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Efeito do treinamento de força com
restrição de fluxo sanguíneo na
reatividade da aorta abdominal em ratos
Wistar

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Nádia Fagundes Garcia

Tese

NÁDIA FAGUNDES GARCIA

EFEITO DO TREINAMENTO DE FORÇA COM RESTRIÇÃO DE FLUXO SANGUÍNEO
NA REATIVIDADE DA AORTA ABDOMINAL EM RATOS *WISTAR*

Versão corrigida

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Orientador: Prof. Dr. Enrico Fuini Puggina

Ribeirão Preto

2021

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GARCIA, Nádia Fagundes

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Aprovado em:

Banca Examinadora

Prof. Dr. _____

Instituição: _____

Julgamento: _____

Prof. Dr. _____

Instituição: _____

Julgamento: _____

Prof. Dr. _____

Instituição: _____

Julgamento: _____

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DEDICATÓRIA

“À minha mãe, por muitas vezes iluminar meus pensamentos, ser minha conselheira e minha melhor amiga.

Ao meu pai, meu primeiro exemplo de professor, de caráter justiça e honestidade.

Ao meu irmão, meu parceiro da vida, que é o maior presente que meus pais puderam me dar.

Minha família, vocês são minha maior força, minha base e o meu porto seguro.

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Por fim, espero ter contribuído para a área da Educação Física, profissão que escolhi seguir, por muito gosto. Durante todo o percurso de estudo, busquei trazer informações relevantes para que a leitura desse trabalho seja agradável a vocês, leitores.

RESUMO

GARCIA, Nádia Fagundes. **Efeito do treinamento de força com restrição de fluxo sanguíneo na reatividade da aorta abdominal em ratos *Wistar***. 2021. 86 p. Tese (Doutorado em Reabilitação e Desempenho Funcional) – Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2021.

O treinamento de força (ST) com restrição do fluxo sanguíneo (BFR) é conhecido por promover aumentos na hipertrofia e na força, às vezes semelhante ao ST tradicional, apesar dos efeitos do BFR arterial nas adaptações musculares não estarem bem estabelecidos. No entanto, as sessões de ST de alta intensidade (acima de 70% de 1 contração voluntária máxima – CVM) podem trazer complicações (respostas cardiovasculares exacerbadas na função vascular, no remodelamento estrutural e também pode levar a complicações ortopédicas, por exemplo) em determinadas populações, como idosos ou pessoas com condições especiais de saúde tornando-se, portanto, inviável. Como alternativa, estudos indicam que o treinamento de força com restrição de fluxo sanguíneo com baixa intensidade de carga (20-50% de 1 CVM), pode produzir adaptações positivas semelhantes ao ST com intensidade igual ou superior a 70 % 1 CVM. Entretanto, poucos são os estudos que investigaram seus efeitos na função vascular. Além disso, há muita divergência na metodologia utilizada em estudos com treinamento de força com restrição do fluxo sanguíneo em humanos e, conseqüentemente, encontramos estudos com diferentes conclusões. Dessa forma, apesar das evidências sugerirem que o treinamento de força com restrição do fluxo sanguíneo (STBFR) é seguro, ainda carecem de maiores investigações, principalmente com relação a saúde vascular. Devido a essas dificuldades, optamos por um protocolo de treinamento com BFR arterial em modelo animal. Para tanto, dois estudos foram propostos para atender aos seguintes objetivos; avaliar se o STBFR é capaz de melhorar as adaptações musculares, o desempenho e a segurança da realização deste método em ratos *Wistar* (manuscrito 1), e, avaliar os efeitos do STBFR sobre a função vascular destes animais (manuscrito 2). O protocolo experimental contou com a utilização de ratos *Wistar* Hannover com 8 semanas de idade, divididos em quatro grupos: sedentários sham (S/S), sedentários com BFR arterial (S/BFR), treinados sham (T/S) e treinados com BFR arterial (T/BFR), sendo que os grupos com BFR foram submetidos a cirurgia arteriovenosa para restrição de fluxo sanguíneo da artéria femoral. O protocolo de treinamento consistiu em quatro semanas de ST composto por 6 séries de 10 subidas de escadas com 50% de 1 CVM. No início e ao final do protocolo foram obtidos dados referentes ao peso corporal, gordura epididimal, peso máximo carregado, força de preensão manual, índice de hipertrofia muscular, pressão arterial sistólica, atividade enzimática da superóxido dismutase, concentração de nitrito/nitrato e fator de necrose tumoral alfa (manuscrito 1). Curvas de concentração-resposta na aorta abdominal à acetilcolina (ACh: 10nM - 100µM) e fenilefrina (PHE: 1nM - 30µM), produção de óxido nítrico (NO) e espécies reativas de oxigênio (ROS), remodelamento vascular (matriz de metaloproteinase – 2) e atividade gelatinolítica (zimografia) (manuscrito 2). A normalidade e a homogeneidade foram verificadas pelos testes de Shapiro-Wilk e Levene, respectivamente. A estatística utilizada foi a ANOVA two-way, seguida de um pós-teste de Bonferroni. Valores de $p < 0,05$ usando programa GraphPad Prism. O grupo T/BFR apresentou aumento da força e hipertrofia muscular. Por outro lado, o T/S foi eficaz para incremento da força, porém sem efeito para a hipertrofia. Além disso, não foram encontradas alterações cardíacas e metabólicas significantes (manuscrito 1). Neste contexto, entende-se que o protocolo de treinamento foi eficaz, pois aumentou a força dos animais T/S e T/BFR nos testes de peso

máximo carregado. Os animais do grupo S/S apresentaram relaxamento reduzido na ACh (Emax 22%) comparado ao S/BFR e o treinamento de força preveniu a redução do relaxamento no grupo T/BFR. Para o grupo T/S, não foi encontrada melhora do relaxamento quando comparado aos pares sedentários. A resposta máxima ao agente alfa-agonista foi aumentada em 80% em animais S/BFR. O treinamento físico não alterou a resposta máxima. Por outro lado, os animais dos grupos T/S e T/BFR apresentaram redução de 4 a 4,5 vezes, respectivamente, na sensibilidade à fenilefrina e nos animais do grupo S/BFR, essa redução foi de 6 vezes. Ainda assim, o grupo T/S apresentou aumento na produção de NO e tanto T/S quanto T/BFR apresentaram redução da produção *in situ* de ROS (62% e 40% respectivamente). Além disso, ambos os grupos treinados apresentaram redução da atividade gelatinolítica da MMP-2 na aorta (manuscrito 2). Em conclusão, o T/BFR é capaz de melhorar as adaptações musculares e o desempenho em ratos, sem causar danos cardiovasculares e metabólicos. Esses resultados são inéditos, promissores e sugerem que, além do treinamento de força, o BFR deve ser considerada como uma alternativa de intervenção clínica tanto para o desempenho físico quanto para a melhora da saúde endotelial.

Palavras-chave: 1. Treinamento de força. 2. Restrição do fluxo sanguíneo. 3. Hipertrofia. 4. Desempenho. 5. Aorta. 6. Ratos.

ABSTRACT

GARCIA, Nádia Fagundes. **Effect of strength training with restriction blood flow on reactivity of the abdominal aorta in *Wistar* rats**. 2021. 86 p. Thesis (Doctr's in Rehabilitation and Functional Performance) – Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, 2021.

Strength training (ST) with blood flow restriction (BFR) promotes increases in hypertrophy and strength, sometimes similar to traditional ST. However, the effects of arterial BFR on muscle adaptations are not well up-to-date. However, the consequences of high-intensity ST (above 70% of 1 maximum voluntary contraction - MVC) can bring complications (exaggerated cardiovascular responses in vascular function, structural remodeling and can also lead to orthopedic complications, for example) in certain prev, such as the elderly or people with special health conditions, making it unfeasible. Alternatively, studies indicate that strength training with blood flow restriction with low load intensity (20-50% of 1 MVC) can produce cheerful, positive adaptations to the ST with intensity or equal to 70% 1 MVC. However, few studies have investigated its effects on vascular function. Furthermore, there is a lot of divergence in the methodology applied in studies with strength training with blood flow restriction in humans, and, consequently, we found studies with different means. Thus, despite the evidence suggesting that strength training with restricted blood flow (STBFR) is safe, further investigation is still needed, especially vascular health. Due to these difficulties, we opted for a training protocol with arterial BFR in an animal model. Therefore, two studies were proposed to meet the following objectives; to evaluate whether STBFR can improve muscle adaptations, performance, and safety of performing this method in *Wistar* rats (manuscript 1) and to evaluate the effects of STBFR on the vascular function of these animals (manuscript 2). The experimental protocol included the use of 8-week-old *Wistar* Hannover rats, divided into four groups: sham sedentary (S/S), sedentary with arterial BFR (S/BFR), sham trained (T/S) and trained with arterial BFR (T/BFR), and the groups with BFR underwent arteriovenous surgery to restrict the blood flow of the femoral artery. The training protocol consisted of four weeks of ST consisting of 6 sets of 10 stair climbs with 50% of 1 MVC. At the beginning and the end of the protocol, data on body weight, epididymal fat, maximum weight carried, handgrip strength, muscle hypertrophy index, systolic blood pressure, superoxide dismutase enzymatic activity, nitrite/nitrate concentration, and factor, alpha tumor necrosis (manuscript 1). Concentration-response curves in the abdominal aorta to acetylcholine (ACh: 10nM - 100µM) and phenylephrine (PHE: 1nM - 30µM), nitric oxide (NO) production and reactive oxygen species (ROS), vascular remodeling (metalloproteinase matrix-2) and gelatinolytic activity (zymography) (manuscript 2). Normality and homogeneity were verified by the Shapiro-Wilk and Levene tests, respectively. The statistic used was the two-way ANOVA, followed by a Bonferroni post-test. P-values < 0.05 using GraphPad Prism program. The T/BFR group showed increased strength and muscle hypertrophy. On the other hand, T/S was effective in increasing strength but with no effect on hypertrophy. Furthermore, no significant cardiac and metabolic alterations were found (manuscript 1). In this context, it is understood that the training protocol was effective, as it increased the strength of the T/S and T/BFR animals in the maximum loaded weight tests. The animals in the S/S group reduced dissipation in ACh (Emax 22%) compared to the S/BFR, and strength training prevented reducing relaxation in the T/BFR group. For the T/S group, no improvement in relaxation was found when compared to the sedentary pairs. The

maximal response to the alpha-agonist agent was increased by 80% in S/BFR animals. Physical training didn't change the maximal response. Still, animals in the T/S and T/BFR groups reduced by 4 to 4.5 times, respectively, insensitivity to PHE, and in the S/BFR group, this reduction was six times. The T/S group showed an increase in NO production, and both T/S and T/BFR decreased in situ ROS production (62% and 40%, respectively). Furthermore, both groups trained reduced the gelatinolytic activity of MMP-2 in the aorta (manuscript 2). In conclusion, T/BFR can improve muscle adaptations and performance in rats without causing cardiovascular and metabolic damage. These results are unprecedented, promising, and improvement that, in addition to strength training, the BFR should be considered an alternative clinical intervention for both physical performance to improve endothelial health.

Keywords: 1. Strength training. 2. Blood flow restriction. 3. Hypertrophy. 4. Performance. 5. Aorta. 6. Rats.

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LISTA DE ABREVIATURAS E SIGLAS

ACh	Acetilcolina
AOP	Arterial occlusion pressure
APJ	Apelin receptor
AT2	Angiotensina 2
ATP	Adenosina Trifosfato
ATR1/ATR2	Ratio of angiotensin II receptors
BFR	Blood flow restriction
BP	Blood pressure
BW	Body weight
CEUA	Ethics Committee on Animal Use in Research
CVM	Contração voluntária máxima
DAF/FM-DA	Determination of intracellular nitric oxide
DCV	Doenças cardiovasculares
DHE	Marker of reactive oxygen species production
DP	Duplo produto
EC50	–Log da concentração do agonista necessária para produzir 50% da resposta máxima
EEFERP	Escola de Educação Física e Esporte de Ribeirão Preto
E _{max}	Resposta máxima eNOS
FC	Frequência cardíaca
FDL	Flexor digitorum deltoid
FDP	Flexor digitorum profunds
FHL	Flexor hallucis longus
IL-6	Interleucina 6
iNOS	Óxido nítrico sintase induzível
LDL -/-	Camundongo knockout para o receptor de LDL
MGS	Manual grip strenght test
MLW	Maximum loaded weight test
mN/mm:	Milnewton por milímetr
MMP-2	Matrix de metaloproteinases 2
mTORC1	Mechanistic target of rapamycin complex 1
MVC	Maximal voluntary contraction

NO	Óxido nítrico
PA	Pressão arterial
PAS	Pressão arterial sistólica
PCr	Creatina fosfato
PHE	Fenilefrina
ROS	Espécie reativa de oxigênio
S/BFR	Sedentário com restrição do fluxo sanguíneo
S/S	Sedentário sham
SBP	Sistolic blood pressure
SOD	Superóxido dismutase
SOD-1	Superóxido dismutase 1
ST	Streght training
T/BFR	Treinado com restrição do fluxo sanguíneo
T/S	Treinado sham
TCC	Trabalho de conclusão de curso
TF	Treinamento de força
TFRF	Treinamento de força com restrição do fluxo sanguíneo
TNF- α	Fator de necrose tumoral alfa

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CAPÍTULO 1 – APRESENTAÇÃO DO DOCUMENTO

1. APRESENTAÇÃO DO DOCUMENTO

A presente tese foi redigida como requisito para a obtenção do título de Doutora em Ciências pelo Programa de Pós-Graduação em Reabilitação e Desempenho Funcional/FMRP-USP. O texto foi dividido em cinco capítulos para facilitar o entendimento do leitor sobre a trajetória de pesquisa e os capítulos estão sob forma de *hiperlinks*, assim o leitor pode clicá-lo e ir direto a parte do texto correspondente ao que foi mencionado. O **Capítulo 1** compreende uma breve apresentação de como foi elaborado o documento, em um esforço de deixar sua leitura mais didática e agradável. Ainda, nesse capítulo foi apresentada a temática da tese e as principais atividades desenvolvidas pela autora ao longo de sua trajetória acadêmica.

O **Capítulo 2** aborda a contextualização e os objetivos dos dois manuscritos, frutos desta tese. O primeiro manuscrito, (**Capítulo 3**), aborda as adaptações musculares e o desempenho nos testes de força de ratos submetidos ao treinamento com ou sem restrição do fluxo sanguíneo e a sua segurança. O manuscrito em questão foi aceito para publicação no periódico *Annals of the Brazilian Academy of Sciences* (anexo I). O segundo manuscrito está apresentado no **Capítulo 4**, no qual foram avaliadas as adaptações vasculares (reatividade vascular) em ratos, a concentração de óxido nítrico e espécies reativas de oxigênio e o marcador do remodelamento vascular a fim de avaliar a saúde endotelial. O manuscrito em questão foi submetido na *Life Science* (anexo II). Por fim, as considerações finais e a conclusão geral do trabalho estão apresentadas no **Capítulo 5**.

É importante ressaltar que o desenvolvimento desses dois manuscritos fez parte do projeto de auxílio regular do laboratório do Prof. Dr. Enrico Fuini Puggina (Processo FAPESP nº (#2017/13348-6), intitulado: “Efeito do treinamento de força, com restrição de fluxo sanguíneo, na reatividade da aorta abdominal em ratos *Wistar*”).

Este trabalho foi confeccionado no modelo “escandinavo”, no qual o conhecimento produzido é apresentado no formato de artigos já aprovados ou aptos para a submissão em periódicos. Modelo de texto este que enfatiza a objetividade, porém, sem prejuízo das informações sobre as atividades complementares desenvolvidas durante o curso. No próximo tópico, apresento informações que estão além dos manuscritos, mas que foram essenciais para a construção da minha formação.

1.1 Ingresso no curso e elaboração da temática

Ainda na graduação, em 2011, iniciei minha participação no Grupo de Estudos e Pesquisa em Exercício Físico para Condições especiais de Saúde coordenado pela Profa. Dra. Camila de Moraes. Posteriormente, iniciamos um projeto com apoio financeiro da CAPES (bolsa iniciação científica) e FAPESP (projeto regular). A decisão em integrar o grupo da professora Camila influenciou toda minha trajetória acadêmica, profissional e pessoal de uma forma transformadora. Durante toda a graduação, participei ativamente das pesquisas desenvolvidas pelo nosso grupo que culminaram no meu TCC. Em 2013, concluí minha graduação na Escola de Educação Física e Esporte (EEFERP) da Universidade de São Paulo, na cidade de Ribeirão Preto. Logo no início de 2014, por indicação da professora Camila, ingressei no mestrado da Universidade Estadual Paulista (UNESP), na cidade de Rio Claro, sob orientação da Profa. Dra. Angelina ZanESCO e coorientação da Profa. Dra. Camila de Moraes.

Em decorrência, me mudei para Rio Claro onde morei durante três meses fazendo parte da rotina do laboratório da professora Angelina. Entretanto, meu projeto de mestrado era com animais e o laboratório só trabalhava com humanos e por isso, fechamos uma parceria com a Profa. Dra. Maria Andréia Delbin e me mudei novamente, mas agora para a cidade de Campinas, onde desenvolvi todo o meu projeto intitulado “Efeito do treinamento físico aeróbico na reatividade vascular da artéria ilíaca em camundongos LDLr-/-”, no Instituto de Biologia da UNICAMP. Foi uma fase de grandes aprendizados, desafios e tive a oportunidade de conhecer e trabalhar com pessoas de todas as áreas. Aprendi como treinar e sacrificar camundongos, coletar sangue por punção cardíaca, a fazer o experimento da reatividade vascular que, por sinal, é muito complexo, dentre outros métodos laboratoriais fundamentais para minha pesquisa.

Desde então, passei a fazer parte da rotina do laboratório sob supervisão da professora Maria Andréia. Em 2016, me tornei mestre em “Ciências da Motricidade” e quis voltar para Ribeirão Preto, para tentar o ingresso no doutorado. Com mais uma indicação da professora Camila, em conversa com o Prof. Dr. Enrico Fuini Puggina, surgiu-nos a ideia de pesquisar sobre o treinamento de força com restrição do fluxo sanguíneo e suas adaptações cardiovasculares. Foi então que escrevi o projeto intitulado “Efeito do treinamento de força com restrição de fluxo sanguíneo na reatividade da aorta abdominal em ratos *Wistar*”.

Mesmo com todos nossos esforços, enfrentamos uma infinidade de problemas desde o início da execução deste projeto, como por exemplo: o atraso na entrega da importação de

equipamentos fundamentais para a pesquisa, a adulteração da ração dos ratos pela empresa fornecedora e a troca da linhagem de ratos. Esses dois últimos resultaram na morte de muitos animais, não só os que já estavam em nosso laboratório, mas também de todo o campus da USP de Ribeirão Preto, e então houve suspensão do seu fornecimento por um período de aproximadamente 90 dias. Com a troca da linhagem de ratos, ainda nos surgiu um outro problema: o anestésico antes utilizado para a cirurgia dos animais (ketamina e xilazina), passou a causar a sua morte. Foram inúmeras tentativas para que conseguíssemos ajustar novamente a dose, voltamos a pesquisar em artigos científicos, buscamos informações em outros laboratórios, até que nos aconselharam a trocar o antigo anestésico utilizado para o Tribromoetanol e, finalmente, conseguimos ajustar a sua dose e prosseguirmos com a cirurgia dos animais. Ainda em março de 2019, os laboratórios que tínhamos parceria na realização de alguns experimentos ficaram fechados por consequência da pandemia da COVID-19. Todas essas adversidades resultaram em um atraso considerável da finalização deste projeto e, consequentemente, da publicação de artigos. Ainda assim conseguimos finalizar conforme a nossa proposta original. Em todas essas adversidades, contei com o apoio dos meus amigos Macário e Fábio e dos meus professores Enrico e Camila, presentes a todo momento, me dando a força e o suporte necessário para que essa tese pudesse ser finalizada. Apesar de muitas vezes ter me sentido desmotivada e insatisfeita com meu rendimento acadêmico, desistir nunca esteve em cogitação.

No início do doutorado, eu planejava participar de congressos fora do país e, talvez, até um doutorado sanduíche. Essa cobrança pessoal me gerou uma certa frustração porque, infelizmente, não foi possível e isso impactou negativamente no meu currículo Lattes, visto que, em concursos públicos para universidades, a pontuação aumenta de acordo com a participação em eventos (principalmente fora do país) e artigos publicados. Além disso, há uma enorme troca de ideias, informações e experiência que acontece quando se vai para o exterior ajudando a aprimorar a maturidade acadêmica dos estudantes.

Ademais, nós estudantes de pós graduação e professores, sabemos da dificuldade que é finalizar uma dissertação e/ou tese. E, a realidade é que quanto mais você estuda, mais você percebe o quanto de informação ainda existe para aprender; o fluxo é gigantesco, todo dia há uma publicação nova, sem contar os outros assuntos fora da sua área de pesquisa. São prazos e cobranças para entregas de projetos e artigos e paralelo a isso, a ansiedade em conseguir boas publicações, bolsas de estudo, aprovações nas bancas de qualificação e defesa e, claro, um emprego digno quando a pós graduação for finalizada.

Em contrapartida, acredito que o maior aprendizado de todos esses anos de doutorado também se estende a minha vida pessoal, pois foram os anos de maiores mudanças e, ao mesmo tempo, de tentar manter o controle emocional em todos os setores. Ao longo do projeto, tivemos que mudar algumas estratégias, nos adaptar, trocar ideias e informações constantemente e aprender a ter muita paciência quando nada parecia dar certo. Pude ter, ainda, mais certeza do quanto caminhar junto é fundamental. Ter amigos, professores e familiares presentes, ajudam a tornar o caminho mais leve, quando a tempestade está formada. Viver um dia de cada vez também, sem querer “abraçar o mundo”, com descobertas inovadoras, em um curto espaço de tempo pode ajudar a controlar a ansiedade e a manter a saúde mental em dia. Por fim, esperamos que com essa pesquisa, possamos contribuir com esse mundo ainda a ser descoberto sobre o treinamento de força com restrição do fluxo sanguíneo e suas adaptações cardiovasculares.

1.2 Atividades desenvolvidas

Logo que ingressei no doutorado, em Maio de 2017, participei do desenvolvimento dos projetos de mestrado dos alunos Macário Arosti Rebelo intitulado “Efeitos do treinamento físico contínuo ou intervalado sobre a função vascular e cognitiva em ratos adultos alimentados com frutose” e José Mallone Gonçalves Pedroso, intitulado “Efeito do treinamento físico aeróbico contínuo e intervalado na reatividade vascular da artéria aorta de ratos Sprague Dawley”, ambos sob orientação da Profa. Dra. Camila de Moraes. Além disso, ajudei na elaboração, correção e desenvolvimento do TCC da aluna Savannah Maria Gertrudis Rosa Peters, intitulado “Efeito do treinamento de força com oclusão vascular na performance de ratos *Wistar* Hannover” e do TCC da aluna Amanda Ehmke Senna e Silva, intitulado “Efeitos agudos de uma sessão de HIIT nos indicadores de dano muscular, inflamação e capacidade de recuperação de indivíduos em diferentes estados de treinamento”, ambos sob orientação do Prof. Dr. Enrico Fuini Puggina.

Participo ativamente das reuniões do Grupo de Estudos em Desempenho Físico e Treinamento Esportivo, coordenado pelo Prof. Dr. Enrico Fuini Puggina, na Escola de Educação Física e Esporte de Ribeirão Preto (EEFERP/USP) e do Grupo de estudo e pesquisa em exercício físico para condições especiais e saúde, cuja coordenadora é a Profa. Dra. Camila de Moraes, também da Escola de Educação Física e Esporte de Ribeirão Preto (EEFERP/USP).

Alguns cursos complementares também foram realizados neste período:

- 8º Simpósio sobre Ética no uso de animais. Ribeirão Preto/SP, nos dias 6 e 7 de novembro de 2017 (16 horas de atividades);
- Curso de Extensão Universitária na Modalidade de Difusão: Biologia Molecular aplicada ao exercício físico. Ribeirão Preto/SP, do dia 09/08/2017 a 27/09/2017 (8 horas de atividades);
- Palestra “Função Vascular e Envelhecimento Arterial Precoce. Ribeirão Preto/SP, no dia 09 de maio de 2018 (2 horas de atividades);
- Curso “Escrita Científica”. Ribeirão Preto/SP, no dia 08 de maio de 2018 (8 horas de atividades).
- Participação e apresentação do trabalho de minha autoria “STRENGTH TRAINING WITH RESTRICTED BLOOD FLOW DECREASES OXIDATIVE STRESS IN RATS” no 55º Congresso Anual SBFIS OnLine | Encontro Luso Brasileiro de Fisiologia | Symposium Miguel Covian | Latin American DOHaD Chapter;
- Participação e apresentação do trabalho de minha autoria “Treinamento de força com restrição do fluxo sanguíneo, não prejudica o remodelamento vascular em ratos *Wistar*”, no XXV Simpósio Brasileiro de Fisiologia Cardiovascular”.

Com relação às produções técnicas, fui avaliadora do “26º Simpósio Internacional de Iniciação Científica e Tecnológica da USP – SIICUSP”; do pôster de TCC do trabalho intitulado “Efeito agudo de uma sessão de HIIT nos indicadores de dano muscular e inflamação e capacidade de recuperação de indivíduos em diferentes estados de treinamento” e do pôster de TCC do trabalho intitulado “Efeito do exercício físico moderado sobre a função vascular de ratos adultos alimentados com frutose”, todos na EEFERP, entre os anos de 2017 e 2018. Em 2021, fui revisora do artigo intitulado “Effect of low-intensity resistance training with blood flow restriction and detraining on psychometric variables in older adult women” pela revista MOTRIZ.

No ano de 2017, o Prof. Dr. Enrico Fuini Puggina foi contemplado com auxílio de projeto regular pela FAPESP (#2017/13348-6) o que permitiu o início do meu projeto de doutorado, bem como o aprofundamento das técnicas inicialmente propostas no estudo. Durante esta trajetória, até o momento, conseguimos escrever cinco manuscritos:

1. EHMKE, A; BINHARDI, P. M; AQUINO, R. L. Q. T; **GARCIA, N. F**; PUGGINA, E. F. Acute effects of a hiit protocol on performance, muscle damage and inflammation indicators, and the ability of recovery of individuals in different states of

- training. *Journal of Sports Medicine and Physical Fitness*. Em processo de submissão. A2
2. PEDROSO, J.M.G; AROSTI, M.R; **GARCIA, N.F**; DE MORAES. Effects of Aerobic Continuous and Interval Training on Vascular Responsiveness Aorta Artery of Sprague Dawley Rats. *Revista Portuguesa de Cardiologia*. Submetido. B2
 3. REBELO, M.A; **GARCIA, N.F**; PUGGINA, E.F; DE MORAES, C. Resistance training with or without blood flow restriction improves short term memory in rats. *Psychoneuroendocrinology*. Em processo de submissão. A1
 4. **Nádia Fagundes Garcia**, Camila de Moraes, Macário Arosti Rebelo, Savanah Maria Gertrudis Rosa Peters, Fábio Marzliak Pozzi de Castro, Enrico Fuini Puggina. Strength training with and without arteriovenous blood flow restriction improves performance, regardless of changes in muscle hypertrophy, in *Wistar* rats. *Annals of the Brazilian Academy of Sciences*. Artigo aceito para publicação - (**Manuscrito 1**); B1
 5. **Nádia Fagundes Garcia**, Camila de Moraes, Macário Arosti Rebelo; Fábio Marzliak Pozzi de Castro; Savanah Maria Gertrudis Rosa Peters, Laena Pernomian; Marcela Maria Blascke de Mello; Michele Mazzaron de Castro; Enrico Fuini Puggina. Low load intensity strength training, associated or without blood flow restriction, increases nitric oxide bioavailability and reduces MMP-2 activity in rat aorta. *Life Sciences*. Artigo submetido (aguardando revisão) - (**Manuscrito 2**); A1

No dia 12 de julho de 2019, no auditório da EEFERP-USP, realizei meu exame de qualificação com a presença dos Professores Doutores Camila de Moraes e Rinaldo Roberto de Jesus Guirro. Após essa importante etapa, as considerações levantadas pela banca auxiliaram no olhar sobre nossos resultados e a abordagem experimental conduzida. Ainda, cursei o Estágio Supervisionado em Docência do Programa de Aperfeiçoamento de Ensino (PAE) na disciplina de REF0079-3 - Exercício Físico em Condições Especiais de Saúde I -, na EEFERP-USP, ministrada pela Profa. Dra. Camila de Moraes. Durante meu estágio, auxiliei na no andamento da disciplina e ofereci apoio aos discentes.

Com relação ao meu histórico escolar, cumpri 22 créditos referentes à seis disciplinas nas quais obtive nota máxima (conceito “A”), exceto em uma delas em que o conceito foi B. Ressalto que além das quatro disciplinas obrigatórias do nosso Programa, participei de disciplinas “optativas” que contribuíram para minha formação, principalmente na atuação prática da minha profissão como a “Aspectos Metodológicos e Científicos do Treinamento Físico” e “Suplementos Alimentares no Exercício Físico”, ambas cursadas na EEFERP-USP.

CAPÍTULO 2 – CONTEXTUALIZAÇÃO

2. CONTEXTUALIZAÇÃO

Os exercícios de força são fundamentais para prevenção e/ou tratamento não farmacológico de distúrbios metabólicos e controle de massa corporal. Seus efeitos protetores são observados em indivíduos com diabetes mellitus, dislipidemias, doenças cardiovasculares, doenças neurodegenerativas dentre outras e, consequentemente, impactam na melhora da qualidade de vida e na diminuição dos índices de morbidade e mortalidade (CORREA et al. 2016; GRØNFELDT et al. 2020). Contudo, para que grandes adaptações morfológicas e funcionais aconteçam, é necessário que a intensidade do exercício seja equivalente ou maior que 70% de uma contração voluntária máxima (CVM). Entretanto, sabe-se que a realização de sessões de treinamento com carga elevada, maior do que 70% de 1CVM, podem provocar respostas cardiovasculares exacerbadas, como, por exemplo, na frequência cardíaca (FC), na pressão arterial sistólica (PAS) - consequentemente no duplo produto (DP) - na função vascular e no remodelamento estrutural (GHORAYEB et al. 2019) e também pode ser impeditiva pra pacientes com complicações ortopédicas (FERRAZ et al. (2017).

Dessa forma, o treinamento de força com cargas elevadas pode ser inviável para determinadas populações com condições especiais de saúde como idosos, pacientes com DCV, pacientes com complicações ortopédicas (ZANETT et al., 2013; SBC 2013; MACEDO et al., 2016). Em contrapartida, nos anos 60, surgiu no Japão um método de treinamento alternativo chamado de treinamento de força com restrição do fluxo sanguíneo (TFRF) (ou oclusão vascular) ou ainda Kaatsu training, que nos últimos anos vem ganhando cada vez mais espaço nas academias e clubes (TEIXEIRA, 2018). A vantagem desse método de treinamento é que exercícios de força com restrição do fluxo sanguíneo realizados em intensidade relativamente baixa (20% - 50% de 1CVM), podem promover adaptações similares ao treinamento de força tradicional com intensidade elevadas (LOENNEKE et al., 2012).

Vários estudos têm demonstrado que o treinamento de força em baixa intensidade (cargas entre 20-50% 1CVM) se realizados com restrição do fluxo sanguíneo, no membro exercitado, levam a hipertrofia muscular semelhante ao treinamento de força tradicional realizado com cargas equivalentes ou maiores do que 70% de 1CVM (TAKARADA et al., 2004; LOENNEKE, 2010; LOENNEKE et al., 2011). Além disso, Rodrigues Neto et al. (2017) em uma recente revisão sistemática, observaram que as elevações agudas da FC, PA e DP durante o TFRF são significativamente menores das que observadas durante o treinamento de força (TF) com cargas elevadas e, no geral, quando prescrito da maneira correta, o TFRF

não prejudica, de forma aguda ou crônica a função vascular e ainda pode promover aumento agudo da angiogênese (HORIUCHI; OKITA, 2012; RODRIGUES NETO et al., 2017).

Os mecanismos adaptativos envolvidos nas respostas ao TFRF ainda carecem de maiores investigações, no entanto, evidências recentes apontam que o estresse metabólico pode ser o gatilho para as adaptações uma vez que a região muscular ocluída sofre hipóxia, ocasionando em maior acúmulo local de metabólitos durante este tipo de treinamento (TANIMOTO et al., 2005; FRY et al., 2010; DE CASTRO, et al. 2017). Este acúmulo gera maior taxa de hidrólise de trifosfato de adenosina (ATP), depleção aumentada de creatina fosfato (PCr) (SUGA et al., 2012), diminuição do pH intramuscular (SUGA et al., 2009), aumento da concentração de lactato (TAKARADA et al., 2000; TAKANO et al., 2005; FUJITA et al., 2007) e de hormônios anabólicos, sistemicamente (TAKARADA et al., 2004; ABE et al., 2005; PIERCE et al., 2006; FRY 2010). Além dos mecanismos citados acima, o TFRF é capaz de inibir alguns mecanismos negativos da hipertrofia muscular. Kawada e Ishii (2005) observaram os efeitos crônicos de uma cirurgia de restrição do fluxo sanguíneo em ratos sobre a expressão de miostatina, uma proteína envolvida em vias de inibição do crescimento celular, e constataram que esse procedimento cirúrgico reduziu significativamente a expressão de miostatina, o que foi correlacionado com o aumento da massa magra dos animais.

Por sua vez, a diminuição da concentração de miostatina parece auxiliar na regulação da via mTORC1. Sendo assim, essa diminuição observada permitiria uma maior ativação da via mTORC1 e assim aumento da massa magra em indivíduos submetidos a esse método de treinamento. O estudo de Gundermann et al (2014) corrobora com esse resultado, no estudo em que jovens saudáveis foram submetidos ao treinamento de força com restrição do fluxo sanguíneo (4 séries de cadeira extensora a 20% de 1CVM) e divididos em grupos controle e rapamicina (inibidora da mTORC1). Ao final do estudo, comprovou-se maior aumento da taxa de síntese proteica, da fosforilação da mTOR (Ser2448) e S6K1 (Thr389) no vasto lateral do grupo controle, quando comparado ao rapamicina. Diante de tudo isso concluiu-se que a ativação da mTORC1 e a síntese de proteína no músculo após o exercício com BFR é inibido na presença de rapamicina.

O estresse de cisalhamento induzido pelo exercício físico é um dos mecanismos responsáveis pelo aumento da produção e da biodisponibilidade de óxido nítrico, pois a hiperemia provocada pelo exercício está relacionada com o aumento da pressão de pulso e da pulsatilidade. Com relação à produção de NO, estudos em humanos mostram que o TFRF parece aumentar a produção e biodisponibilidade de NO causando vasodilatação. Hunt et al.

(2012), demonstraram que quatro semanas de treinamento com *handgrip* aumentou o diâmetro da artéria braquial em resposta à vasodilatação mediada pelo fluxo em homens que realizaram o treino com restrição do fluxo sanguíneo, mas não em homens que não tiveram o membro exercitado ocluído. Os autores sugerem a importância de se realizar estudos com o músculo liso e a função endotelial em organismos expostos ao TFRF.

Em modelo animal não foram encontrados, até o momento, estudos que utilizaram treinamento de força realizado com restrição do fluxo sanguíneo e avaliaram a reatividade vascular e, portanto, ainda não estão esclarecidas (HUNT et al., 2012), assim como os mecanismos envolvidos nesse fenômeno. Apesar da limitação no que tange a extrapolação dos resultados observados em modelo animal para humanos, faz-se necessária a realização de protocolos experimentais em animais, para que se possa avaliar de maneira ampla as possíveis alterações nos parâmetros vasculares e os mecanismos envolvidos. Por isso, a vantagem do uso de modelo animal, nesse caso, pode superar as limitações e proporcionar a investigação de forma mais complexa, pois é de grande vantagem para elucidar os mecanismos subcelulares envolvidos nas possíveis alterações da função vascular (KAWADA, ISHII, 2005).

Dessa forma, o objetivo deste estudo é avaliar os efeitos da realização do treinamento de força com restrição do fluxo sanguíneo, com carga de 50% de 1CVM, sobre a reatividade vascular da aorta abdominal de ratos *Wistar*, submetidos à cirurgia para restrição do fluxo sanguíneo.

Frente ao exposto, os manuscritos tiveram como principais objetivos preencher as lacunas encontradas na literatura sobre:

- (i) O treinamento de força com restrição do fluxo sanguíneo arterial é capaz de melhorar as adaptações musculares dos membros posteriores e o desempenho em testes de força em ratos *Wistar* submetidos à cirurgia com restrição arteriovenosa da artéria femoral? (**Manuscrito 1**);
- (ii) De acordo com a avaliação dos parâmetros cardiovasculares (pressão arterial (PA) e concentração de nitrito / nitrato), inflamatórios (superóxido dismutase (SOD) e fator de necrose tumoral alfa (TNF- α), o TFRF pode ser considerado um método seguro em ratos *Wistar* submetidos a cirurgia com restrição arteriovenosa da artéria femoral? (**Manuscrito 1**);
- (iii) Qual o efeito do TFRF sobre a reatividade vascular submetidas a cirurgia com restrição arteriovenosa da artéria femoral? (**Manuscrito 2**)

Essas questões abordadas serão norteadoras do desenvolvimento dos dois manuscritos descritos nos próximos capítulos. Importante destacar que as referências do [Manuscrito 2](#) ainda não estão no formato da revista porque segundo a mesma, isso só será necessário caso o manuscrito seja aceito para publicação.

2.1 Objetivo Geral

Avaliar se o treinamento de força com restrição do fluxo sanguíneo da artéria femoral de ratos *Wistar* é capaz de provocar hipertrofia dos membros posteriores e melhorar o desempenho em testes de força, além de ser um método seguro de treinamento através da avaliação de parâmetros cardiovasculares, inflamatórios e reatividade vascular da aorta abdominal.

2.2 Objetivos Específicos

- Quantificar a hipertrofia dos músculos sóleo, plantar e gastrocnêmio ([Manuscrito 1](#))
- Comparar o desempenho nos testes de preensão manual e teste do peso máximo carregado do treinamento de força com ou sem restrição do fluxo sanguíneo ([Manuscrito 1](#))
- Medir Pressão Arterial ([Manuscrito 1](#));
- Mensurar as concentrações de nitrito/nitrato; Superóxido dismutase (SOD); Fator de necrose tumoral alfa (TNF- α) ([Manuscrito 1](#));
- Determinar as curvas concentração-resposta na aorta abdominal ([Manuscrito 2](#));
- Determinação de óxido nítrico intracelular por análise de fluorescência DAF/FM-DA ([Manuscrito 2](#));
- Quantificar a produção de espécies reativas de oxigênio (ROS) por meio de ensaio por fluorescência de dihidroetida (DHE) ([Manuscrito 2](#));
- Medir a matriz de metaloproteinase (MMP-2) por imunofluorescência ([Manuscrito 2](#)) e
- Determinar a atividade gelatinolítica (zimografia *in situ*) ([Manuscrito 2](#)).

CAPÍTULO 3 – MANUSCRITO 1

3. MANUSCRITO 1

Strength training with and without arteriovenous blood flow restriction improves performance, regardless of changes in muscle hypertrophy, in *Wistar* rats

3.1 Abstract

Strength training (ST) with blood flow restriction (BFR) is known to promote increases in hypertrophy and strength sometimes similar to traditional ST despite the effects of the arterial BFR on muscle adaptations and safety are not well established. The aim of this study was to assess whether ST with arterial BFR is able to improve muscular adaptations, performance and its safety in *Wistar* rats. Animals aging 8 weeks were divided in four groups: sedentary sham (S/S), sedentary with arterial BFR (S/BFR), trained sham (T/S), and trained with arterial BFR (T/BFR). Training protocol consisted of four weeks of ST composed by six sets of 10 ladder climbing with 50% of 1 maximal voluntary contraction. Body weight, epididymal fat, maximum loaded weight, manual grip strength, muscular hypertrophy index, systolic blood pressure, enzyme activity of superoxide dismutase, nitrite/nitrate concentration and tumor necrosis factor alpha were analyzed. The BFR rate was between 36% and 38%. T/BFR was effective to promote strength and hypertrophy. T/S is an alternative to improve strength, but it did not promote hypertrophy. Furthermore, we found no significant cardiac and metabolic changes. Thus, T/BFR is able to improve muscle adaptations and performance in rats, without causing cardiovascular and metabolic damage.

Keywords: blood flow restriction, strength, hypertrophy, rats, strength training, safety.

3.2 Introduction

Blood flow restriction (BFR) exercise is a method which has been studied exhaustively in the last 20 years and consists of applying pressure through an external device, such as a tourniquet or blood pressure cuff around the limbs, to promote blood pooling distally from the occlusion point while performing exercise (Slysz et al. 2016). Although BFR alone can attenuate disuse atrophy, it is normally employed with some exercise stimulus for enhanced muscular development (Scott et al. 2015). The advantage of this training method is the use of strength exercises performed at relatively low loads (20% - 50% of 1 maximal voluntary contraction (MVC)) (Grønfeldt et al. 2020, Lixandrão et al. 2018, Wernbom & Aagard 2020). It has been demonstrated that low intensity exercise with BFR exhibit greater increases in muscle mass and strength than the low intensity exercise alone (Slysz et al. 2016) but controversial results about strength when compared to high load strength training (ST) have been reported with similar (Grønfeldt et al. 2020) or lower increases in strength despite similar hypertrophy (Lixandrão et al. 2018). Nevertheless, because high load ST is contraindicated for certain individuals, such as the elderly, persons with chronic disease, or rehabilitating and recovering athletes, low load ST with BFR is suggested as an interesting possibility (Slysz et al. 2016).

It is known that the adaptations from BFR training are dependent on both BFR stimulus itself, which is accepted to be high enough to occlude venous return from the muscles but low enough to maintain arterial inflow into the muscle, and the exercise protocol performed (Scott et al. 2015). Recently, it has been suggested that the pressure employed in the BFR training be based on the arterial occlusion pressure (AOP) (Scott et al. 2015, Patterson et al. 2019). However, there is evidence that the blood flow response between upper (Mouser et al. 2017) and lower limbs (Mouser et al. 2018) to various relative pressures is different. While the blood flow in posterior tibial artery decreases in a linear manner across increasing pressures with many participants still having blood flow through that artery at 100% of their pretesting AOP (Mouser et al. 2018), the application of different pressures decreased the brachial blood flow in a nonlinear, stepped fashion with the blood flow decreased at 10% AOP and remained constant until decreasing again at 40%, where it remained until 90% AOP (Mouser et al. 2017). Thus, this different response between lower and upper limbs may influence the adaptations from training.

A study comparing muscular adaptations between low (40% AOP) and high (90% AOP) pressure did not find differences between conditions in elbow flexors (Counts et al.

2016). These results are totally in accordance and can be easily explained by the data mentioned above. Nevertheless, and in the authors' knowledge, no similar study has been conducted on lower limbs and the effects of a high degree of arterial occlusion on adaptations from training is unknown.

It is recommended that limbs with larger circumferences be occluded using wide cuffs because the larger the limb circumference, the higher is the pressure required (Scott et al. 2015). However, wide cuffs may produce discomfort or pain as well as reduced exercise performance (number of repetitions) compared to narrow cuffs for the same relative (Spitz et al. 2019) or absolute pressure (Rossow et al. 2012). Likewise, the pressure employed affect both the feelings from BFR exercise (Mattocks et al. 2017, Soligon et al. 2018, Spitz et al. 2019) and performed repetitions (Mattocks et al. 2017, Spitz et al. 2019) with high pressures showing the worse outcomes.

Although there is no conclusive dose-response relationship between volume and adaptations for BFR training, it is suggested that 75 repetitions, across four sets (30, 15, 15, 15) or 3-5 sets to concentric failure promotes an efficient volume to lead to adaptations in most people (Patterson et al. 2019). Taken together, because of the requirement of a high pressure or wide cuffs to cause arterial BFR on lower limbs and the negative impacts of these factors on exercise performance, the total exercise volume cannot be reached, as shown by Rossow et al. (2012) and in some trials carried in our lab with 50% AOP (data not published). Because these difficulties, we opted for an arterial BFR in animal model, which was employed previously (Lloyd et al. 2001) to study the effects of arterial ischemic training on muscle adaptations. Thus, the aim of the present study was to assess whether ST with arterial BFR is able to improve muscular adaptations in the hindlimbs and the performance in strength tests in *Wistar* rats.

As an additional goal of this study, cardiovascular (blood pressure (BP) and nitrite/nitrate concentration) and inflammatory (superoxide dismutase (SOD) and tumor necrosis factor alpha (TNF- α) parameters were analyzed. Despite evidences suggesting that BFR training is safe (Patterson et al. 2019), doubts about its safety, mainly on vascular health, remain (da Cunha Nascimento et al. 2020).

3.3 Materials and Methods

Animals

All procedures were reviewed and approved by the Ethics Committee on Animal Use in Research (CEUA/EEFERP protocol number 2016.5.80.90.4) in compliance with the

“Principles of laboratory animal care” (NIH publication N° 86-23, revised 1985) and the national law (CONCEA publication N° 11.794, 2008).

Male *Wistar* Hannover rats (8 weeks old, weighting 250 - 270g) were divided into four groups: sedentary sham (S/S (16)), sedentary with BFR (S/BFR (12)), trained sham (T/S (12)), and trained with BFR (T/BFR(12)). The animals were housed in collective cages (3 or 4 animals/cage), and kept under a 12h light/dark cycle and controlled temperature (22°C to 24°C). All animals had free access to standard chow (Nuvilab, Quimtia) and drinking water (tap water). The body weight (BW) was measured weekly, from the beginning to the end of the study.

Arteriovenous restriction surgery

Surgery for arteriovenous occlusion was performed as previously described (Lloyd et al. 2001) in animals in the S/BFR and T/BFR groups. The group of sham animals (S/S and T/S) underwent the same procedure, but was not occluded. The purpose of the surgery was to restrict arterial flow and venous return in the segment (right rear leg). Therefore, the animals were fasted for 12 hours, had the inguinal region sterilized with iodine and shaved to remove hair. A pre-surgery measurement of blood flow in the right rear leg was also performed using a non-invasive blood flow meter (Laser Doppler Blood FlowMeter, ADInstruments) coupled with a signal amplifier (PowerLab 400™, ADInstruments, Australia). Subsequently, the animals were anesthetized with 2,2,2 - 97% tribromoethanol (Sigma-Aldrich Co. LLC, USA), 1 mg/kg of animal intraperitoneally.

The superficial right femoral artery and the right femoral vein were isolated and blood flow was restricted approximately 5 mm distally to the inguinal ligament using a 4-0 nylon thread. The skin was closed with an intradermal suture with 4-0 nylon thread and the effectiveness of arteriovenous restriction surgery was confirmed by measuring blood flow. The rate of BFR was between 36% and 38%. The area was then cleaned with iodopolividone (1%). After surgery, animals were maintained in heated cages until the anesthesia effect was abolished. A post-surgery treatment was carried out using anti-inflammatory (ketoprofen 3 mg/kg, subcutaneously) every 12 hours for 5 days and antibiotic (pentabiotic Fort Dodge, 40,000 IU/kg, intramuscularly) every 48 hours for 3 days.

Familiarization with the strength training protocol, maximum loaded weight test and Manual Grip Strength Test

This protocol was adapted Neves et al. 2016. After two weeks of the surgical procedure, all animals were adapted to the ST protocol, which consists of climbing a vertical ladder (110 cm high, 18 cm wide, 2 cm grid, 80° inclination) that has a housing (20 x 20 x 20 cm) located at the top used as a shelter during the rest period (figure 1). During two days (Monday and Wednesday) of the first week, they were familiarized with the equipment and the task they should perform in the exercise sessions. Thus, the familiarization consisted of climbing the ladder with a load equipment (conic tube), without weight, tied to the animal's tail (Neves et al. 2016). The number of sets on the first day of familiarization (Monday) was 3 and of repetitions, 10. On the second day of familiarization (Wednesday), the number of sets and repetitions was 6 and 10, respectively.

On Friday, the maximum loaded weight (MLW) was determined for all groups of animals. For the initial climb, they were loaded with 75% of the animal's BW. Then, a load of 30g was added until reaching the maximum load, considered when the animal fails to climb the ladder after 3 consecutive stimuli on the tail (using tweezers). The largest load that the animal carries along the entire length of the ladder, added to the animal's BW on the day of the test was defined as the MLW (Neves et al. 2016).

This test was performed at the beginning of the training protocol, at the end of the second and fourth weeks of the experimental protocol to adjust the training load and determine the effectiveness of the ST protocol, respectively. At the end of the second week (training load adjustment), only this reassessment was made, that is, there was no training. Similarly, the last evaluation was carried out replacing the last training day.

Figure 1. Vertical ladder used for strength training protocol.

The Manual grip strength test (MGS) was performed at the beginning and at the end fourth weeks of the experimental protocol to determine the effectiveness of physical training. The last evaluation was also carried out replacing the last training day. The MGS was performed four hours after the end of the MLW test, a guaranteed time for full recovery of the animal, using a gripping force system (model: DFE-002, San Diego Instruments, San Diego, Cal, USA). Three attempts were made to grasp with the front and rear legs without the values being considered. Each attempt consisted of placing the animal on the grid and, as soon as it is stable, the researcher carried out a traction by the tail so that both paws passed through all the wires of the grid and the rodent disengaged from it (figure 2). Subsequently, valid attempts were made and the three grip values applied to the grids were recorded (Anderson et al. 2004). The test result was determined by calculating the arithmetic mean of the three attempts. It is important to note that for both tests (MLW and MGS), only the pre and post values were considered for analysis.

Figure 2. Manual grip strength test.

Strength training protocol

After the adaptation and testing protocols, animals from T/S and T/BFR groups underwent ST during 4 weeks, once a day, and three days a week. The training session consisted of six sets of 10 ladder climbing, 1-minute rest between sets, carrying a load fixed to the tail (50% of 1 MVC obtained through the MLW test more the BW of each animal) to mimicking a muscular hypertrophy training (Neves et al. 2016). The average session time obtained in a pilot study was 20 to 30 minutes as well as the time under tension measured as the sum of the rise times in each series was 60 seconds.

The load was prepared using zinc fishing weights inside conical tubes that are fixed to the proximal part of the animal's tail using Scotch rubber tape (Scotch 3M, São Paulo, Brazil) (Neves et al. 2016).

Euthanasia

Forty-eight hours after the last exercise session animals were decapitated under anesthesia with ketamine/xylazine (40-2 mg/kg intraperitoneally). The animals remained 12 hours fasting before the euthanasia. After this procedure, the skin of the animal's abdominal region was removed and samples of white adipose tissue from the epididymal region were collected and weighed immediately. Skeletal muscles (soleus, plantar and gastrocnemius) were collected and weighed immediately for analysis. The animal's tibia was dissected and its length measured with a tape measure.

Muscular hypertrophy

Muscular hypertrophy was determined as previously described (Do Carmo et al. 2011). Briefly, a ratio between muscle weight and the length of the tibia was calculated. This ratio was calculated for soleus, plantar and gastrocnemius muscles separately and presented as mg/mm.

Hemodynamic measurements

BP measurement of the animals was performed before surgery, in the second week of recovery from surgery and one week before the animals were euthanized, using the tail cuff technique. For this, awake animals were placed in a container and a cuff was placed at the base of the animal's tail, as well as the pressure sensors. After this procedure, the cuff was automatically inflated and deflated and the systolic BP (SBP) signal was captured by the

sensors that send the information to the signal acquisition system amplifier (PowerLab 400™, ADInstruments, Australia).

Measurement activity of the superoxide dismutase antioxidant enzyme

Serum samples were collected without using an anticoagulant and clotted for 30 minutes. Then it was centrifuged at 2,000 x g for 15 minutes at 4°C. The test was performed following the manufacturer's recommendations (Cayman Chemical Company, Ann Arbor, MI, EUA).

Measurement nitrite/nitrate concentration

Plasma samples were collected and nitrate/nitrite levels (index of nitric oxide (NO)) were measured calorimetrically by commercially available kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, EUA).

Measurement tumor necrosis factor alpha concentration

Serum samples were collected all reagents, samples and standards were prepared as instructed. Then, 100 µl of the standard or sample was added to each well of the plate. 2.5 hours were incubated at room temperature. Thereafter, an additional 100 µl of biotin antibody prepared in each well was added. Incubate for 1 hour at room temperature 100 µl of prepared streptavidin solution was added and incubated again for 45 minutes at room temperature. Another 100 µl TMB One-Step Substrate Reagent to each well and another 30 minutes of incubation at room temperature. Finally, 50 µl Stop Solution to each well was added. Reading at 450 nm immediately (R&D System, Inc., Minneapolis, USA).

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (S.D) for n experiments. Shapiro-Wilk and Levene tests were used to check normality and homogeneity of variances in our data respectively. When the normality and/or homogeneity assumptions were not met, a data transformation technique (Log) was applied in an attempt to get around this problem. One-way ANOVA was used for hypertrophy, epididymal fat, TNF- α , nitrite/nitrate and SOD while for the data of BW, MLW, MGS and BP the mixed ANOVA tests were used and, when not indicated a parametric test, the Kruskal-Wallis test. The post-hocs were Bonferroni for parametric and Mann-Whitney tests corrected by Bonferroni's correction for non-parametric.

The Graph Pad Prism 5.0 program (GraphPad Software, CA, USA) was used for figures and the SPSS (Statistical Package for Social Sciences) 17.0 was used to run the statistical analysis. Statistical significance was considered at 5% ($p < 0,05$).

3.4. Results

Body weight and epididymal fat

Table 1 shows the parameters related to BW and the amount of epididymal fat of animals in the S/S, S/BFR, T/S and T/BFR groups. There was a time-group interaction [$F(3, 48) = 5,960$; $p = 0,002$] for the BW. At the end of the experimental protocol, the T/S group showed lower BW compared to the other groups ($p = 0,001$). All groups differed from pre to post ($p < 0,001$). No statistical difference was found for epididymal fat [$F(3, 47) = 1,117$; $p = 0,352$].

Table 1. Initial weight, final weight and epididymal fat of rats from the sedentary (S/S) groups, sedentary with blood flow restriction (S/BFR), trained (T/S), and trained with blood flow restriction (T/BFR).

	S/S (n=16)	S/BFR (n=12)	T/S (n=12)	T/BFR (n=12)
Initial	273,31±5,85	262,91±3,98	257,16±8,54	260,5±6,46
Weight (g)				
Final	378,56±6,42	380,83±7,21	339,91±6,32 ^{*, &}	381,66±6,32
Weight (g)				
Epididymal	7± 0,72	5,63±0,43	5,35±0,41	5,78±0,68
Fat (g)				

*different to S/S, ⁺different to S/BFR and [&]different to T/BFR. $p = 0,001$.

Physical performance: Maximum loaded weight test and Manual Grip Strength Test

The physical performance at the beginning was not different among groups [$\chi^2(3) = 6,079$; $p = 0,108$ for MGS and; $\chi^2(3) = 6,126$; $p = 0,106$ for MLW]. However, there was an effect of groups on both MLW [$\chi^2(3) = 36,416$; $p < 0,001$] and MGS [$\chi^2(3) = 38,302$; $p < 0,001$] final values. BFR did not impair physical performance of S/BFR compared to S/S. On the other hand, the ST protocol was effective to improve both physical performance tests in either T/S or T/BFR rats. T/S had increased their MLW in 31% and T/BFR in 34% when

compared to their initial values (figure 3A and 3B) and they were greater than S/S and S/BFR ($p < 0,001$). Regarding MGS, T/S increased MGS in 28% that was greater than S/S and S/BFR ($p < 0,001$) and T/BFR in 37% that was greater than all groups ($p < 0,001$ to S/S, S/BFR and T/S) (figure 3C and 3D).

Figure 3. Maximum loaded weight initial test (panel A); maximum loaded weight final test (panel B); manual grip strength initial test (panel C) and manual grip strength final test (panel D) of groups: S/S, S/BFR, T/S and T/BFR. *different to both S/S and S/BFR and +different to T/S. $p < 0,001$.

Muscular Hypertrophy Index

Effects of group on muscular hypertrophy index of both plantar [$F(3, 48) = 3,836$; $p = 0,015$] and gastrocnemius [$F(3, 48) = 2,940$; $p = 0,042$] were found. There was no effect of group on muscular hypertrophy index of soleus [$F(3, 48) = 2,322$; $p = 0,087$]. T/BFR showed an increase in the plantar muscle hypertrophy index in relation to the groups S/S ($p = 0,027$) and T/S ($p = 0,028$) while S/BFR showed an increase in the gastrocnemius muscle hypertrophy index in relation to T/S ($p = 0,043$) (figure 4).

Figure 4. Skeletal muscle hypertrophy index of the paw with restriction surgery (right) soleus (panel A), plantar (panel B) and gastrocnemius muscles (panel C), corrected by the length of the tibia of groups: S/S, S/BFR, T/S and T/BFR. *different to S/S; #different to T/S. $p < 0,05$.

Blood pressure

There was no time main effect [$F(2, 94) = 1,452$; $p = 0,239$] as well as no effect of groups [$F(3, 47) = 1,470$; $p = 0,235$] for the BP. Also, an interaction effect between time and groups was not found [$F(6, 94) = 0,887$; $p = 0,507$]. Data not show (S/S = $138\text{mmHg} \pm 1.5$; S/BFR = $135\text{mmHg} \pm 1.6$; T/F = $137\text{mmHg} \pm 1.5$; T/BFR = $133\text{mmHg} \pm 1,1$)

Antioxidant activity of superoxide dismutase

There was no effect of groups for SOD antioxidant activity [$F(3, 28) = 2,443$; $p = 0,085$]. Data not show (S/S = $1.8 \text{ U/mL} \pm 0.2$; S/BFR = $2.1 \text{ U/mL} \pm 0.1$; T/S = $1.6 \text{ U/mL} \pm 0.1$; T/BFR = $1.5 \text{ U/mL} \pm 0.2$)

Concentration of Nitrite/Nitrate

For the data of nitrite and nitrate no effect of groups was found [$F(3, 28) = 1,883$; $p = 0,164$]. Data not show (S/S = $35.2 \text{ n/M} \pm 2.2$; S/BFR = $48.7 \text{ n/M} \pm 4.7$; T/S = $38.5 \text{ n/M} \pm 3.2$; T/BFR = $39.4 \text{ n/M} \pm 1.5$).

Tumor necrosis factor alfa

Effect of groups was found for TNF- α values [$F(3, 24) = 13,026$; $p < 0,001$] with S/S showing lower values compared to the values obtained in the S/BFR ($p = 0,005$) and T/BFR groups ($p < 0,001$) as well as lower values in the T/S group compared to the T/BFR group ($p = 0,002$) (Figure 5).

Figure 5. Measurement of the concentration of TNF- α in serum. Sedentary groups (S/S), sedentary with blood flow restriction (S/BFR), strength trained (T/S) and strength trained with blood flow restriction (T/BFR). Data are expressed as mean $\pm$ SEM. Experimental n is 12 to 16. *different to S/S, $p < 0.001$; #different to T/S, $p = 0.002$.

3.5. Discussion

The main results of this study showed that the ST protocol was able to improve physical performance, as demonstrated by the increased strength in the MLW and MGS tests for T/S or T/BFR and that the hypertrophy index was mainly influenced by the arteriovenous BFR with the greater hypertrophy index of the plantar muscles in response to T/BFR than S/S and T/S while significant difference in the hypertrophy index of the gastrocnemius muscle being observed in response to S/BFR compared to T/S. Also, arteriovenous BFR with and without ST seems to have no significant effect on cardiovascular health since no changes were found in SBP, as well as in the blood levels of SOD and nitrite/nitrate compared to S/S group. On the other hand, serum level of TNF- α was increased in response to BFR and enhanced by associated ST.

All the animals independent of the group increased the BW during the protocol. Because the animals were young, the effects of maturation on BW were evident, as seen in S/S group, and BFR, as observed in S/BFR group, did not interfere with animals' growth (Kawada & Ishii 2008).

It is known that physical exercise increases the energy expenditure and it is an important factor that can affect the weight gain (Coqueiro et al. 2017, Coqueiro et al. 2018). Interestingly, male rats, but not female, undergone forced exercise program usually decrease or not change energy intake different from voluntary exercise (running wheels) (Titchenal 1988). In this way, when rats were subjected to ST reduced food intake was found (Coqueiro et al. 2017, Coqueiro et al. 2018). Moreover, optimized lipid oxidation can contribute for lower BW and improved body composition (Coqueiro et al. 2017, Gollisch et al. 2009). However, in spite of epididymal fat assessment in the present study, it does not translate accurately the body fat mass despite being part of the total body fat content (Coqueiro et al. 2017). Thus, it is possible that the reduction on body fat has occurred although no significant changes in epididymal fat mass have been verified. These facts can explain the lower final BW in T/S group despite no assessment of these parameters had been conducted in the present study and, this fat loss may have been made up for a possible hypertrophy in the T/BFR group (see below).

Both MLW and MGS increased in trained animals with higher values for T/BFR group in MGS test. According to the authors knowledge, there is no study that assessed the effects of ST with BFR in rats. Nevertheless, a lot of studies have shown the positive effects of ST on different strength tests in rodents (Do Carmo et al. 2011, Cassilhas et al. 2012, Hornberger et al. 2004, Neves et al. 2016, Coqueiro et al. 2018, Philippe et al. 2019). Loads of 45–50% of 1 MVC (and less) have shown to increase dynamic muscular strength in untrained individuals (American College of Sports Medicine 2009). Because the animals evaluated were naive to ST, it is possible that the loads used were sufficient to cause gains in strength. This fact can be explained, at least partially, by the learning process in rats which is similar to neural adaptations that occurs in early strength gains in humans (Hornberger et al. 2004). However, for T/BFR group, the impact of hypertrophy on strength gain can be huge (Loenneke et al. 2012) and it is likely that the hypertrophy was the advantage obtained by T/BFR over T/S, mainly in MGS test, since the hypertrophy may occur in the muscles which are not under direct influence of BFR but that was minimally stimulated (Madaramé et al. 2008) such as the muscles involved in the grabbing ability in the forelimbs.

We must recognize that the MGS test used in our study has some limitations. First, the MGS used to assess the grip strength is dependent on examiner manipulation (Hays et al. 2013). For example, the traction force magnitude employed by researcher in the tail of the animal may be different from one trial to another one. In the same way, the angle of force traction application may be different between two trials. These aspects related to examiner handling can affect the grab capacity of the animals and can interfere with the test results. However, despite this, it is a validated, simple, cheap and widely used test in studies with rats.

No evidence for muscle hypertrophy following ST alone was found in the present investigation contradicting other studies (Hornberger et al. 2004, Do Carmo et al. 2011, Cassilhas et al. 2012, Coqueiro et al. 2017, Philippe et al. 2019). These differences between our data and previous results may be explained by methodological aspects. The ST protocol used can offer an efficient stimulus for a muscle group but not for another one (Hornberger et al. 2004). For example, ladder climbing can be an effective stimulus for flexor hallucis longus (FHL) or flexor digitorum longus (FDL), highly involved in the grabbing ability which is suggested to limit the climbing ability of the animals (Hornberger et al. 2004). However, rats undergone the squat in a special apparatus obtained hypertrophy in plantar flexors muscles (plantar and gastrocnemius) (Do Carmo et al. 2011). Hypertrophy has been observed in FDL (Cassilhas et al. 2012), deltoid and flexor digitorum profundus (FDP) (Philippe et al. 2019) and tibialis muscle (Coqueiro et al. 2017) but not in plantar flexors (Hornberger et al. 2004, Neves et al. 2016) following ST composed by ladder climbing. Because we evaluated plantar, soleus and gastrocnemius muscles, it is possible that the hypertrophy which has occurred in another muscle group that was hard involved in the ladder climbing movement, such as FDL or FHL, was not evidenced. Therefore, it does not permit us to discard totally the occurrence of muscle hypertrophy and the effects of that as a factor contributing to the increased strength in T/S group.

Only both BFR groups exhibited significant differences in hypertrophy index in the assessed muscles. Initially, the greater hypertrophy index for gastrocnemius in S/BFR group compared to T/S group, can be attributed to a trend of lower hypertrophy index in T/S group than greater hypertrophy in S/BFR group. As mentioned earlier, rats subjected to ST can reduce the food intake (Coqueiro et al. 2017, Coqueiro et al. 2018) and it is known that energy deficit results in loss of skeletal muscle even when higher protein diet is consumed (Philippe et al. 2019). Thus, hypothetically speaking, the low stimulation of gastrocnemius

muscle in the exercise protocol together with an energy deficit created by reduced food intake and higher energy expenditure could have attenuated the muscle development in T/S group.

Moreover, both gastrocnemius (Kawada & Ishii 2005) and plantar muscles (Kawada & Ishii 2005, Kawada & Ishii 2008) have been shown to respond positively to BFR alone in rats. However, difference in occlusion protocols might affect these adaptations from BFR. The occlusion model in the studies cited above (vena saphena magna, vena saphena accessoria medialis, vena circumflexa ilium superficialis, and vena epigastrica superficialis) could have obtained a more severe BFR than our model (femoral artery and vein) although the blood flow in the limbs occluded was not checked by the authors of these studies. If it was the case, greater hypoxia was generated in the muscles triggering a more intense activity of the mechanisms related to muscle adaptations (De Castro et al. 2017). Also, because plantar muscle is smaller than gastrocnemius muscle, maybe the minimum stimulation promoted by exercise with BFR was efficient to trigger the hypertrophy in this muscle while no hypertrophy was observed for gastrocnemius muscle. In practice, bodybuilders generally employ greater workloads through higher volumes and intensities in large muscle groups compared to small muscle groups what may indicate that larger muscle groups require higher stimulation level.

Soleus hypertrophy index was not different among groups in the present study. Evidences reported in the literature showed no significant difference between rats undergone BFR or sham operation (Kawada & Ishii 2005, Pour et al. 2017, Bahreinipour et al. 2018) but when low intensity endurance exercise with BFR is analyzed increased soleus hypertrophy index were found (Pour et al. 2017, Bahreinipour et al. 2018). The difference between our results and those previously mentioned may reflect the training protocol employed. Strength exercise differ from endurance exercise with the former being intermittent efforts interspersed with rests between the sets while the latter is characterized by long continuous periods of exercise that is dependent on oxidative muscle fibers which are resistant to fatigue. Soleus is a muscle group with high percentage type I fibers which may became this muscle group less responsive to the occlusion suggesting that the effects of occlusion may depend on the muscle-fiber composition (Kawada & Ishii 2005). Therefore, neither occlusion alone nor ST with and without BFR may be an adequate stimulation to promote muscle adaptation in the soleus muscle.

Regarding cardiovascular assessments, our results showed that there was a maintenance of SBP levels during experimental protocol in all groups. It is known that SBP is not altered in both hypertensive and normotensive rats after a ST protocol compared to

their respective sedentary peers (Gomes et al. 2017). This fact indicate that ST is safe even in hypertensive condition. In fact, a systematic review carried out in humans (Cardoso et al. 2010) recommends ST as an important strategic tool in maintaining cardiovascular health because active individuals are less likely to develop high BP.

As mentioned earlier, no studies have been found in the literature on animals submitted to ST with BFR. However, in humans, Sardelli et al. (2017) demonstrated that all groups (high load, low load, low load with BFR and control) exhibited no different values of SBP before, after and 30 minutes after 45° leg press exercise in healthy elderly people what led the authors to conclude that ST with BFR seems to be a safe method. In addition, a systematic review by Neto et al. (2016), in humans, also corroborates these results and recommends ST with BFR as a safe and viable method for special populations, such as the elderly and cardiac patients, among others, because it promotes increased muscle strength and hypertrophy without negatively altering the hemodynamic response.

Regarding the concentration of SOD, an important antioxidant enzyme that can prevent muscle stress and increase tolerance to intense or ischemic exercise, we did not find any changes between the groups. Corroborating this result, Cumming et al. (2016) found that SOD concentration remain unchanged at the end of the protocol in both leg trained in high load ST, and the contralateral limb trained in low load ST with BFR. However, more studies should be concerned with analyzing the SOD concentrations in the exercised muscle.

Another important biomarker involved in cardiovascular health is nitrite/nitrate, an indirect marker of the production of NO. In our study, the results did not differ between groups. Acutely, a study by Ozaki et al. (2012), in healthy young people, also found no difference in nitrite/nitrate concentration between groups (high load ST, low load ST and low load ST with BFR). Likewise, a study by Ramis et al. (2020) found no differences between high load ST and low load ST with BFR after 8 weeks, but comparing the pre and post intragroup moments, they found an increased bioavailability of NO which indicates a similar effect of flow mediated dilatation in both groups. Thus, our results indicate that T/BFR had neither beneficial nor negative effects on endothelial health.

This study was also the pioneer in evaluating the concentration of TNF- α in animals submitted to ST with BFR. It is known that aerobic training with adequate intensity and volume, is capable of producing anti-inflammatory substances, improving systemic inflammation, which in turn can protect against problems associated with metabolic syndrome, such as arterial hypertension, diabetes, among others, and consequently preventing the development of cardiovascular diseases (Franklin et al. 2015, Dorneles et al. 2019,

Mcleod et al. 2019). With regard to ST, the results found in the literature also indicate such beneficial effects (Williams 2008, Mcleod et al. 2019, Da Silva et al. 2020).

Wistar rats undergone a ST protocol for 8 weeks, performed 1 time every 3 days, with a minimum load of 50% of the BW demonstrated a decrease in serum TNF- α concentrations pointing that this type of training is an important intervention strategy for the prevention and treatment of the inflammatory state of obesity and associated chronic diseases (Speretta et al. 2012). In our study, ST did not alter the blood concentration of TNF- α but, when associated with BFR, there was an increase in its concentration compared to the control group. Although TNF- α has not caused any harm in increasing muscle strength and hypertrophy, it is directly related to the components of the metabolic syndrome and can predict cardiovascular risk.

On the other hand, TNF- α is also related to the activity of mTOR (Laplane & Sabatini 2012). Thus, this could help explain the fact that TNF- α increased in S/BFR and even more in T/BFR groups and that greater muscle hypertrophy index was found in the BFR groups, more clearly in the T/BFR group. The neutrophils and macrophages responsible for tissue remodeling secrete both TNF- α and IL-6. However, the role of inflammatory activity in vascular occlusion is still unclear. Therefore, more studies are needed on this topic.

We found only one study that evaluated TNF- α concentration in high load ST (80% 1RM) and low load with BFR (30% 1RM) and the results corroborate ours (Da Silva et al. 2020). The BFR attenuated the beneficial adaptations of ST in overweight men, concentration of TNF- α , since high load ST group maintained the pre values, after 8 weeks of training. The authors argue that acute hypoxia suggests the migration of inflammatory cells to the exercised muscle. Thus, tissue inflammation and metabolic stress are linked to muscle adaptation to this training method (Dorneles et al. 2016, Da Silva et al. 2020). In this way, more studies are necessary to understand the adaptive mechanism of the inflammatory process, since the increase in this cytokine did not affect the increase in strength and muscle hypertrophy in the T/S and T/BFR groups. On the contrary, the T/BFR group had an even greater increase in manual pressure strength, and was the only group to increase plantar muscle hypertrophy values, when compared to its sedentary and trained counterparts.

Lastly, some considerations need to be made. In humans, BFR causes ischemia-reperfusion during the restriction and release of the cuff, respectively. In this situation, an increase in arterial flow and venous return occur which, in turn, potentiate the shear stress and it can have physiological impacts such as in NO production (Horiuchi & Okita 2012). However, unlike the human model, flow restriction in animals is continuous (throughout the

protocol) and there is a decrease in arterial flow and venous return which could impact less NO production compared to the human model. Also, these methodological differences in occlusion protocol between humans and animals may take part in explaining why many studies (Kawada & Ishii 2005, Kawada & Ishii 2008, Pour et al. 2017) not all (Bahreinipour et al. 2018) have shown that glycolytic muscles (plantar, gastrocnemius or extensor digitorum longus (EDL)) get hypertrophy following BFR alone in rats, but not in humans where BFR can attenuate the disuse atrophy but not cause hypertrophy (Takarada et al. 2000, Kubota et al. 2008).

In conclusion, the used chronic arteriovenous occlusion model was efficient to increase strength and hypertrophy in rats without negative impacts on cardiovascular health. However, some caution is necessary to extrapolate the results from the present investigation to humans due to methodological differences. Because of the discomfort and pain caused by BFR in lower limbs, it is necessary for the individuals have a high pain threshold. Finally, more research is needed to understand why and if the oxidative and glycolytic muscles share the same adaptations mechanism or not following BFR training.

3.6. Conclusion

Low load arteriovenous BFR ST is an effective and safety approach to improve strength and hypertrophy in untrained rats while low load ST without BFR is an alternative to improve strength but it does not promote hypertrophy, at least in short periods such as that used in the present study. This is similar what happens in untrained humans in the first weeks of training. From this study was not possible to detect how much of the strength gain was due to muscle hypertrophy what deserves attention in future studies together with comparative studies between ST with either arterial occlusion and venous occlusion on muscle adaptations and adaptive mechanisms. In addition, we found no significant cardiac and metabolic changes. Thus, ST with arteriovenous BFR is able to improve muscle adaptations and performance in rats, without causing cardiovascular and metabolic damage.

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CAPÍTULO 4 – MANUSCRITO 2

4 MANUSCRITO 2

Low load intensity strength training, associated or without blood flow restriction, increases nitric oxide bioavailability and reduces MMP-2 activity in rat aorta

4.1 Abstract

Physical exercise is one of the main lifestyle changes that can significantly reduce cardiovascular events. Strength training (ST) performed at high intensity (greater than 70% of 1 maximum voluntary contraction - MVC) induced morphological and functional adaptations as a result of metabolic stress in both, skeletal and cardiac muscles and has been widely used. However, high intensity ST sessions may cause impairments in certain people such as elderly or physically disabled people. As an alternative, studies have indicated that ST with blood flow restriction (BFR) with low load intensity (20-50% of 1 MVC), can produce positive adaptations similar to ST with an intensity equal to or greater than 70% 1 MVC. Furthermore, recent studies have been dedicated to investigate the effects of STBFR on muscle adaptations, thus, there are few studies that investigated the effects of STBFR on vascular function. In this context, the objective of the present study was to evaluate the effects of a low load intensity ST program on the vascular function of *Wistar* rats. Male rats were divided into four groups: sedentary sham (S/S), sedentary with blood flow restriction (S/BFR), trained sham (T/S) and trained with blood flow restriction (T/BFR). The animals in the S/BFR and T/BFR groups underwent surgery to BFR in the femoral artery and vein. After one week, the trained groups of animals started the training protocol during four weeks of ST composed by six sets of 10 ladder climbing with 50% of 1 MVC. Maximum loaded weight (MLW) were analyzed. Abdominal aortic artery reactivity was analyzed using concentration-response curves to acetylcholine (ACh: 10nM - 100µM) and phenylephrine (PHE: 1nM - 30µM). The production of nitric oxide (NO), and reactive oxygen species (ROS) and the vascular remodeling marker (MMP-2) were also evaluated in other experiments. The ST was effective as it increased the strength of the T/S and T/BFR animals in the tests of MLW. The animals in the S/BFR group showed reduced relaxation at ACh (E_{max} 22%) and ST prevented decreased relaxation in the T/BFR group. For the T/S group, it was not found increased relaxation when compared to sedentary pairs. Still, the T/S group showed an increase in the production of NO and both T/S and T/BFR showed a reduction in the *in situ* production of ROS (62% and 40% respectively). In addition, both trained groups had a reduction in the gelatinolytic activity of MMP-2 in the aorta. In conclusion, these results are unprecedented, promising and suggest that in addition to strength training, BFR should be considered as an alternative for clinical intervention for both physical performance and to improve endothelial health.

Keywords: strength training; blood flow restriction, vascular reactivity, vascular remodeling, oxidative stress, aorta, rat.

4.2 Introduction

Strength exercises are essential for the prevention and/or non-pharmacological treatment of metabolic disorders and body mass control. Its protective effects were observed in individuals with diabetes mellitus, dyslipidemia, cardiovascular disease, neurodegenerative diseases, which impacts on the improvement of quality of life and on the reduction of morbidity and mortality rates (CORREA et al. 2016; Grønfeldt et al. 2020). However, to obtain a greater magnitude of morphological and functional adaptations it is necessary that the intensity of the exercise is equivalent to or greater than 70% of 1 maximum voluntary contraction (MVC) (Lixandrão et al. 2018, Wernbom & Aagard 2020). However, it is widely known that training sessions performed at high loads (greater than 70% of 1 MVC) may produce exacerbated cardiovascular responses, such as increased heart rate (HR) and systolic blood pressure (SBP), consequently in the double product (DP). In addition, it can also lead to orthopedic complications such as ligament and muscle ruptures, among others. Thus, strength training (ST) with high loads may not be indicated for certain populations with special health conditions such as the elderly, patients with cardiovascular diseases and patients with orthopedic complications (ZANETTI et al., 2013; SBC 2013; MACEDO et al., 2016).

On the other hand, in the 1960s, an alternative training method called strength training (ST) with restricted blood flow (BRF), or vascular occlusion, or even KAATSU training emerged in Japan in gyms and clubs (LOENNEKE et al., 2012). The characteristic of this training method is that strength exercises are performed at relatively low intensities (20% - 50% of 1 MVC), resulting in similar adaptations to traditional strength training performed at high intensity (LOENNEKE et al., 2012). In addition, Neto et al. (2017) in a recent systematic review, noted that acute elevations of HR, PAS and DP during TBRF are significantly lower than those observed during ST with high loads. Additionally, promotes increased strength and muscle hypertrophy, therefore, can be considered as a safe and viable method for the elderly and populations with special health conditions.

The adaptive mechanisms involved in skeletal muscle responses to strength training with restricted blood flow (STBFR) must be investigated. Recent evidence points out that metabolic stress, generated by the greater local accumulation of metabolites due to hypoxia, may be the trigger for adaptations (TANIMOTO et al., 2005; FRY et al., 2010; DE CASTRO, et al. 2017). In this condition, there is a higher rate of hydrolysis of adenosine triphosphate (ATP), increased depletion of creatine phosphate (PCr), decreased intramuscular pH (SUGA et al., 2009), increased lactate concentration (TAKARADA et al., 2000;

TAKANO et al., 2005; FUJITA et al., 2007) and anabolic hormones systemically (TAKARADA et al., 2004; ABE et al., 2005; PIERCE et al., 2006; FRY 2010). The vascular smooth muscle tissue, which suffers adaptations induced by physical exercise mediated by shear stress, seems to undergo adaptations in response to strength training with blood flow restriction (FARIA et al. 2017; MACEDO et al. 2016; LOPES et al. 2019). Thus, the aim of this study was to evaluate the effects of STBFR program, with a 50% load of 1 MVC, on the vascular reactivity of the abdominal aorta of *Wistar* rats with arteriovenous blood flow restriction of the femoral artery.

4.3 Materials and Methods

Animals

All procedures followed the Ethics Committee on the Use of Animals in Research (protocol CEUA / EEFERP number 2016.5.80.90.4), in accordance with the “Principles of care for laboratory animals” (NIH publication No. 86- 23, revised in 1985) and the national regulations (CONCEA publication, no. 11.794.2008).

Male *Wistar* Hannover rats (8 weeks, weighing 250 - 270g) were randomly divided into four groups: sedentary sham (S/S, n = 16), sedentary with blood flow restriction (S/BFR, n = 12), trained sham (T/S, n = 12) and trained with blood flow restriction (T/BFR, n = 12). They were kept in collective boxes (3 or 4 animals/boxes) and maintained in a 12-hour light/dark cycle and controlled temperature (22°C to 24°C). All animals had free access to standard feed (Nuvilab, Quimtia) and water. They were weighed weekly, from beginning to end of the protocol.

All potentially exciting tissues were extracted for future investigations, and after sacrifice, the animals were frozen and discarded in an appropriate place. The study was carried out in compliance with the ARRIVE guidelines.

Arteriovenous restriction surgery

Blood flow restriction surgery was performed in animals of the S/BFR and T/BFR groups as described by Lloyd et al. (2001). Sham groups (S/S and T/S) underwent the surgical procedure, but didn't have restricted flow. The aim of the surgery was to restrict arterial flow and venous return from the superficial femoral artery (right hind leg). For this, the animals were fasted for 12 hours, had the inguinal region sterilized with iodine and shaved to remove hair. A pre-surgical blood flow (perfusion) measurement on the right rear

leg was also performed using a non-invasive blood flow meter (Laser Doppler Blood FlowMeter, ADInstruments) coupled to a signal amplifier (PowerLab 400 TM, ADInstruments). Subsequently, the animals were anesthetized with 2,2,2-Tribromoethanol 97% (Sigma-Aldrich Co. LLC, USA), 1 mg/kg intraperitoneally. Superficial right femoral artery and the right femoral vein were isolated and blood flow was restricted approximately 5 mm distally to the inguinal ligament using a 4-0 nylon thread. The skin was sutured with 4-0 nylon thread and the effectiveness of the arteriovenous restriction surgery was confirmed through a new blood flow measurement. BFR rate should be between 36% and 38%. The area was then cleaned with an antiseptic solution and the animals were kept in heated boxes until the effect of anesthesia wore off. In addition, they received anti-inflammatory (ketoprofen 3 mg/kg) every 12 hours for 5 days subcutaneously and antibiotics (veterinary pentabiotic Fort Dodge, 40,000 IU/kg) 48 hours for 3 days intramuscularly.

Performance Tests, Familiarization and strength training protocol

Familiarization sessions, performance tests and strength training protocol were adapted from NEVES et al. 2016. Two weeks after the surgery, all animals were familiarized to the strength training protocol, which consisted of climbing a vertical ladder (110 cm high, 18 cm wide, 2 cm grid, 80° inclination) with an accommodation (20 x 20 x 20 cm) located at the top used as a shelter during the rest periods. During two non-consecutive days (Monday and Wednesday) of the first week, they were familiarized with the equipment and the task to be performed in the exercise sessions. The familiarization consisted of climbing the ladder with a load equipment (conical tube), without weight, attached to the animal's tail (NEVES et al. 2016). The number of sets (climbs) on the first day of familiarization (Monday) was 3 and 10 repetitions. On the second day of familiarization (Wednesday), the number of sets and repetitions was 6 and 10, respectively.

To evaluate performance, on Friday, the maximum loaded weight (MLW) was determined for all groups of animals. For the initial climb, 75% of the animal's body weight was used. Then, a load of 30g was added until reaching the maximum load, considered when the animal was unable to continue the climb after 3 consecutive stimuli on the tail (using tweezers). The largest load that the animal carried along the entire length of the ladder, added to the animal's body weight on the day of the test, was defined as MLW (Neves et al. 2016). This test was performed at the beginning, at the end of the second week to adjust the training load and in the fourth week to determine the effectiveness of the strength training protocol.

After the adaptation and testing protocols, the animals in the T/S and T/BFR groups were submitted to strength training for 4 weeks, once a day, 3 non-consecutive days a week (Mon, Wed, Fri). The training session consisted of 6 sets of 10 stair climbs (repetitions), 1 minute rest between sets, carrying a load fixed to the tail (50% of 1 CVM obtained by the MLW test). The load was prepared with lead weights inside conical tubes, fixed in the proximal part of the animal's tail, with rubber adhesive tape (Scotch 3M, São Paulo, Brazil) (Neves et al. 2016).

Euthanasia

Forty-eight hours after the last training session, the animals were decapitated under ketamine/xylazine anesthesia (40-2 mg/kg intraperitoneally). They were fasted for 12 hours before euthanasia. After this procedure, the skin of the animal's abdominal region was removed and samples of white adipose tissue from the epididymal region were collected and weighed immediately. Abdominal aorta rings were also removed immediately for analysis of vascular reactivity. Other fragments of the abdominal aorta were frozen in cryoprotection medium (Tissue Tek - OCT, Sakura) and kept at -20 °C for later quantification of the production of nitric oxide (NO), reactive oxygen species (ROS) and metalloproteinase 2 (MMP-2).

Concentration-response curve in abdominal aorta

Vascular reactivity was evaluated in abdominal aortic rings (3 mm), without perivascular adipose tissue, suspended between two wire hooks, mounted in an isolated organ bath system and maintained in a solution containing Krebs (pH 7.4) at 37°C and continuously aerated with 95% O₂ and 5% CO₂ under a resting tension of 1.5 g. The isometric tension of the tissues was recorded by a force displacement transducer (UgoBasile, Varese, Italy) connected to a PowerLab 400 TM data acquisition system (ADInstruments Pty Ltd., Castle Hill, Australia). After 1h of stabilization, the aorta rings with intact endothelium were pre-contracted with phenylephrine (2 µM) and the endothelium-dependent relaxation was evaluated by concentration-response curves for acetylcholine (ACh, 10 nM-100 µM). The contractile response was assessed by concentration-response curves to phenylephrine (PHE, 1 nM - 3 µM).

Determination of intracellular nitric oxide (NO): assay by DAF-FM/DA fluorescence

The intracellular formation of NO was evaluated in cross sections of abdominal aorta (5 μ m) isolated from rats in the S/S, S/BFR, T/S and T/BFR groups. Therefore, the sections were hydrated for 5 minutes in Hanks' solution, with the following formulation (in mM): 1.6 CaCl₂, 1.0 MgCl₂, 120.0 NaCl, 5.0 KCl, 0.5 NaH₂PO₄, 10.0 dextrose, and 10.0 HEPES (pH 7.4). Then, the sections were incubated with the DAF-FM/DA intracellular NO fluorescent marker (10 μ M; Thermo Fisher Scientific; # D23844), at room temperature, for 30 min. Subsequently, the sections were washed with Hanks' solution and quickly exposed to a DAPI solution (2 μ g/mL; ThermoFisher Scientific; # D1306) for marking cell nuclei, followed by washing with Hanks' solution and slide assembly. The negative control was performed by exposing the abdominal aorta sections of rats to Hanks' solution, at room temperature, for 30 min, followed by exposure to DAPI. The images were acquired in 40x objectives. The fluorescence intensity emitted by DAF-FM/DA in abdominal aortic sections was acquired using a fluorescence microscope (Carls Zeiss Microscopy Ltd, Cambridge, England) using the GFP/FITC filter and the fluorescence intensity emitted by DAPI was evaluated using the UV/DAPI filter. The quantification of fluorescence by positive marking to DAF-FM/DA was performed in the ImageJ program, based on the quantification average of 30 rectangles of 0.75 x 1.00 in area, in three photos in 16 bits taken from the same section of each experimental *n*.

Determination of reactive oxygen species (ROS): assay by dihydroetide fluorescence (DHE)

The production of intracellular ROS was evaluated in cross sections of abdominal aorta (5 μ m) isolated from rats from groups S/S, S/BFR, T/S and T/BFR, incubated with the intracellular ROS marker Dihydroetide (DHE; 10 μ M; Sigma Aldrich; # D7008), at room temperature, for 30 min. The negative control was obtained by exposing the left ventricular sections of rats to phosphate-buffered saline (PBS), at room temperature, for 30 min. The images were acquired in 40x objectives. The fluorescence intensity emitted by DHE in the abdominal aorta sections was acquired using a fluorescence microscope (Carls Zeiss Microscopy Ltd, Cambridge, England) using the Cy5 filter. The quantification of the fluorescence of cell nuclei by positive marking to DHE was performed in the ImageJ program, based on the average quantification of 40 rectangles of 0.50 x 0.47 area, in three photos taken from the same section of each experimental *n*.

Immunofluorescence for MMP-2

Protein expression and tissue location of MMP-2 was evaluated in cuts of abdominal aorta (5µm) isolated from rats in the groups S/S, S/BFR, T/S and T/BFR. Therefore, the sections were hydrated and fixed in a 4% paraformaldehyde solution for 15 min and then incubated with blocking and permeabilization solution containing 1% bovine serum albumin, 0.3 mol/L glycine and PBS-Tween 0.1% for 1h. Subsequently, the sections were incubated for 18h with the primary anti-human MMP-2 antibody produced in mice (1: 200; Millipore; mab3308), at 4 ° C. Then, the cuts were washed with PBS solution and incubated with the secondary anti-mouse IgG antibody conjugated to Texas red produced in horse (1: 500; Vector; # TI2000), for 1h, at room temperature. Fluoroshield™ with DAPI (Sigma Aldrich; # F6057) was used for the marking of cell nuclei and as a means of assembling the slides, which after drying at 4°C, were sealed. The negative control was obtained following all the steps previously described, except for the application of the primary anti-MMP-2 antibody. The experimental images were acquired in a 20x objective and the negative fluorescence control was acquired in a 10x objective. The fluorescence intensity emitted by the Texas red fluorophore was acquired in a fluorescence microscope (Carls Zeiss Microscopy Ltd, Cambridge, England), using the Rhodamine filter and the fluorescence intensity emitted by the DAPI was evaluated using the UV/DAPI filter. The quantification of fluorescence by positive marking to Texas red was performed in the ImageJ program, based on the average quantification of 30 squares of 0.50 x 0.50 area, in three photos in 16 bits taken from the same section of each experimental *n*.

Zymography *in situ*

Gelatinolytic activity was evaluated in abdominal aorta sections (5µm) isolated from S/S, S/BFR, T/S and T/BFR rats, incubated with the fluorescent substrate DQTM Gelatin (ThermoFisher Scientific; # D12054). Therefore, the abdominal aorta sections of rats were hydrated with PBS pH7.4 solution for 10 min and then the fluorescent substrate DQTM Gelatin (1:10) diluted in Tris-HCl/CaCl₂/NaCl (Tris- 50mM HCl; 5mM CaCl₂.H₂O; 150mM NaCl; pH 7.6) for 1h at 37°C, protected from light. Subsequently, the sections were fixed with a 4% paraformaldehyde solution for 20 min at room temperature, followed by washing with PBS. Cell nucleus staining was performed with rapid exposure to the DAPI solution (2 µg/mL; ThermoFisher Scientific; #1306), followed by washing with PBS and slide assembly. The negative control was performed by exposing the abdominal aorta sections of rats only to the Tris-HCl/CaCl₂/NaCl solution for 1h, at 37 ° C, followed by rapid exposure to DAPI.

The images were acquired in 40x objectives. The fluorescence intensity emitted by the fluorescent substrate DQTM Gelatin was evaluated using a fluorescence microscope (Carl Zeiss Microscopy Ltd, Cambridge, England) using the GFP/FITC filter, and the fluorescence intensity emitted by the DAPI was evaluated using the filter UV/DAPI. Quantification of fluorescence by positive marking to DAF-FM/DA was performed in the ImageJ program, based on the quantification average of 40 rectangles of 0.75 x 1.25 area, in three photos in 16 bits taken from the same section of each experimental n .

Statistical analysis

The results obtained in the experiments were analyzed using the statistical program Graph Pad Prism 5.0 (GraphPad Software, CA, USA) for Windows and were presented as mean $\pm$ standard deviation (SD). Normality and homogeneity were checked by Shapiro-Wilk and Levene's test, respectively. The statistic used was two-way ANOVA (factors: flow restriction and training), followed by a Bonferroni post-test. Values of $p < 0.05$ were adopted as significant. Nonlinear regression analysis to determine $E_{\max}$, $\log EC_{50}$ and n were performed using GraphPad Prism (GraphPad software, San Diego, CA, USA) with the constraint that $\Phi = \text{zero}$.

4.4 Results

Physical performance at the beginning did not differ between groups. However, at the end of the experimental protocol, the performance improvement was proven in the MLW test. The T/S and T/BFR groups increased the test load by 31% and 34%, respectively, when compared to their sedentary pair, thus proving the effectiveness of physical training. BFR didn't impair the physical performance S/BFR compared to S/S (table 1.1).

Table 1.1. Initial test of the maximum weight loaded (MLW) for the groups: sedentary (S/S), sedentary with blood flow restriction (S/BFR), trained in strength (T/S) and strength trained with blood flow restriction (T/BFR).

	S/S	S/BFR	T/S	T/BFR
MLW inicial (g)	651 $\pm$ 9	665 $\pm$ 11	696 $\pm$ 22	696 $\pm$ 14
MLW final (g)	722 $\pm$ 10	735 $\pm$ 11	1010 $\pm$ 24*	1052 $\pm$ 23*

Data are expressed as mean $\pm$ SD. Experimental n is 12-16. *different S/S and S/BFR.

Regarding the reactivity of the abdominal aorta, it is observed that BFR reduced endothelium-dependent relaxation in the S/BFR group by 22% compared to S/S. Strength training prevented decreased relaxation in the T/BFR group and, for the T/S group, didn't promote increased relaxation when compared to sedentary pair. The tissue's sensitivity to acetylcholine wasn't modified by BFR or physical exercise (figure 1.1).

Figure 1.1. Concentration response curves to Acetylcholine in aorta rings with intact endothelium pre-contracted with PHE. Groups: sedentary (S/S), sedentary groups with blood flow restriction (S/BFR), strength trained (T/S) and strength trained with blood flow restriction (T/BFR). Data are expressed as mean ± SD. Experimental *n* is represented in parentheses. *different S/S.

The maximum response to alpha-agonist agent was increased by 80% in S/BFR animals compared to the group S/BFR. Physical training didn't change the maximum response. On the other hand, animals in the T/S and T/BFR groups showed 4 to 4.5-fold reduction, respectively, in sensitivity to phenylephrine (EC50) and in the animals in the S/BFR group, this reduction was 6-times compared to the group S/S (figure 2.1).

Figure 2.1. Concentration response curves to Phenylephrine in abdominal aorta with intact endothelium. Groups: sedentary (S/S), sedentary groups with blood flow restriction (S/BFR), strength trained (T/S) and strength trained with blood flow restriction (T/BFR). Data are expressed as mean $\pm$ SD. Experimental n is represented in parentheses. *different S/S.

The results showed that the animals in the groups with BFR showed a tendency to increase the production of NO. A significant result was observed for the T/S group, which showed 71% increase in agent production compared to the S/S group. The quantification results and their photographic representation of the immunofluorescence for DAF-2 are shown in figures 3.1A and 3.1B.

The production of ROS was reduced in animals in the groups submitted to physical training. This decrease was 62% in the T/S group and 40% in the T/BFR group (figures 4.1A and 4.1B).

Figure 3.1. Representative/quantitative analysis for 4,5-diaminofluoresceína (DAF-2), a marker of nitric oxide production (panel A) and photographic representation of immunofluorescence for DAF-2 (panel B) in abdominal aorta with intact endothelium in all groups. DAF-2 using the DAF-FM/DA fluorescent marker (for intracellular NO) and DAPI (for cell nuclei). Data are expressed as mean $\pm$ SD. Experimental n is 3 to 4. *different S/S.

Figure 4.1. Representative/quantitative analysis for dihydroethidium (DHE) a marker of reactive oxygen species production (panel A) and photographic representation of immunofluorescence for DHE (panel B) in abdominal aorta with intact endothelium in all groups. DHE using DAPI (for cell nuclei). Data are expressed as mean $\pm$ SD. Experimental n is 3 to 4. *different S/S; ⁺different S/BFR.

Vascular remodeling was investigated to quantifying metalloproteinase 2 (MMP-2) and its activity. The immunofluorescence of MMP-2 wasn't different between groups (figures 5.1A and 5.1B). However, the proteolytic activity, which means, the enzyme activity for MMP-2 was reduced by 10.6% in animals from S/BFR compared to S/S and the values of the trained groups proved to be much lower than those of the S/S and S/BFR group (figure 5.1C).

Figure 5.1. Intraluminal incubation of abdominal aorta with MMP-2 for 30 min increases gelatinolytic activity in all layers of the vessel. (A) Representative MMP-2 concentration in abdominal aorta. (B) Representative confocal photomicrographs of abdominal aorta sections incubated with DAPI and anti-MMP-2 antibody. DAPI's blue fluorescence indicates the nuclei and red fluorescence indicates the staining with anti-MMP-2 antibody (this antibody reacts only with the recombinant MMP-2 protein and not with endogenous rabbit MMP-2). (C) Aortic gelatinolytic activity quantified by in situ zymography in aortas exposed to intraluminal incubation with MMP-2. Data are expressed as mean $\pm$ SD. Experimental n is 3 to 4. *different S/S.

4.5 Discussion

Results show that the training protocol used was effective in maintaining endothelium-dependent relaxation in T/S group, a response that was reduced in animals with BFR and sedentary, in addition to attenuating hyperresponsiveness to the alpha-agent adrenergic. This improved function in trained animals may be related to the greater bioavailability of NO, less production of ROS and increased production of NO *in situ*. Still, less activity of MMP-2 was observed in both groups of trained animals. Thus, the great differential of this work concerns the vascular responses induced by the strength training associated or without the local BFR.

It is known that physical exercise promotes important adaptations in the vasculature, contributing to the prevention of cardiovascular events such as myocardial infarction and stroke, in addition to minimizing the harmful effects on the vessels caused by diabetes mellitus and hypercholesterolemia (GREEN; SMITH, 2018). Much of the beneficial effects observed on the vasculature are induced by the shear stress caused by the increase in pulsatile blood flow during physical effort that triggers increased NO bioavailability (GREEN et al., 2017). These conclusions were drawn from studies that observed vascular function in individuals submitted to physical, aerobic or strength exercise, without using BFR method. However, the effect of strength training with blood flow restriction on the vascular response still needs further investigation (ZHAO; LIN; JIAO, 2020; EARLY et al. 2020; RAMIS et al. 2020).

There are few studies that have observed the effect of physical exercise associated with BFR method, most of them used aerobic exercise protocols for physical training. Sprague Dawley rats that had restricted femoral artery flow surgically and performed 2 weeks of running training, which consisted of 2 weekly sessions until exhaustion showed improvements in endothelium-dependent relaxation in collateral vessels (COLLERAN et al., 2010).

Naderi-boldaji et al. (2018) also investigated the effect of aerobic training, with BFR in the femoral artery, on the hemodynamic function of elderly *Wistar* rats (22-24 months of life). The training protocol (running on the treadmill, average intensity of 50% of VO_{2max} , for 60 minutes, 5 times a week) was performed for 10 weeks and, at the end, no differences were found between the trained and sedentary groups. In another study, elderly rats (22-24 months old), also submitted to low load intensity aerobic training for 10 weeks, 5 times a week, at a speed of 15 m/min, with or without BFR in the bilateral femoral artery, concluded that BFR

alone may be associated with impaired vascular remodeling in the aorta, unlike the group trained without BFR that increased the number Apelin and its receptor, APJ, and angiotensin II receptors (AT₂) and modulated the ratio of angiotensin II receptors (ATR1/ATR2). However, when associated with training and BFR, the beneficial effect of exercise on the aortic structure was partially preserved by increasing aortic distensibility and compliance, improving the elastin/collagen ratio (BEJESHK et al., 2018).

Specifically with regard to vascular adaptations, Macedo et al. (2016) observed that eight weeks of strength training, using the squat apparatus, with a 40% load of 1 MVC, improved the endothelium-dependent relaxing response in mesenteric artery rings of *Wistar* rats. The results also show that the trained animals had higher expression of nitric oxide endothelial synthase (eNOS) and NO production in situ. Elderly Fischer rats trained for six weeks, using the same apparatus as in this study (climbing ladder) showed improvement in endothelium-dependent relaxation in the femoral artery, however, there was no increase in the expression of phosphorylated eNOS or eNOS in the arteries of trained animals (HARRIS et al., 2010).

Acute training protocol made by Faria et al. 2017 also investigated the expression of eNOS, nitric oxide phosphorylated endothelial synthase (p-eNOS1177) and nitric oxide inducible endothelial synthase (iNOS) to assess the response to ACh and PHE in the thoracic aorta of hypertensive rats undergoing training of acute force by electrical stimulation (20 volts lasting 0.3 seconds). The repetitions were performed at 3-second intervals, with 1 minute of rest between sets, totaling 20 sets with 15 repetitions and 50% intensity of 1 MVC. The control group was submitted to the same protocol, but without load. This type of exercise simulated squatting in humans. A single exercise session was able to increase relaxation to ACh and decrease the maximum response to PHE, compared to the control group. However, the exercise didn't alter the expression of eNOS and iNOS, but increased the p-eNOS. Thus, these results seem to be mediated by an increase in NO production by shear stress, indicating an improvement in endothelial function.

The responses of arterial relaxation and vascular adaptation observed after strength training, with light to moderate loads, are similar to those observed in studies that used aerobic exercise of moderate intensity (DE MORAES et al., 2008, OHAROMARI et al., 2015; GARCIA et al., 2017). These similar adaptations observed for the two different types of training would be related to the shear stress generated by the increase in pulsatile blood flow that occurs during the effort (GREEN et al., 2017).

To the best of our knowledge, the present study is the first to demonstrate the effect of strength training associated with BFR on the vascular response in animals. The results showed that the training protocol used was effective to maintain endothelium-dependent relaxation in animals that underwent to surgical restriction of arteriovenous flow in the femoral artery. It is interesting to note that in animals that maintained their femoral flow intact, the training protocol didn't improve the relaxing response evoked by acetylcholine in the aorta. Thus, there is evidence that the strength training protocol used can be considered effective for maintaining or improving arterial relaxation if used in association with BFR.

The surgical restriction of the femoral arteriovenous flow led to increased aortic contractility to the alpha-adrenergic agonist in S/BFR compared to the animal S/S. The reason for this finding is not clear and may be related to other mechanisms of vascular tone control such as adrenergic signaling. It is interesting to note that physical exercise partially reestablished this response in T/BFR animals, but the opposite response was observed in T/S animals that showed a tendency to increase the maximum response, but significant loss of sensitivity to the alpha-adrenergic agent. The control of vascular tone seems to be modified by physical exercise through changes in autonomic regulation as observed by Macedo et al. (2016), who showed an attenuated response to phenylephrine in the mesenteric artery due to decreased sympathetic activity and improved baroreflex sensitivity. Another hypothesis that may be influencing this response in trained animals is the lower presence of perivascular adipose tissue, which has an anti-contractile action, in aortas of trained animals (ARAÚJO et al., 2015).

The bioavailability of NO was increased in trained animals, due to the increased production of this agent in the T/S group, but mainly by the decrease in the production of ROS in the aorta of animals from the T/S and T/BFR groups. The improvement of the vascular antioxidant apparatus seems to be one of the most striking responses induced by physical exercise that leads to the improvement of NO-dependent relaxation. This finding can be confirmed by previous studies in which trained rats that showed improvement in relaxation evoked by acetylcholine and increased expression of SOD-1 in aortas (DE MORAES et al., 2008). A similar result was observed by Oharomari et al. (2015) in trained rats and/or supplemented with antioxidant Taurine that showed greater endothelium-dependent relaxation, greater expression of SOD and lower production of ROS in the aorta.

There was no change in the concentration of MMP-2, but the gelatinolytic activity of this metalloproteinase was reduced in aortas of trained animals, being, to date, the only study that investigated this effect of strength training in vessels. Considering that, the

increased metalloproteinase activity is observed in animals that develop atherosclerosis, contribute to instability, plaque rupture and cardiovascular disease (GALIS et al., 2002). We can infer that the strength training used, associated or without BFR, contributes for the prevention of vascular remodeling. Reduction of MMP-2 activity in the heart was observed in *Wistar* rats trained for 6 weeks (wheel-running) and subsequently undergoing ischemia-reperfusion surgery of the heart (PÓSA et al., 2015). Probably the lower gelatinolytic activity of MMP-2 observed in trained animals is due to the lower production of ROS, and consequently to a possible lower formation of peroxynitrite in the aorta of these animals (VIAPPIANI et al., 2009).

4.6 Conclusion

In conclusion, these results are unprecedented and promising and suggest that, in addition to strength training, blood flow restriction should also be considered as an alternative clinical intervention for both physical performance and improved endothelial health.

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CAPÍTULO 5 – CONSIDERAÇÕES FINAIS

5 CONSIDERAÇÕES FINAIS

Os achados sobre as adaptações do treinamento de força, com restrição do fluxo sanguíneo, nas adaptações musculares nos membros posteriores e o desempenho em teste de força em ratos *Wistar* pode contribuir com o conhecimento acerca deste método de treinamento em comparação ao treinamento de força. Além disso, apesar de ainda existirem dúvidas sobre a sua segurança com relação à saúde vascular, nosso trabalho encontrou evidências de que o TFRF é um método seguro de treinamento. Entretanto, mais estudos são necessários, uma vez que pesquisas dedicadas a avaliar estes parâmetros são escassos e ainda mais quando se trata de treinamento de força comparado ao aeróbio. Dessa forma, para contribuir com o conhecimento acerca desses processos, a presente tese teve como objetivo avaliar a hipertrofia nos membros posteriores de ratos *Wistar* e o desempenho dos testes de força, além dos efeitos da realização do treinamento de força com restrição do fluxo sanguíneo, com carga de 50% de 1CVM, sobre a reatividade vascular da aorta abdominal de ratos *Wistar* submetidos à cirurgia para restrição do fluxo sanguíneo.

No primeiro estudo ([Manuscrito 1](#)), objetivou-se avaliar se o TF com BFR arterial é capaz de melhorar as adaptações musculares nos membros posteriores e o desempenho em testes de força em ratos *Wistar*. Como um objetivo adicional deste estudo, os parâmetros cardiovasculares (pressão arterial (PA) e concentração de nitrito/nitrato) e inflamatórios (superóxido dismutase (SOD) e fator de necrose tumoral alfa (TNF- α)) também foram analisados. Como conclusão, o treinamento de força é uma alternativa para melhorar a força, mas não promoveu hipertrofia. Além disso, não encontramos alterações cardíacas e metabólicas significativas. E, o TFRF é capaz de melhorar as adaptações musculares e o desempenho em ratos, sem causar danos cardiovasculares e metabólicos.

O segundo estudo ([Manuscrito 2](#)) teve como objetivo avaliar os efeitos do TFRF, com carga de 50% de 1 CVM, sobre a reatividade vascular da aorta abdominal de ratos *Wistar* com restrição do fluxo sanguíneo arteriovenoso da artéria femoral. Em conclusão, esses resultados são inéditos, promissores e sugerem que, além do treinamento de força, o TFRF deve ser considerado como uma alternativa de intervenção clínica tanto para o desempenho físico quanto para a melhora da saúde endotelial.

5.1 Conclusões da tese

Frente aos resultados obtidos nos diferentes experimentos, conclui-se que o treinamento de força é uma alternativa para melhorar a força, mas não para a hipertrofia muscular em função do protocolo de treino utilizado. Além disso, não encontramos alterações cardíacas e metabólicas significativas. Adicionalmente, o TFRF é capaz de melhorar as adaptações musculares e o desempenho em ratos, sem causar danos cardiovasculares e metabólicos. Com relação aos parâmetros vasculares na aorta abdominal, os resultados sugerem que, além do treinamento de força, o TFRF deve ser considerado como uma alternativa de intervenção clínica também para a melhora da saúde endotelial.

5.2 Limitações do estudo

Há muitas divergências na metodologia utilizada em estudos com treinamento de força com restrição do fluxo sanguíneo em humanos e, conseqüentemente, encontramos estudos com diferentes conclusões. Algumas das divergências metodológicas encontradas dizem respeito ao tamanho do manguito de oclusão utilizado, ao tempo total da restrição do membro em exercício (contínuo ou intermitente), à pressão de oclusão, volume total de repetições e séries, intervalo de descanso, porcentagem da carga de 1CVM utilizada, dentre outros. Dessa forma, apesar das evidências sugerirem que o treinamento de força com restrição do fluxo sanguíneo é seguro, ainda carecem de maiores investigações, principalmente com relação à saúde vascular. Devido a essas dificuldades, optamos por um BFR arterial em modelo animal.

Apesar dos resultados encontrados nos dois manuscritos serem favoráveis ao uso deste método de treinamento e de haver grande semelhança fisiológica, celular e molecular entre os modelos animais e humanos, não podemos deixar de frisar que a extrapolação total destes resultados para a prática em humanos deve ser feita com cautela. Além disso, a oclusão realizada nos animais foi feita de forma cirúrgica e permaneceu durante todo o protocolo experimental, e não somente durante a realização do exercício, como ocorre em humanos.

Entretanto, pesquisas em fisiologia básica, como o presente estudo, se faz de fundamental importância para o entendimento das adaptações a nível celular, uma vez que questões éticas e metodológicas inviabilizam o estudo em humanos.

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ANEXO I – Comprovante de aceite do manuscrito 1

22/07/2021

E-mail de Universidade de São Paulo - Annals of the Brazilian Academy of Sciences - Decision on Manuscript ID AABC-2020-1147.R2

Nadia Fagundes Garcia <nadia.garcia@usp.br>

Annals of the Brazilian Academy of Sciences - Decision on Manuscript ID AABC-2020-1147.R2

6 mensagens

Igor Luis Kaefer <onbehalf@manuscriptcentral.com>

13 de fevereiro de 2021 22:04

Responder a: kaefer@ufam.edu.br

Para: nadia.garcia@usp.br, nadia.fgarcia@hotmail.com

13-Feb-2021

Dear Ms. Garcia:

It is a pleasure to accept your manuscript entitled "Strength training with and without arteriovenous blood flow restriction improves performance, regardless of changes in muscle hypertrophy, in Wistar rats" in its current form for publication in the Annals of the Brazilian Academy of Sciences. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Annals of the Brazilian Academy of Sciences, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Igor Luis Kaefer

Editor-in-Chief, Annals of the Brazilian Academy of Sciences

kaefer@ufam.edu.br

Associate Editor

Comments to the Author:

(There are no comments.)

27/10/2021 20:48

E-mail de Universidade de São Paulo - Pre-proof of accepted manuscript AABC-2020-1147

Nadia Fagundes Garcia <nadia.garcia@usp.br>

Pre-proof of accepted manuscript AABC-2020-1147

6 mensagens

Yraima Cordeiro <yraima@pharma.ufrj.br>

21 de outubro de 2021 13:48

Para: nadia.garcia@usp.br

Cc: Maria Lucia Maioli <mlucia@abc.org.br>, Daniel Lopes Lima Sant'Anna <dlopes@abc.org.br>, Ana Claudia Ribeiro <editor@e-papers.com.br>

Dear Dr. Garcia,

We hope this message finds you well.

We are currently with a huge backlog of accepted manuscripts to be published in our journal. Your paper AABC-2020-1147 'Strength training with and without arteriovenous blood flow restriction improves performance, regardless of changes in muscle hypertrophy, in Wistar rats' is within this backlog. To overcome this issue, we are trying to implement the publication of accepted papers as pre-proofs, with the respective DOI. Your manuscript has been selected to be readily available as a pre-proof (the accepted version, without journal editing) in the Scielo website. Please let us know (by replying to this message) if you agree that the pdf file of the accepted version of your manuscript is made available as a pre-proof until the final edited version is published.

Best regards,

Yraima Cordeiro

Associate Editor

Annals of the Brazilian Academy of Sciences

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Yraima Cordeiro

Professor Associado/ Associate Professor

BioTecFar, Faculdade de Farmácia

Av. Carlos Chagas Filho 373, CCS, Bloco Bss, sala 17

21941-902, Rio de Janeiro, RJ, Brasil

ANEXO II – Comprovante de submissão do manuscrito 2

30/10/2021 17:13

E-mail de Universidade de São Paulo - Submission Confirmation

Nadia Fagundes Garcia <nadia.garcia@usp.br>

Submission Confirmation

1 mensagem

Life Sciences <em@editorialmanager.com>
Responder a: Life Sciences <support@elsevier.com>
Para: Nádia Fagundes Garcia <nadia.garcia@usp.br>

30 de outubro de 2021 17:12

Dear Mrs Fagundes Garcia,

Your submission entitled "Low load intensity strength training, associated with or without blood flow restriction, increases nitric oxide bioavailability and reduces MMP-2 activity in rat aorta" has been received by Life Sciences

You will be able to check on the progress of your paper by logging on to Editorial Managers as an author. The URL is <https://www.editorialmanager.com/lfs/>.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

Kind regards,

Life Sciences

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