et al*. (2023b) found this activity to be unchanged in untrained subjects up to 8 h following an RT bout, but the lack of agreement might be due to the different time points of muscle biopsies between studies. Curiously, Calpain-1 and Calpain-2 protein expression presented an acute downregulation (Fig. 3c/d). However, it is possible for enhanced activity to be compatible with reduced protein expression since factors beyond the abundance of calpain proteases control calpain activity. For instance, calcium availability is the primary regulator for activating the proteolytic function of calpain, alongside the calpastatin protein, which acts as an inhibitor when bound to activated calpains (GOLL *et al.*, 2003; HYATT *et al.*, 2020). Regarding the untrained and trained states, no differences were observed in calpain activity and calpain-1 protein expression. However, for calpain-2 protein expression, higher values were observed in the trained state than in the untrained state. To the best of our knowledge, a comparison of calpain pathway markers in the untrained and trained states has been examined only in a transcriptome study by Damas *et al*. (2018b). Contrary to our results, those authors reported a higher upregulation of genes related to the calpain pathway in the untrained state compared to the trained state, suggesting an attenuation in calpain pathway-related acute responses. The lack of agreement may be due to posttranslational processes affecting the translation of mRNAs into proteins. In this regard, previous evidence has shown that mRNA levels explain approximately 40% of the variability in protein levels (SCHWANHÄUSSER *et*

al., 2011), so further research concomitantly assessing transcriptome and proteome markers may aid in understanding the topic.

Proteasome activity and the protein expression of the proteins belonging to the 20S proteasome were upregulated from Pre to 24 h after an acute bout of RT in both the untrained and trained states (Fig. 4a/c). However, there was an effect of condition on proteasome activity, indicating attenuated values in the trained state. Our findings corroborate previous observations regarding an acute upregulation in 20S protein expression in untrained subjects (WILLOUGHBY *et al.*, 2003). Regarding the untrained versus trained states, our results do not support the hypothesis that the acute upregulation of UPP markers – i.e., the change from Pre to 24 h – would be lower in the trained state. Previous studies have reported an attenuation in the response of UPP-related markers after two repeated bouts of exercise stimulus (WILLOUGHBY *et al.*, 2003; NEDERGAARD *et al.*, 2007; MASCHER *et al.*, 2008). The lack of agreement may be due to methodological differences since these studies have: (i) measured mRNA expression and not protein and enzymatic activity levels, and (ii) used a two-bout design that may not reflect muscle adaptations that occur further along the process of RT-induced muscle growth. Regarding the protein expression of poly-ubiquitinated proteins, our results show no differences between timepoints (Fig. 4d). This finding aligns with previous results showing that the protein expression of ubiquitin ligases (MURF-1, FOXO1, and FOXO3) remains unchanged 22 h after an RT bout performed in the trained state (STEFANETTI *et al.*, 2015). This finding suggests there may not be a tight relationship between ubiquitinated protein content and proteolysis via the proteasome pathway and/or protein polyubiquitination may have occurred earlier following exercise bouts. More research is needed to shed light on the processes of ubiquitination and deubiquitination of proteins, as well as non-ubiquitin-dependent proteasome activity.

Regarding MMP activity, our results indicate an acute downregulation following a bout of RT in both the untrained and trained states. This was accompanied by increased MMP-9 and a preservation of MMP-14 abundance (Fig. 5). While the decrease in activity level is surprising, these results reflect the global activity of MMPs. Thus, it may not necessarily align with the activities of individual MMPs. MMP-9 protein expression acutely increased from Pre to 24 h. The acute upregulation is aligned with previous data indicating MMP-9 muscle protein levels are significantly higher immediately following exercise and remain elevated for at least 120 minutes following an exercise bout (RULLMAN *et al.*, 2007). MMP-14 protein expression was unchanged from Pre to 24 h, but values were lower in the trained state (Fig. 5d). To the best of our knowledge, no studies have explored the acute response of MMP-14. However, Godwin *et al*. (2023a) showed that the basal expression of MMP-14 is significantly downregulated following 10 weeks of RT. This contrasts with a recent study that reported that several genes associated with ECM remodeling, including MMP-14, were enriched after 14 weeks of progressive RT in older adults (KULKARNI *et al.*, 2020). This lack of agreement may be due to the different populations investigated since ECM adaptations appear to become more critical for muscle hypertrophy in older adults (ROBERTS *et al.*, 2023). However, this warrants further examination. Finally, the protein expression of TIMP-1 was acutely reduced in both the untrained and trained states, while the protein expression of TIMP-2 was unchanged (Fig. 5e/f). While acute changes induced by RT in TIMP-2 protein levels have not been investigated elsewhere, it has been demonstrated, at least in plasma, that TIMP-1 appears to decrease after exercise (KIM *et al.*, 2020). Four known TIMPs act in the ECM, and while all of them can inhibit all types of MMPs, they do so with different affinity levels (BREW *et al.*, 2010). TIMP-1 has the most restricted inhibitory range, as it has a low affinity for MMP-14 but has a higher affinity for MMP-9. On the other hand, TIMP-2 may inhibit all MMPs but selectively interacts with MMP-14 (BREW *et al.*, 2010; ALAMEDDINE, 2012; LO PRESTI *et al.*, 2017). Thus, the

acute downregulation observed in TIMP-1 is aligned with the acute upregulation in MMP-9. Likewise, the absence of acute changes in TIMP-2 aligns with the acutely unchanged levels of MMP-14. Exploring these ECM remodeling factors concomitantly with markers of ECM abundance could provide more insight.

We also investigated whether biological sex could be an influencing factor in the response of these biomarkers. An audit-based review study demonstrated that consensus statements and position stands in the field of RT are comprised of 70% male participants (PANDIT *et al.*, 2023). It is plausible to assume that the scenario is similar – if not more inequitable – when it comes to studies on the RT-induced response of molecular pathways, and the findings are extrapolated from male to female populations. Of the 20 human experimental studies referenced and discussed above, 18 had samples comprised exclusively of men, one had a mixed sample of older women and men, and one had a mixed sample of young women and men. Out of the studies with a mixed sample, only Stupka *et al*. (2001) compared the results of women and men and report no significant sex-based differences in proteolytic responses; nonetheless, these authors argued that their study was underpowered to assess possible effects. Using a 2.4 times greater sample size comprised equally of women and men, our study advances this topic and confirms that there were no sex-based differences in proteolytic responses. Regarding ECM remodeling-related markers, to the best of our knowledge, the present study was the first to investigate and show that there are no sex-based differences in ECM remodeling factors in response to RT.

The present study is not without limitations. We were unable to control for the phase of the menstrual cycle during which measurements were taken. However, studies suggest that menstrual cycle phases do not significantly impact muscle (THOMPSON *et al.*, 2021) or fiber (SUNG *et al.*, 2014) cross-sectional area, nor protein and collagen synthesis (MILLER *et al.*, 2006). Yet, the potential influence of the cycle phase on changes in muscle proteolysis and

extracellular matrix remodeling induced by resistance training remains unknown. Although dietary intake was not controlled in the present study, we recommended that participants maintained their usual dietary habits throughout the study. Importantly, the impact of regulating dietary intake on muscle hypertrophic response may not be substantial in healthy individuals, as indicated by a recent review wherein self-reported dietary patterns, including energy intake and even protein consumption, did not differ between distinct levels of responders to muscle hypertrophy (ROBERTS *et al.*, 2018). Our sample is composed exclusively of healthy young adults, thus limiting the extrapolation to the context of diseases or older participants, and our inferences would benefit from more investigated targets, including muscle damage markers. Finally, including additional biopsy time points following the acute bouts of RT and adding acute session assessments during the mid-RT program would provide greater insight into the dynamics of response throughout the training period.

CONCLUSION

Proteolysis-related and ECM remodeling biomarkers responses to a bout of RT are largely not attenuated in the trained state, and this suggests that proteolysis and remodeling responses are likely sustained to further promote skeletal muscle hypertrophy and tissue remodeling. However, our findings do not exclude the possibility of attenuation in activity following the first few bouts of an RT program. In this regard, the activities of these markers may be directed mainly to repairing the structural damage during the first few bouts. Whether these activities will stay unattenuated with the continuation of RT for a more extended period remains to be investigated.

SUPPLEMENTAL MATERIAL

Table S1 – Vastus lateralis muscle cross-sectional area and Type I and Type II fibers cross-sectional area

	Women (W)			Men (M)			ES (95% CI)	Magnitude of ES
	Baseline	Post	ΔW	Baseline	Post	ΔM	ΔM vs. ΔW	
mCSA (cm^2)	18.63 ± 3.52	20.85 ± 5.13	2.23 ± 2.28	23.79 ± 4.36	25.86 ± 4.46	2.08 ± 2.18	-0.07 (-0.70 to 0.56)	Small
Type I fCSA (μm^2)	4253 ± 744	4378 ± 983	214 ± 963	4825 ± 953	4855 ± 1180	27 ± 1100	-0.18 (-0.80 to 0.45)	Small
Type II fCSA (μm^2)	3885 ± 818	4195 ± 816	360 ± 877	5066 ± 1057	5561 ± 1513	426 ± 1355	0.06 (-0.54 to 0.65)	Small

Legend: Vastus lateralis muscle cross-sectional area (mCSA), and type I and type II mean fiber cross-sectional areas (fCSA) of women (W) and men (M) before (Baseline) and after (Post) a 10-week period of resistance training. Values are mean ± SD. Δ = absolute change from Baseline to Post; ES = effect size; CI = confidence interval.

Table S2 – ANCOVA Type III tests for muscle cross-sectional area and Type I and Type II fibers cross-sectional area

Variables	SEX		COVARIATE	
	F Value	Pr > F	F Value	Pr > F
ΔmCSA	0.39	0.537	0.67	0.420
ΔType I fCSA	0.03	0.856	3.66	0.064
ΔType II fCSA	1.85	0.183	3.66	0.065

Legend: Analysis of Covariance (ANCOVA) Type III tests, indicating the effect of sex on the absolute change observed in the vastus lateralis muscle cross-sectional area (mCSA), and type I and type II fiber cross-sectional areas (fCSA), after controlling for the respective covariate (Baseline values). Δ = absolute change from Baseline to Post-training values.

Table S3 – Muscle proteolysis and ECM remodeling biomarkers in the untrained state

	WOMEN (W)			MEN (M)			ES (95% CI)	Magnitude
	Pre	24h	ΔW	Pre	24h	ΔM	ΔM vs. ΔW	of ES
Enzymatic Activity								
Calpain (RLU)	9229 ± 6064	17318 ± 6597	8089 ± 9808	8946 ± 2120	19313 ± 6273	10540 ± 7038	0.28 (-0.17 to 0.74)	Medium
Proteasome (RLU)	23566 ± 14545	30133 ± 14303	6566 ± 16385	25140 ± 8816	32396 ± 15160	8080 ± 13844	0.10 (-0.35 to 0.55)	Small
Global MMP (RFU)	5398 ± 1323	4028 ± 1297	-1369 ± 1200	5904 ± 1028	4067 ± 1368	-1727 ± 1136	-0.30 (-0.76 to 0.15)	Medium
Protein content (AU)								
Calpain-1	0.37 ± 0.09	0.28 ± 0.08	-0.10 ± 0.10	0.37 ± 0.07	0.29 ± 0.06	-0.09 ± 0.08	0.11 (-0.34 to 0.56)	Small
Calpain-2	3.91 ± 1.15	3.00 ± 1.11	-0.91 ± 1.39	3.60 ± 0.93	2.63 ± 0.83	-0.97 ± 0.93	-0.05 (-0.50 to 0.40)	Small
20S Proteasome	0.34 ± 0.14	0.37 ± 0.11	0.03 ± 0.13	0.34 ± 0.11	0.46 ± 0.26	0.13 ± 0.28	0.45 (-0.01 to 0.90)	Medium
Polyubiquitinated proteins	6.40 ± 0.54	6.31 ± 0.68	-0.09 ± 0.79	6.27 ± 0.45	6.17 ± 0.49	-0.10 ± 0.43	-0.02 (-0.47 to 0.43)	Small
MMP-9	0.24 ± 0.08	1.21 ± 0.95	0.97 ± 0.91	0.22 ± 0.09	1.40 ± 0.95	1.18 ± 0.95	0.22 (-0.23 to 0.67)	Medium
MMP-14	0.29 ± 0.06	0.28 ± 0.05	-0.01 ± 0.06	0.27 ± 0.04	0.26 ± 0.03	-0.01 ± 0.05	-0.10 (-0.55 to 0.35)	Small
TIMP-1	4.05 ± 1.14	3.51 ± 0.86	-0.55 ± 0.77	3.57 ± 0.98	2.94 ± 0.91	-0.72 ± 0.51	-0.26 (-0.71 to 0.19)	Medium
TIMP-2	0.79 ± 0.37	0.69 ± 0.24	-0.10 ± 0.32	0.74 ± 0.27	0.58 ± 0.20	-0.19 ± 0.19	-0.33 (-0.79 to 0.12)	Medium

Legend: Enzyme activity levels and protein expression of assayed muscle proteolysis and extracellular matrix remodeling (ECM) markers measured Pre and 24h after a resistance training session of women (W) and men (M) in the untrained state. Values are mean ± SD. RLU = relative luminescence units; RFU = relative fluorescence units; AU = arbitrary units; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = absolute change from Pre to 24h; ES = effect size; CI = confidence interval.

Table S4. ANCOVA Type III tests for muscle proteolysis and ECM remodeling biomarkers in the untrained state

	SEX		COVARIATE	
	F Value	**Pr > F**	**F Value**	**Pr > F**
Enzymatic Activity				
ΔCalpain	0.79	0.382	28.47	<.0001
ΔProteasome	0.18	0.677	7.78	0.008
ΔGlobal MMP	0.35	0.558	4.51	0.041
Protein Content				
ΔCalpain-1	0.23	0.636	24.10	<.0001
ΔCalpain-2	0.76	0.388	21.19	<.0001
Δ20S Proteasome	2.08	0.158	6.80	0.013
ΔPolyubiquitinated proteins	0.20	0.660	10.16	0.003
ΔMMP-9	0.56	0.461	0.66	0.422
ΔMMP-14	2.84	0.101	28.09	<.0001
ΔTIMP-1	2.57	0.118	13.38	0.001
ΔTIMP-2	2.80	0.103	38.64	<.0001

Legend: Analysis of Covariance (ANCOVA) Type III tests, indicating the effect of sex on the absolute change observed in muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers in the untrained state, after controlling for the respective covariate (Pre values). Δ = absolute change from Pre to 24 h after a bout of resistance training in the untrained state.

Table S5. Muscle proteolysis and remodeling biomarkers in the trained state

	WOMEN (W)			MEN (M)			ES (95% CI)	Magnitude of ES
	Pre	24h	ΔW	Pre	24h	ΔM	ΔM vs. ΔW	
Enzymatic Activity								
Calpain (RLU)	9762 ± 2985	17698 ± 7989	7935 ± 6564	10401 ± 2696	19113 ± 6399	8798 ± 7229	0.12 (-0.33 to 0.57)	Small
Proteasome (RLU)	19915 ± 10335	28199 ± 13632	8283 ± 12080	19875 ± 7109	30480 ± 8740	10907 ± 12033	0.22 (-0.24 to 0.67)	Medium
Global MMP (RFU)	5179 ± 1168	3945 ± 1288	-1234 ± 1611	5641 ± 1269	4605 ± 1009	-1088 ± 1089	0.10 (-0.35 to 0.55)	Small
Protein content (AU)								
Calpain-1	0.38 ± 0.06	0.30 ± 0.07	-0.08 ± 0.09	0.38 ± 0.06	0.30 ± 0.07	-0.05 ± 0.05	0.35 (-0.10 to 0.81)	Medium
Calpain-2	4.71 ± 1.14	3.53 ± 1.15	-1.17 ± 1.23	4.71 ± 1.14	3.53 ± 1.15	-0.49 ± 0.69	0.69 (0.22 to 1.15)	Medium
20S Proteasome	0.35 ± 0.14	0.41 ± 0.14	0.06 ± 0.19	0.35 ± 0.14	0.41 ± 0.14	0.05 ± 0.27	-0.06 (-0.51 to 0.39)	Small
Polyubiquitinated proteins	6.27 ± 0.48	6.16 ± 0.49	-0.11 ± 0.33	6.27 ± 0.48	6.16 ± 0.49	-0.17 ± 0.58	-0.12 (-0.57 to 0.33)	Small
MMP-9	0.25 ± 0.09	0.82 ± 0.59	0.57 ± 0.58	0.25 ± 0.09	0.82 ± 0.59	0.81 ± 0.69	0.37 (-0.08 to 0.82)	Medium
MMP-14	0.26 ± 0.02	0.26 ± 0.05	0.00 ± 0.04	0.26 ± 0.02	0.26 ± 0.05	0.00 ± 0.01	-0.01 (-0.46 to 0.44)	Small
TIMP-1	3.74 ± 0.8	3.26 ± 0.84	-0.48 ± 0.71	3.74 ± 0.80	3.26 ± 0.84	-0.10 ± 0.76	0.50 (0.05 to 0.96)	Medium
TIMP-2	0.77 ± 0.23	0.67 ± 0.25	-0.10 ± 0.22	0.77 ± 0.23	0.67 ± 0.25	-0.03 ± 0.22	0.32 (-0.13 to 0.77)	Medium

Legend: Enzyme activity levels and protein expression of assayed muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers measured Pre and 24h after a resistance training session of women (W) and men (M) in the trained state (i.e., after 10 weeks of resistance training). Values are mean ± SD. RLU = relative luminescence units; RFU = relative fluorescence units; AU = arbitrary units; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase; Δ = absolute change from Pre to 24h; ES = effect size; CI = confidence interval.

Table S6. ANCOVA Type III tests for muscle proteolysis and ECM remodeling biomarkers in the trained state

	SEX		COVARIATE	
	F Value	**Pr > F**	**F Value**	**Pr > F**
Enzymatic Activity				
ΔCalpain	0.18	0.677	0.26	0.612
ΔProteasome	0.43	0.514	7.72	0.009
ΔGlobal MMP	1.74	0.197	19.55	0.0001
Protein content				
ΔCalpain-1	0.25	0.622	24.59	<.0001
ΔCalpain-2	0.16	0.692	20.66	<.0001
Δ20S Proteasome	0.46	0.503	40.10	<.0001
ΔPolyubiquitinated proteins	0.01	0.912	16.53	0.0003
ΔMMP-9	1.18	0.285	0.09	0.768
ΔMMP-14	0.07	0.797	0.44	0.513
ΔTIMP-1	1.24	0.274	5.68	0.023
ΔTIMP-2	0.20	0.654	6.50	0.015

Legend: Analysis of Covariance (ANCOVA) Type III tests, indicating the effect of sex on the absolute change observed in muscle proteolysis and extracellular matrix (ECM) remodeling biomarkers in the trained state, after controlling for the respective covariate (Pre values). Δ = absolute change from Pre to 24 h after a bout of resistance training in the trained state.

REFERÊNCIAS

AHMAD, K.; SHAIKH, S.; CHUN, H. J. *et al.* Extracellular matrix: the critical contributor to skeletal muscle regeneration—a comprehensive review. **Inflammation and Regeneration**, 43, n. 1, p. 58, 2023/11/27 2023.

AHTIAINEN, J. P.; WALKER, S.; PELTONEN, H. *et al.* Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages. **Age (Dordr)**, 38, n. 1, p. 10, Feb 2016.

ALAMEDDINE, H. S. Matrix metalloproteinases in skeletal muscles: Friends or foes? **Neurobiology of Disease**, 48, n. 3, p. 508-518, 2012/12/01/ 2012.

ANGLERI, V.; DAMAS, F.; PHILLIPS, S. M. *et al.* Resistance training variable manipulations are less relevant than intrinsic biology in affecting muscle fiber hypertrophy. **Scand J Med Sci Sports**, 32, n. 5, p. 821-832, May 2022a.

ANGLERI, V.; UGRINOWITSCH, C.; LIBARDI, C. A. Individual Muscle Adaptations in different Resistance Training Systems in Well-Trained Men. **Int J Sports Med**, 43, n. 1, p. 55-60, Jan 2022b.

BAEHR, L.; TUNZI, M.; BODINE, S. Muscle hypertrophy is associated with increases in proteasome activity that is independent of MuRF1 and MAFbx expression. **Front Physiol**, 5, 2014.

BAKDASH, J. Z.; MARUSICH, L. R. Repeated Measures Correlation. **Front Psychol**, 8, p. 456, 2017.

BAMMAN, M. M.; PETRELLA, J. K.; KIM, J. S. *et al.* Cluster analysis tests the importance of myogenic gene expression during myofiber hypertrophy in humans. **J Appl Physiol**, 102, n. 6, p. 2232-2239, Jun 2007.

BAMMAN, M. M.; ROBERTS, B. M.; ADAMS, G. R. Molecular Regulation of Exercise-Induced Muscle Fiber Hypertrophy. **Cold Spring Harb Perspect Med**, 8, n. 6, Jun 1 2018.

BELLAMY, L. M.; JOANISSE, S.; GRUBB, A. *et al.* The Acute Satellite Cell Response and Skeletal Muscle Hypertrophy following Resistance Training. **PLoS One**, 9, n. 10, p. e109739, 2014.

BREW, K.; NAGASE, H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. **Biochim Biophys Acta**, 1803, n. 1, p. 55-71, Jan 2010.

BROWN, L. E.; WEIR, J. P. ASEP procedures recommendation I: accurate assessment of muscular strength and power. **J Exerc Physiol Online**, 4, n. 3, p. 1-21, 2001.

CHAVES, T. S.; SCARPELLI, M. C.; BERGAMASCO, J. G. A. *et al.* Effects of resistance training overload progression protocols on strength and muscle mass. **International Journal of Sports Medicine**, 2024a.

CHAVES, T. S.; SCARPELLI, M. C.; BERGAMASCO, J. G. A. *et al.* Effects of resistance training overload progression protocols on strength and muscle mass. **Int J Sports Med**, Jan 29 2024b.

COHEN, J. **Statistical power analysis for the behavioral sciences**. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum, 1988.

CONCEIÇÃO, M. S.; VECHIN, F. C.; LIXANDRÃO, M. *et al.* Muscle Fiber Hypertrophy and Myonuclei Addition: A Systematic Review and Meta-analysis. **Med Sci Sports Exerc**, 50, n. 7, p. 1385-1393, Jul 2018.

DAMAS, F.; BARCELOS, C.; NOBREGA, S. R. *et al.* Individual Muscle Hypertrophy and Strength Responses to High vs. Low Resistance Training Frequencies. **J Strength Cond Res**, 33, n. 4, p. 897-901, Apr 2019.

DAMAS, F.; LIBARDI, C. A.; UGRINOWITSCH, C. The development of skeletal muscle hypertrophy through resistance training: the role of muscle damage and muscle protein synthesis. **Eur J Appl Physiol**, 118, n. 3, p. 485-500, Mar 2018a.

DAMAS, F.; PHILLIPS, S. M.; LIBARDI, C. A. *et al.* Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage. **J Physiol**, 594, n. 18, p. 5209-5222, Sep 15 2016a.

DAMAS, F.; PHILLIPS, S. M.; LIXANDRÃO, M. E. *et al.* Early resistance training-induced increases in muscle cross-sectional area are concomitant with edema-induced muscle swelling. **Eur J Appl Physiol**, 116, n. 1, p. 49-56, 2016/01/01 2016b.

DAMAS, F.; UGRINOWITSCH, C.; LIBARDI, C. A. *et al.* Resistance training in young men induces muscle transcriptome-wide changes associated with muscle structure and metabolism refining the response to exercise-induced stress. **Eur J Appl Physiol**, 118, n. 12, p. 2607-2616, 2018/12/01 2018b.

DANKEL, S. J.; LOENNEKE, J. P.; LOPRINZI, P. D. Participation in muscle-strengthening activities as an alternative method for the prevention of multimorbidity. **Prev Med**, 81, p. 54-57, Dec 2015.

DANKEL, S. J.; LOENNEKE, J. P.; LOPRINZI, P. D. Determining the Importance of Meeting Muscle-Strengthening Activity Guidelines: Is the Behavior or the Outcome of the Behavior (Strength) a More Important Determinant of All-Cause Mortality? **Mayo Clin Proc**, 91, n. 2, p. 166-174, Feb 2016a.

DANKEL, S. J.; LOENNEKE, J. P.; LOPRINZI, P. D. Dose-dependent association between muscle-strengthening activities and all-cause mortality: Prospective cohort study among a national sample of adults in the USA. **Arch Cardiovasc Dis**, Aug 31 2016b.

DAVIDSEN, P. K.; GALLAGHER, I. J.; HARTMAN, J. W. *et al.* High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. **J Appl Physiol (1985)**, 110, n. 2, p. 309-317, Feb 2011.

DAVIS, M. E.; GUMUCIO, J. P.; SUGG, K. B. *et al.* MMP inhibition as a potential method to augment the healing of skeletal muscle and tendon extracellular matrix. **J Appl Physiol (1985)**, 115, n. 6, p. 884-891, Sep 2013.

DEFREITAS, J. M.; BECK, T. W.; STOCK, M. S. *et al.* An examination of the time course of training-induced skeletal muscle hypertrophy. **Eur J Appl Physiol**, 111, n. 11, p. 2785-2790, Nov 2011.

EVANS, J. D. **Straightforward Statistics for the Behavioral Sciences**. Brooks/Cole Publishing Company, 1996. 9780534231002.

FÉASSON, L.; STOCKHOLM, D.; FREYSSENET, D. *et al.* Molecular adaptations of neuromuscular disease-associated proteins in response to eccentric exercise in human skeletal muscle. **J Physiol**, 543, n. Pt 1, p. 297-306, Aug 15 2002.

GILLIES, A. R.; LIEBER, R. L. Structure and function of the skeletal muscle extracellular matrix. **Muscle Nerve**, 44, n. 3, p. 318-331, Sep 2011.

GODWIN, J. S.; SEXTON, C. L.; KONTOS, N. J. *et al.* Extracellular matrix content and remodeling markers do not differ in college-aged men classified as higher and lower responders to resistance training. **J Appl Physiol (1985)**, 134, n. 3, p. 731-741, Mar 1 2023a.

GODWIN, J. S.; TELLES, G. D.; VECHIN, F. C. *et al.* Time Course of Proteolysis Biomarker Responses to Resistance, High-Intensity Interval, and Concurrent Exercise Bouts. **J Strength Cond Res**, Jul 28 2023b.

GOLL, D. E.; THOMPSON, V. F.; LI, H. *et al.* The Calpain System. **Physiological Reviews**, 83, n. 3, p. 731-801, 2003.

HADDAD, F.; ZALDIVAR, F.; COOPER, D. M. *et al.* IL-6-induced skeletal muscle atrophy. **J Appl Physiol**, 98, n. 3, p. 911-917, Mar 2005.

HAUN, C. T.; VANN, C. G.; MOBLEY, C. B. *et al.* Pre-training Skeletal Muscle Fiber Size and Predominant Fiber Type Best Predict Hypertrophic Responses to 6 Weeks of Resistance Training in Previously Trained Young Men. **Front Physiol**, 10, p. 297, 2019.

HEDGES, L. V.; OLKIN, I. Estimation of a Single Effect Size: Parametric and Nonparametric Methods. *In*: HEDGES, L. V. e OLKIN, I. (Ed.). **Statistical Methods for Meta-Analysis**. San Diego: Academic Press, 1985. p. 75-106.

HÖHFELD, J.; BENZING, T.; BLOCH, W. *et al.* Maintaining proteostasis under mechanical stress. **EMBO Reports**, 22, n. 8, p. e52507, 2021.

HOIER, B.; NORDSBORG, N.; ANDERSEN, S. *et al.* Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. **J Physiol**, 590, n. 3, p. 595-606, Feb 1 2012.

HOPKINS, W. G. Precision of Measurement. *In*: **A New View of Statistics**. Auckland, NZ: Internet Society for Sport Science, 2001.

HYATT, H. W.; POWERS, S. K. The Role of Calpains in Skeletal Muscle Remodeling with Exercise and Inactivity-induced Atrophy. **Int J Sports Med**, 41, n. 14, p. 994-1008, 2020/07/17 2020.

KIM, D.; KIM, H. J.; SONG, T. J. Association of Body Composition Indices with Cardiovascular Outcomes: A Nationwide Cohort Study. **Am J Clin Nutr**, Feb 24 2024.

KIM, G.; LEE, S. E.; LEE, Y. B. *et al.* Relationship Between Relative Skeletal Muscle Mass and Nonalcoholic Fatty Liver Disease: A 7-Year Longitudinal Study. **Hepatology**, 68, n. 5, p. 1755-1768, Nov 2018.

KIM, J.; LEE, J. Plasma MMP-9, TIMP-1, and TGF-beta1 Responses to Exercise-Induced Muscle Injury. **Int J Environ Res Public Health**, 17, n. 2, p. 566, Jan 16 2020.

KITAJIMA, Y.; TASHIRO, Y.; SUZUKI, N. *et al.* Proteasome dysfunction induces muscle growth defects and protein aggregation. **J Cell Sci**, 127, n. Pt 24, p. 5204-5217, Dec 15 2014.

KITAJIMA, Y.; YOSHIOKA, K.; SUZUKI, N. The ubiquitin–proteasome system in regulation of the skeletal muscle homeostasis and atrophy: from basic science to disorders. **The Journal of Physiological Sciences**, 70, n. 1, p. 40, 2020/09/16 2020.

KJAER, M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. **Physiol Rev**, 84, n. 2, p. 649-698, Apr 2004.

KRAEMER, W. J.; RATAMESS, N. A. Fundamentals of resistance training: progression and exercise prescription. **Med Sci Sports Exerc**, 36, n. 4, p. 674-688, Apr 2004.

KULKARNI, A. S.; PECK, B. D.; WALTON, R. G. *et al.* Metformin alters skeletal muscle transcriptome adaptations to resistance training in older adults. **Aging (Albany NY)**, 12, n. 20, p. 19852-19866, Oct 18 2020.

LASEVICIUS, T.; UGRINOWITSCH, C.; SCHOENFELD, B. J. *et al.* Effects of different intensities of resistance training with equated volume load on muscle strength and hypertrophy. **Eur J Sport Sci**, 18, n. 6, p. 772-780, Jul 2018.

LIU, M.; ZHANG, Z.; ZHOU, C. *et al.* Predicted fat mass and lean mass in relation to all-cause and cause-specific mortality. **J Cachexia Sarcopenia Muscle**, 13, n. 2, p. 1064-1075, Apr 2022.

LIXANDRÃO, M. E.; BAMMAN, M.; VECHIN, F. C. *et al.* Higher resistance training volume offsets muscle hypertrophy nonresponsiveness in older individuals. **J Appl Physiol (1985)**, 136, n. 2, p. 421-429, Feb 1 2024.

LIXANDRAO, M. E.; UGRINOWITSCH, C.; BOTTARO, M. *et al.* Vastus lateralis muscle cross-sectional area ultrasonography validity for image fitting in humans. **J Strength Cond Res**, 28, n. 11, p. 3293-3297, Nov 2014.

LO PRESTI, R.; HOPPS, E.; CAIMI, G. Gelatinases and physical exercise: A systematic review of evidence from human studies. **Medicine (Baltimore)**, 96, n. 37, p. e8072, Sep 2017.

MACKEY, A. L.; KJAER, M. Connective tissue regeneration in skeletal muscle after eccentric contraction-induced injury. **J Appl Physiol (1985)**, 122, n. 3, p. 533-540, Mar 1 2017.

MACKEY, A. L.; KJAER, M.; CHARIFI, N. *et al.* Assessment of satellite cell number and activity status in human skeletal muscle biopsies. **Muscle Nerve**, 40, n. 3, p. 455-465, Sep 2009.

MANN, T. N.; LAMBERTS, R. P.; LAMBERT, M. I. High Responders and Low Responders: Factors Associated with Individual Variation in Response to Standardized Training. **Sports Medicine**, 44, n. 8, p. 1113-1124, 2014/08/01 2014.

MARUSICH, L.; BAKDASH, J. rmcorrShiny: A web and standalone application for repeated measures correlation [version 2; peer review: 2 approved]. 10, n. 697, 2021.

MASCHER, H.; TANNERSTEDT, J.; BRINK-ELFEGOUN, T. *et al.* Repeated resistance exercise training induces different changes in mRNA expression of MAFbx and MuRF-1 in human skeletal muscle. **Am J Physiol Endocrinol Metab**, 294, n. 1, p. E43-51, Jan 2008.

MCDONALD, J. H. **Handbook of Biological Statistics**. 3rd ed. Baltimore, MD: Sparky House Publishing, 2014.

MILLER, B. F.; HANSEN, M.; OLESEN, J. L. *et al.* No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle. **Am J Physiol Endocrinol Metab**, 290, n. 1, p. E163-E168, Jan 2006.

MILLWARD, D. J. A protein-stat mechanism for regulation of growth and maintenance of the lean body mass. **Nutr Res Rev**, 8, n. 1, p. 93-120, Jan 1995.

MITCHELL, C. J.; CHURCHWARD-VENNE, T. A.; WEST, D. W. *et al.* Resistance exercise load does not determine training-mediated hypertrophic gains in young men. **J Appl Physiol (1985)**, 113, n. 1, p. 71-77, Jul 2012.

MOBLEY, C. B.; HAUN, C. T.; ROBERSON, P. A. *et al.* Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training. **PLoS One**, 13, n. 4, p. e0195203, 2018.

MURTON, A. J.; CONSTANTIN, D.; GREENHAFF, P. L. The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy. **Biochim Biophys Acta**, 1782, n. 12, p. 730-743, Dec 2008.

NABA, A.; CLAUSER, K. R.; DING, H. *et al.* The extracellular matrix: Tools and insights for the "omics" era. **Matrix Biol**, 49, p. 10-24, Jan 2016.

NAKAGAWA, S.; CUTHILL, I. C. Effect size, confidence interval and statistical significance: a practical guide for biologists. **Biol Rev Camb Philos Soc**, 82, n. 4, p. 591-605, Nov 2007.

NEDERGAARD, A.; VISSING, K.; OVERGAARD, K. *et al.* Expression patterns of atrogenic and ubiquitin proteasome component genes with exercise: effect of different loading patterns and repeated exercise bouts. **J Appl Physiol**, 103, n. 5, p. 1513-1522, Nov 2007.

NOBREGA, S. R.; UGRINOWITSCH, C.; PINTANEL, L. *et al.* Effect of Resistance Training to Muscle Failure vs. Volitional Interruption at High- and Low-Intensities on Muscle Mass and Strength. **J Strength Cond Res**, 32, n. 1, p. 162-169, Jan 2018.

OGASAWARA, R.; AKIMOTO, T.; UMENO, T. *et al.* MicroRNA expression profiling in skeletal muscle reveals different regulatory patterns in high and low responders to resistance training. **Physiol Genomics**, 48, n. 4, p. 320-324, Apr 2016.

OGASAWARA, R.; THIEBAUD, R. S.; LOENNEKE, J. P. *et al.* Time course for arm and chest muscle thickness changes following bench press training. **Interv Med Appl Sci**, 4, n. 4, p. 217-220, Dec 2012.

OJIMA, K.; ONO, Y.; OTTENHEIJM, C. *et al.* Non-proteolytic functions of calpain-3 in sarcoplasmic reticulum in skeletal muscles. **J Mol Biol**, 407, n. 3, p. 439-449, Apr 1 2011.

OSBURN, S. C.; VANN, C. G.; CHURCH, D. D. *et al.* Proteasome- and Calpain-Mediated Proteolysis, but Not Autophagy, Is Required for Leucine-Induced Protein Synthesis in C2C12 Myotubes. **Physiologia**, 1, n. 1, p. 22-33, 2021.

PANDIT, A.; TRAN, T. B.; LETTON, M. *et al.* Data Informing Governing Body Resistance-Training Guidelines Exhibit Sex Bias: An Audit-Based Review. **Sports Med**, 53, n. 9, p. 1681-1691, Sep 2023.

PASIAKOS, S. M.; CARBONE, J. W. Assessment of skeletal muscle proteolysis and the regulatory response to nutrition and exercise. **IUBMB Life**, 66, n. 7, p. 478-484, Jul 2014.

PETRELLA, J. K.; KIM, J. S.; CROSS, J. M. *et al.* Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. **Am J Physiol Endocrinol Metab**, 291, n. 5, p. E937-946, Nov 2006.

PETRELLA, J. K.; KIM, J. S.; MAYHEW, D. L. *et al.* Potent myofiber hypertrophy during resistance training in humans is associated with satellite cell-mediated myonuclear addition: a cluster analysis. **J Appl Physiol**, 104, n. 6, p. 1736-1742, Jun 2008.

RAASTAD, T.; OWE, S. G.; PAULSEN, G. *et al.* Changes in calpain activity, muscle structure, and function after eccentric exercise. **Med Sci Sports Exerc**, 42, n. 1, p. 86-95, Jan 2010.

RAJ, D. S.; MOSELEY, P.; DOMINIC, E. A. *et al.* Interleukin-6 modulates hepatic and muscle protein synthesis during hemodialysis. **Kidney Int**, 73, n. 9, p. 1054-1061, May 2008.

RASBAND, W., 2011, **ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA**.

ROBERTS, M. D.; HAUN, C. T.; MOBLEY, C. B. *et al.* Physiological Differences Between Low Versus High Skeletal Muscle Hypertrophic Responders to Resistance Exercise Training: Current Perspectives and Future Research Directions. **Front Physiol**, 9, p. 834, 2018.

ROBERTS, M. D.; MCCARTHY, J. J.; HORNBERGER, T. A. *et al.* Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: current understanding and future directions. **Physiol Rev**, 103, n. 4, p. 2679-2757, Oct 1 2023.

RULLMAN, E.; NORRBOM, J.; STRÖMBERG, A. *et al.* Endurance exercise activates matrix metalloproteinases in human skeletal muscle. **J Appl Physiol**, 106, n. 3, p. 804-812, 2009.

RULLMAN, E.; RUNDQVIST, H.; WÅGSÄTER, D. *et al.* A single bout of exercise activates matrix metalloproteinase in human skeletal muscle. **J Appl Physiol**, 102, n. 6, p. 2346-2351, 2007.

SARTORI, R.; ROMANELLO, V.; SANDRI, M. Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. **Nat Commun**, 12, n. 1, p. 330, Jan 12 2021.

SCARPELLI, M. C.; NÓBREGA, S. R.; SANTANIELO, N. *et al.* Muscle Hypertrophy Response Is Affected by Previous Resistance Training Volume in Trained Individuals. **J Strength and Cond Res**, 36, n. 4, p. 1153-1157, 2022.

SCHNEIDER, C. A.; RASBAND, W. S.; ELICEIRI, K. W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, 9, n. 7, p. 671-675, 2012/07/01 2012.

SCHWANHÄUSSER, B.; BUSSE, D.; LI, N. *et al.* Global quantification of mammalian gene expression control. **Nature**, 473, n. 7347, p. 337-342, May 19 2011.

SMITH, M. A.; SEXTON, C. L.; SMITH, K. A. *et al.* Molecular predictors of resistance training outcomes in young untrained female adults. **J Appl Physiol (1985)**, 134, n. 3, p. 491-507, Mar 1 2023.

SRIKANTHAN, P.; KARLAMANGLA, A. S. Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the third National Health and Nutrition Examination Survey. **J Clin Endocrinol Metab**, 96, n. 9, p. 2898-2903, Sep 2011.

STEC, M. J.; KELLY, N. A.; MANY, G. M. *et al.* Ribosome biogenesis may augment resistance training-induced myofiber hypertrophy and is required for myotube growth in vitro. **Am J Physiol Endocrinol Metab**, 310, n. 8, p. E652-e661, Apr 15 2016.

STEFANETTI, R. J.; LAMON, S.; WALLACE, M. *et al.* Regulation of ubiquitin proteasome pathway molecular markers in response to endurance and resistance exercise and training. **Pflugers Arch**, 467, n. 7, p. 1523-1537, Jul 2015.

STUPKA, N.; TARNOPOLSKY, M. A.; YARDLEY, N. J. *et al.* Cellular adaptation to repeated eccentric exercise-induced muscle damage. **J Appl Physiol**, 91, n. 4, p. 1669-1678, Oct 2001.

SUNG, E.; HAN, A.; HINRICHS, T. *et al.* Effects of follicular versus luteal phase-based strength training in young women. **Springerplus**, 3, p. 668, 2014.

THOMPSON, B. M.; HILLEBRANDT, H. L.; SCULLEY, D. V. *et al.* The acute effect of the menstrual cycle and oral contraceptive cycle on measures of body composition. **Eur J Appl Physiol**, 121, n. 11, p. 3051-3059, Nov 2021.

WEN, Y.; MURACH, K. A.; VECHETTI, I. J., Jr. *et al.* MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry. **J Appl Physiol (1985)**, 124, n. 1, p. 40-51, Jan 1 2018.

WILLOUGHBY, D. S.; TAYLOR, M.; TAYLOR, L. Glucocorticoid receptor and ubiquitin expression after repeated eccentric exercise. **Med Sci Sports Exerc**, 35, n. 12, p. 2023-2031, Dec 2003.

WOLFE, R. R. The underappreciated role of muscle in health and disease. **Am J Clin Nutr**, 84, n. 3, p. 475-482, Sep 2006.

ZAMIR, O.; HASSELGREN, P.-O.; HIGASHIGUCHI, T. *et al.* Tumour necrosis factor (TNF) and interleukin-1 (IL-1) induce muscle proteolysis through different mechanisms. **Mediators of Inflammation**, 1, p. 802823, 1900/01/01 1992.

RELATÓRIO DE ATIVIDADES EXTRACURRICULARES

Artigos publicados

1. CHAVES, T. S.; **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; SILVA, D. G.; MEDALHA JUNIOR, R. A.; DIAS, N. F.; BITTERNCOURT, D.; CARELLO FILHO, P. C.; ANGLERI, V.; NÓBREGA, S. R.; ROBERTS, M. D.; UGRINOWITSCH, C.; LIBARDI, C. A. Effects of resistance training overload progression protocols on strength and muscle mass. *International Journal of Sports Medicine*, Jan 2024. https://doi.org/10.1055/a-2256-5857

2. NÓBREGA, S. R.; **SCARPELLI, Maíra C.**; BARCELOS, C.; CHAVES, T. S.; LIBARDI, C. A. Muscle hypertrophy is affected by volume load progression models. *Journal of Strength and Conditioning Research*, 37(1): 62–67, Jan 2023. https://doi.org/10.1519/JSC.0000000000004225

3. **SCARPELLI, Maíra C.**; NÓBREGA, S. R.; SANTANIELO, N.; ALVAREZ, I. F.; OTOBONI, G. B.; UGRINOWITSCH, C.; LIBARDI, C. A. Muscle hypertrophy response is affected by previous resistance training volume in trained individuals. *Journal of Strength and Conditioning Research*, 36(4): 1153–1157, Apr 2022. https://doi.org/10.1519/JSC.0000000000003558

4. **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; ARRUDA, E. A. B.; COOK, S. B.; LIBARDI, C. A. Resistance training with partial blood flow restriction in a 99-year-old individual: a case report. *Frontiers in Sports and Active Living*, 3–2021: 671–764, June 2021. https://doi.org/10.3389/fspor.2021.671764

5. SANTANIELO, N.; NÓBREGA, S., R.; **SCARPELLI, Maíra C.**; ALVAREZ, I. F.; OTOBONI, G. B.; PINTANEL, L.; LIBARDI, C. A. Effect of resistance training to muscle failure vs non-failure on strength, hypertrophy and muscle architecture in trained individuals. *Biology of Sport*, 37(4): 333–341, 2020. https://doi.org/10.5114/biolsport.2020.96317

Artigos submetidos para revisão

1. **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; GODWIN, J.; MESQUITA, P. H. C.; CHAVES, T. S.; SILVA, D. G.; BITTERNCOURT, D.; DIAS, N. F.; MEDALHA JUNIOR, R. A.; CARELLO FILHO, P. C.; ANGLERI, V.; COSTA, L.; KAVAZIS, A.; UGRINOWITSCH, C.; ROBERTS, M. D.; LIBARDI, C. A. Resistance training-induced changes in muscle proteolysis and extracellular matrix remodeling biomarkers in the untrained and trained states. Under review at *European Journal of Applied Physiology*.

2. **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; GODWIN, J.; MESQUITA, P. H. C.; CHAVES, T. S.; SILVA, D. G.; BITTERNCOURT, D.; DIAS, N. F.; MEDALHA JUNIOR,

R. A.; CARELLO FILHO, P. C.; ANGLERI, V.; COSTA, L.; KAVAZIS, A.; UGRINOWITSCH, C.; ROBERTS, M. D.; LIBARDI, C. A. Individual muscle hypertrophy is affected by the overload progression model and is associated with changes in satellite cell content. Under review at *Journal of Applied Physiology*.

3. BERGAMASCO, J. G. A.; **SCARPELLI, Maíra C.**;; GODWIN, J.; MESQUITA, P. H. C.; CHAVES, T. S.; SILVA, D. G.; BITTERNCOURT, D.; DIAS, N. F.; MEDALHA JUNIOR, R. A.; CARELLO FILHO, P. C.; ANGLERI, V.; COSTA, L.; MICHEL, M.; VECHIN, F. C.; KAVAZIS, A.; UGRINOWITSCH, C.; ROBERTS, M. D.; LIBARDI, C. A. Acute and chronic changes in muscle androgen receptor markers are not associated with myofiber hypertrophy in women and men. Under review at *Medicine & Science in Sports & Exercise*.

4. SILVA, D. G.; SILVA, D.; ANGLERI, V.; **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; NÓBREGA, S. R.; DAMAS, F.; CHAVES, T. S.; CAMARGO, H. A.; UGRINOWITSCH, C.; LIBARDI, C. A. Application of artificial intelligence to automate the reconstruction of muscle cross-sectional area obtained by ultrasound. Under review at *Medicine & Science in Sports & Exercise*.

5. DIAS, N. F.; BERGAMASCO, J. G. A.; **SCARPELLI, Maíra C.**; SILVA, D. G.; CHAVES, T. S.; BITTERNCOURT, D.; MEDALHA JUNIOR, R. A.; CARELLO FILHO, P. C.; DE SOUZA, E. O.; UGRINOWITSCH, C.; LIBARDI, C. A. Changes in muscle cross-sectional area during two menstrual cycles cannot be exclusively attributed to resistance training. Under review at *Applied Physiology, Nutrition, and Metabolism*.

Resumos publicados em anais de congressos

1. LIBARDI, C. A., CHAVES, T. S., **SCARPELLI, Maíra C.**, BERGAMASCO, J. G. A., SILVA, D. G., MEDALHA JÚNIOR, R. A., DIAS, N. F., CARELLO FILHO, P. C., BITTENCOURT, D., ANGLERI, V. Comparison between volume load progression models in resistance training on muscle hypertrophy: A randomized controlled trial, 2023, Paris - France. 28th Annual Congress of the European College of Sports Science (ECSS) – Book of Abstracts, 2023. v. 1. p. 721

2. SILVA, D. G. ; ANGLERI, V. ; **SCARPELLI, Maíra C.** ; NOBREGA, S. R. ; CHAVES, T. S. ; BERGAMASCO, J. G. A. ; UGRINOWITSCH, C. ; LIBARDI, C. A. Uso da inteligência artificial na reconstrução automatizada da área de secção transversa muscular a partir de imagens sequenciais de ultrassonografia, 2023, Londrina - PR. Anais do IX Congresso Brasileiro de Metabolismo, Nutrição e Exercício (CONBRAMENE), 2023. v. 1. p. 482-485.

3. DIAS, N. F. ; **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A. ; SILVA, D. G. ; CHAVES, T. S. ; MEDALHA JUNIOR, R. A. ; UGRINOWITSCH, C. ; LIBARDI, C. A. . Análise da área de secção transversa muscular durante as fases de dois ciclos menstruais, 2023, Londrina - PR. Anais do IX CONBRAMENE, 2023. v. 1. p. 317-317.

4. CHAVES, T. S. ; DIAS, N. F. ; **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A. ; SILVA, D. G. ; MEDALHA JUNIOR, R. A. ; CARELLO FILHO, P. C. ; LIBARDI, C. A. . Delineamento experimental intra-sujeito como abordagem para avaliar as verdadeiras respostas individuais na hipertrofia muscular induzida pelo treinamento de força: um estudo piloto, 2023, Londrina - PR. Anais do IX CONBRAMENE, 2023. v. 1. p. 364.

5. CAMARGO, J. B. B. ; CHAVES, T. S. ; **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A. ; SILVA, D. G. ; MEDALHA JUNIOR, R. A. ; CARELLO FILHO, P. C. ; LIBARDI, C. Efeitos de diferentes modelos de progressão do volume total na força muscular de jovens não-treinados: um estudo randomizado, intra-sujeito, 2023, Londrina - PR. Anais do IX CONBRAMENE, 2023. v. 1. p. 363.

6. **SCARPELLI, Maíra C.**; NOBREGA, S. R.; BARCELOS, C.; CHAVES, T. S.; LIBARDI, C. A. A hipertrofia muscular é afetada pela taxa de progressão do volume da carga de treinamento de força, 2021, Online. Anais do VIII CONBRAMENE, 2021. v. 1. p. 76.

7. NOBREGA, S. R.; **SCARPELLI, Maíra C.**; BARCELOS, C.; CHAVES, T. S.; LIBARDI, C. Indivíduos com maior progressão do volume da carga ganham mais força e área de secção transversa muscular, 2021, Online. Anais do VIII CONBRAMENE, 2021. v. 1. p. 78.

8. **SCARPELLI, Maíra C.**; BERGAMASCO, J. G. A.; ARRUDA, E. A. B.; LIBARDI, C. A. Estudo de Caso: Treinamento de Força com Restrição de Fluxo Sanguíneo em Nonagenário, 2019, João Pessoa - PB. Anais do II Simpósio Brasileiro de Ciências do Exercício e do Esporte, 2019.

Orientações

1. Francisco Sabará Belizário. Efeitos do treinamento aeróbico realizado em bicicleta sobre a hipertrofia muscular: uma revisão sistemática. 2021. Trabalho de Conclusão de Curso. (Graduação em Educação Física) - Universidade Federal de São Carlos.

2. Paulo César Carello Filho. Efeito do volume prévio de treinamento sobre os ganhos de força muscular de homens jovens treinados. 2021. Iniciação Científica. (Graduando em Educação Física) - Universidade Federal de São Carlos.

3. André Francisco Valsecki Veneroso e Erick Thesolin Pereira. O impacto do distanciamento social na qualidade de vida de praticantes de treinamento de força. 2020. Monografia. (Aperfeiçoamento/Especialização em Ciência do Treinamento de Força) - Universidade Federal de São Carlos.

4. Erick Thesolin Pereira. O impacto do distanciamento social na saúde mental e rotina de treino de praticantes de treinamento de força. 2020. Trabalho de Conclusão de Curso. (Graduação em Educação Física) - Universidade Federal de São Carlos.