Universidade Estadual de Londrina

THÂMARA ALVES

# ALTERAÇÕES TEMPORAIS DE MARCADORES SISTÊMICOS INFLAMATÓRIOS E OXIDATIVOS EM DOIS MODELOS EXPERIMENTAIS ENVOLVENDO EXERCÍCIO EM HUMANOS SAUDÁVEIS E COM SÍNDROME METABÓLICA

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Tese apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina como requisito para obtenção do título de Doutor.

Orientadora: Profa. Dra. Flávia Alessandra Guarnier

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Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Alves, Thâmara .
Alterações temporais de marcadores sistêmicos inflamatórios e oxidativos em dois modelos experimentais envolvendo exercício em humanos saudáveis e com síndrome metabólica / Thâmara Alves - Londrina, 2017.
82 f. : il.

Orientadora: Flávia Alessandra Guarnier.
Tese (Doutorado em Patologia Experimental) - Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Patologia Experimental, 2017.
Inclui bibliografia.

1. Inflamação - Tese. 2. Estresse oxidativo - Tese. 3. Músculos - Tese. 4. Síndrome metabólica – Tese. I. Guarnier, Flávia Alessandra. II. Universidade Estadual de Londrina. Centro de Ciências Biológicas. Programa de Pós-Graduação em Patologia Experimental. III. Título.

CDU: 616-092

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**BANCA EXAMINADORA**

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Londrina, 28 de Julho de 2017

## AGRADECIMENTOS

Agradeço a Deus, por me permitir realizar esta pesquisa e por me salvar todos os dias das minhas fraquezas.

À minha família, pelo apoio e carinho em todos os momentos. À minha mãe Bácima Eliana Alves Simão, minha irmã Ymara Alves Pimenta, minha avó Salma Simão Cruz, meu avô Isaías Alves da Cruz (*in memorian*), à minha tia Elizabete Regina da Cruz Lemes, ao meu tio Isaías Alves da Cruz Júnior (*in memorian*) e aos meus primos Ícaro Araújo Simão Alves e Ana Giulia Alves Cruz, muito obrigada por tudo. Sem o apoio de vocês, eu não teria conseguido chegar até aqui. Amo muito todos vocês.

À minha orientadora, Profa. Dra. Flávia Alessandra Guarnier, muito obrigada pela convivência, pela calma, pelos ensinamentos e por todas as contribuições com a minha formação, desde o início do meu Mestrado até agora, muito obrigada, de coração.

À banca examinadora, Profa. Dra. Solange de Paula Ramos, Prof. Dr. Rubens Cecchini, Prof. Dr. Rodrigo Cabral Luiz e Prof. Dr. Emerson José Venâncio, muito obrigada por terem aceitado prontamente o convite. À Profa. Dra. Solange de Paula Ramos e ao Prof. Dr. Jayme Netto Junior, pela participação na banca de qualificação. A todos, muito obrigada pelas excelentes contribuições.

Aos técnicos do Departamento de Ciências Patológicas da UEL, Jesus A. Vargas; nosso querido Zui, e Pedro Sebastião Dionízio, muito obrigada pela convivência e pela prontidão em ajudar no que fosse preciso. Ao Zui, muito obrigada pela ajuda nos experimentos e principalmente pela alegria! Ela me contagiou todos os dias, até nos mais difíceis.

Aos amigos dos Laboratórios de Fisiopatologia das Adaptações Musculares, Fisiopatologia dos Radicais Livres e Patologia Molecular, da Universidade Estadual de Londrina: Thamara Nishida Xavier Silva, Jean Lucas Kremer, Laís Sábio, Poliana Marinello, Walison Brito, Lúcio Caldeira e Victor Freitas. Aos amigos do Laboratório de Fisioterapia Desportiva, da UNESP de Presidente Prudente, Ítalo Ribeiro Lemes e Maria Paula Figueiredo. Muito obrigada a todos pelo companheirismo, apoio e ajuda nas coletas de dados e experimentos. Obrigada por todos os momentos alegres e difíceis que dividimos.

Aos professores Dr. Jayme Netto Júnior e Dr. Carlos Marcelo, do Laboratório de Fisioterapia Desportiva, da UNESP de Presidente Prudente, pela contribuição com o trabalho, com a minha formação e amizade, desde a época do meu mestrado.

A todos os voluntários (as) que participaram dos estudos que compõem a presente tese. Sou imensamente grata a todos vocês. Muito obrigada pela paciência e tempo dedicado a esta pesquisa, pelas coletas, muitas vezes desconfortáveis e longas, muito obrigada, de coração.

À CAPES e à Fundação Araucária, pelo apoio financeiro.

ALVES, Thâmara. **Alterações temporais de marcadores sistêmicos inflamatórios e oxidativos em dois modelos experimentais envolvendo exercício em humanos saudáveis e com síndrome metabólica**. 2017. 82 páginas. Tese. Universidade Estadual de Londrina, Londrina, 2017.

## RESUMO (CAPÍTULO 1)

Após a realização de uma sessão de exercício não habitual, são observados sinais e sintomas de processo inflamatório local, como dor muscular tardia, edema, e perda de função (redução de força muscular e amplitude de movimento). Alguns autores utilizam, além desses sinais e sintomas, a quantificação sistêmica de citocinas proinflamatórias e marcadores de estresse oxidativo, a fim de avaliar a presença e/ou magnitude do dano muscular gerado por exercício. O tipo de contração utilizada, preferencialmente, nesses estudos, é a excêntrica, que notadamente promove danos mais expressivos em miofibrilas, em virtude do alongamento das fibras durante a contração. No entanto, a associação entre os parâmetros sistêmicos supracitados e a ocorrência de inflamação local induzida por exercício não é completamente elucidada na literatura. O objetivo do presente estudo foi investigar se existe relação entre dano muscular gerado por exercício não habitual e a presença de marcadores sistêmicos de inflamação e estresse oxidativo. Para isso, sete adultos jovens (idade: 25,7 ± 5,7 anos; massa corpórea: 76,3 ± 14,4 kg; altura: 176,0 ± 8,0 cm; IMC: 24.76 ± 4.89 kg/m$^2$) realizaram uma sessão aguda de exercício pliométrico (5 séries de 20 saltos em profundidade). Amostras de sangue e informações como dor muscular, limiar de dor à pressão, circunferência da coxa e salto vertical foram coletadas antes, imediatamente após, 0,5; 24, 48 e 72 horas após o protocolo de exercícios. Adicionalmente, foi realizado teste de contração isométrica voluntária máxima uma semana antes da realização do protocolo, 24, 48 e 72 horas após o mesmo. As amostras de sangue foram utilizadas para análise de marcadores sistêmicos de inflamação normalmente usados na literatura corrente (TNF-α, IL-6 e IL-10) e parâmetros de avaliação do balanço redox (quimioluminescência estimulada por tert-butil, consumo de oxigênio, conteúdo de proteínas carboniladas, produtos avançados de oxidação de proteínas, e catalase). O protocolo de exercícios foi capaz de gerar dano muscular significante, em virtude do aumento da percepção de dor [aumento significante às 24 (P=0,03), 48 (P=0,004) e 72 horas (P=0,015) após o exercício, em comparação ao momento basal] e perda de função [o salto vertical apresentou diminuição significante às 48 horas após a sessão (P=0,03) e a contração isométrica máxima foi menor às 24 (P=0.015) e 48 horas (P=0.03), em comparação ao momento pré-exercício]. Apesar de terem sido observados sinais de dano muscular induzido por exercício, os níveis sistêmicos das citocinas inflamatórias analisadas e os parâmetros de avaliação de estresse oxidativo não foram modificados ao longo do período de avaliação pós-exercício. Isso mostra que o dano muscular induzido por exercício não é necessariamente acompanhado por aumento sistêmico de citocinas inflamatórias e marcadores de estresse oxidativo, mostrando que é necessário ter cautela quando estas variáveis são utilizadas para avaliar a presença e/ou magnitude do dano muscular induzido por exercício.

**Palavras-chave**: dano muscular induzido por exercício; exercício pliométrico; dor muscular; estresse oxidativo; inflamação.

ALVES, Thâmara. **Time-course changes in systemic inflammatory and oxidative stress markers after two different exercise protocols applied in healthy humans and metabolic syndrome patients**. 2017. 82 pages. Thesis. Universidade Estadual de Londrina, Londrina, 2017.

## ABSTRACT (CHAPTER 1)

After a session of unaccustomed exercise, it is observed loss of muscle function and range of motion, soreness and swelling. Other markers, such as proinflammatory cytokines and reactive oxygen species are also utilized to evaluate exercise induced muscle damage (EIMD). However, there is not a conclusion about the association between the presence of inflammation caused by EIMD and the release of systemic markers of inflammation and oxidative stress after unaccustomed exercise. The aim of the present study was to investigate if there is a relationship among EIMD, presence of systemic inflammatory markers and alterations on systemic redox status. Seven healthy young males (age: 25.7 ± 5.7 years; body mass: 76.3 ± 14.4 kg; body height: 176.0 ± 8.0 cm; BMI: 24.76 ± 4.89 kg/m2) performed an acute session of plyometric exercise (5 sets of 20 drop jumps). Blood samples and information such as soreness, pressure pain threshold (PPT), thigh circumference and countermovement jump (CMJ) were collected before, immediately after, 0.5, 24, 48 and 72 hours post-exercise. Maximal isometric voluntary contraction (MIVC) was analyzed before exercise, 24, 48 and 72 hours post-exercise. Blood samples were collected for analysis of inflammatory cytokines (TNF-α, IL-6 e IL-10) and oxidative stress parameters [chemiluminescence (CL) stimulated by tert-butyl, oxygen uptake, carbonyl protein content and advanced protein oxidation products). It was observed that 100 drop jumps were able to produce significant amount of muscle damage, as it was observed by increased muscle soreness [significant higher at 24 (P=0.03), 48 (P=0.004) and 72 hours (P=0.015) after exercise, when compared to the baseline], and loss of function [CMJ presented a significant reduction at 48 hours after bout (P=0.03), and MIVC was smaller at 24 (P=0.015) and 48 hours (P=0.03), when compared to the baseline]. In spite of the presence of some classical sigs of local inflammation following an unaccustomed exercise session, systemic levels of proinflammatory cytokines and most of oxidative stress markers were not modified by the protocol. Therefore, the present study demonstrated that EIMD is not always accompanied by an increase of systemic proinflammatory cytokines or oxidative stress markers. Due to its complexity, the events involved in EIMD should be analyzed individually according to each protocol of exercise before selection of the best parameters to evaluate magnitude of EIMD.

**Keywords:** exercise-induced muscle damage, plyometric exercise, delayed-onset muscle soreness, oxidative stress, inflammation.

ALVES, Thâmara. **Alterações temporais de marcadores sistêmicos inflamatórios e oxidativos em dois modelos experimentais envolvendo exercício em humanos saudáveis e com síndrome metabólica**. 2017. 82 páginas. Tese. Universidade Estadual de Londrina, Londrina, 2017.

## RESUMO (CAPÍTULO 2)

A síndrome metabólica (SM) é considerada uma condição grave que se caracteriza pela presença de um conjunto de fatores de risco cardiovascular, presença de estresse oxidativo e inflamação sistêmica. Exercícios físicos são uma das melhores estratégias de tratamento, porém não há consenso sobre o melhor método de treino para essa população, além de existir um desafio na prescrição de exercícios para portadores de (SM): redução de massa corporal sem reduzir massa muscular. O exercício resistido regular é capaz de aumentar as defesas antioxidantes, além de promover efeitos anti-inflamatórios no organismo. Novos métodos de treinamento, como os exercícios funcionais, tem se mostrado eficientes no desenvolvimento de habilidades físicas como força muscular e coordenação motora, o que seria interessante para a população em questão, haja vista a maior incidência de SM em faixas etárias mais avançadas. Em vista disso, o objetivo do presente estudo foi investigar o impacto de dois programas de exercício resistido (convencional *vs*. funcional) no quadro de estresse oxidativo e inflamatório sistêmico, além de avaliar o risco cardiovascular antes e após a realização dos programas de exercícios. O estudo incluiu 54 adultos, de ambos os sexos (50,87±5,67 anos, 83,14±14,19kg, 166±10,4cm), randomizados em 3 grupos: treino funcional (TF) ou convencional (TC) e grupo controle (GC), que não realizou nenhum tipo de exercício durante o período. TF e TC realizaram 12 semanas de treino sistematizado, 3 vezes por semana e o grupo controle foi instruído a não modificar sua rotina. Os fatores de risco cardiovascular, força muscular, marcadores de estresse oxidativo e inflamação foram avaliados antes e após o período de exercícios. Após análise dos resultados, observou-se que a força muscular aumentou em ambos os tipos de treinamento, quando comparada ao grupo controle ($P<0,05$), mostrando eficiência de ambos os tipos de treinamento no incremento de força muscular dos participantes. O treinamento convencional apresentou um aumento significante da atividade das enzimas superóxido dismutase (SOD) e catalase ao final do período de treinamento e uma diminuição, também significante, na lipoperoxidação sistêmica ($P<0.05$), quando comparado ao controle. O treino funcional promoveu redução na pressão sistólica e aumento na atividade da enzima catalase ($P<0.05$). O grupo controle apresentou piora nos níveis de HDL após o periodo analisado. Ambos os programas de exercício aumentaram a força muscular, a defesa antioxidante e mantiveram os níveis de HDL, mas não houve diferença significante entre os grupos em relação aos parâmetros analisados. Conclui-se que o treino resistido (funcional ou convencional) pode ser considerado uma estratégia não farmacológica de longo prazo no tratamento da SM.

**Palavras-chave**: síndrome metabólica; treinamento resistido; treinamento funcional; estresse oxidativo; inflamação; fatores de risco cardiovascular.

ALVES, Thâmara. **Time-course changes in systemic inflammatory and oxidative stress markers after two different exercise protocols applied in healthy humans and metabolic syndrome patients.** 2017. 82 pages. Thesis. Universidade Estadual de Londrina, Londrina, 2017.

## ABSTRACT (CHAPTER 2)

Metabolic syndrome is considered a serious condition that comprises a number of cardiovascular risk factors, oxidative stress and inflammation. Physical exercise practicing is one of the best treatment and prevention options, although there is no consensus about the best exercise program for patients with SM and there is a challenge in prescription of exercises for these patients: reducing mass without affecting lean body mass. Resistance exercise has anti-inflammatory properties and can increase antioxidants defenses and functional training is able to improve strength and motor coordination, which is important for elderly (the highest prevalence of metabolic syndrome is in the elderly people). The purpose of this study was to investigate the impact of two resistance exercise programs (conventional *vs*. functional) on inflammatory and oxidative status and cardiovascular risk factors of volunteers diagnosed with MS. 54 (adults were included in the present study, both sex, (50.87±5.67 years, 83.14±14.19kg, 166±10.4cm), randomized assigned to an exercise group or remained sedentary (control group). Functional (FT) and conventional (CT) groups realized 12 weeks of systematized training, 3 times per week and control (CTRL) was instructed to do not change habitual activities. Cardiovascular risk factors, muscular strength, inflammatory and stress oxidative markers were analyzed before and after training. Muscular strength was improved in both training groups when compared to CTRL ($P<0.05$). CT presented an increase of SOD and catalase at the end of training and a significant decrease in tert-butyl hydroperoxide-initiated chemiluminescence ($P<0.05$) after intervention, when compared to CTRL. FT presented significantly reduction in systolic blood pressure and increase in catalase activity ($P<0.05$). CTRL presented worsening HDL levels at the end of the period. It was observed that both training programs improved muscular strength, antioxidant defense and maintained HDL levels, but it was not possible to conclude if one method is better than the other one. Therefore, resistance training (conventional or functional) can be considered an long-term non-pharmacological therapy for abnormalities that comprise metabolic syndrome.

.

**Keywords**: metabolic syndrome; resistance training; functional training; oxidative stress; inflammation; cardiovascular risk factors.

## LISTA DE TABELAS E FIGURAS – CAPÍTULO 1

### Tabelas



### Figuras

## LISTA DE TABELAS E FIGURAS – CAPÍTULO 2

### Tabelas



### Figuras

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| ANOVA | Análise de variância |
| AOPP | *Advanced oxidation protein* |
| ATP | Adenosina trifosfato |
| BMI | *body mass index* |
| CL | *Tert-butyl hydroperoxide-initiated chemiluminescence* |
| CP | *Carbonyl proteins* |
| CMJ | *Countermovement jump* |
| CT | *Conventional training* |
| CTb | *Conventional training – before training* |
| CTf | *Conventional training – after training* |
| CTRL | *Control group* |
| CTRLb | *Control group - before training* |
| CTRLf | *Control group - after training* |
| DM2 | Diabetes *Mellitus* tipo 2 |
| DOMS | *Delayed onset muscle soreness* |
| EIMD | *Exercise induced muscle damage* |
| ERO | Espécies reativas de oxigênio |
| FID | Federação Internacional do Diabetes |
| FT | *Functional training* |
| FTb | *Functional training - before training* |
| FTf | *Functional training - after training* |
| GSH | Glutationa reduzida |
| HA | Hipertensão Arterial |
| IDF | International Diabetes Federation |
| IL-1β | Interleucina 1-beta |
| IL-6 | Interleucina-6 |
| IL-10 | Interleucina-10 |
| IPAC | Questionário Internacional de Atividade Física |
| IMC | Índice de massa corpórea |
| MIVC | *Maximal isometric voluntary contraction* |
| NF- κB | *Factor nuclear-kappa B* |
| OMS | Organização Mundial da Saúde |
| PPT | *Pressure pain threshold* |

| | |
|---|---|
| QL | Quimioluminescência |
| RI | Resistência à insulina |
| RNM | Ressonância Nuclear Magnética |
| SM | Síndrome Metabólica |
| SOD | Superóxido dismutase |
| TNF-α | Fator de necrose tumoral alfa |
| t-BHP | *tert-butyl hydroperoxide* |
| USOD | Unidade arbitrária de superóxido dismutase |
| 1RM | Uma repetição máxima |

**Sumário**

## Apresentação

A presente tese foi dividida em dois capítulos:

O primeiro capítulo foi construído com o objetivo de investigar o dano muscular induzido por exercício e sua relação com as variações de marcadores sistêmicos comumente relacionados à inflamação e estresse oxidativo.

O segundo capítulo foi construído com o objetivo de modificar, por meio de diferentes protocolos de exercício resistido, os padrões sistêmicos de inflamação e estresse oxidativo presentes na Síndrome Metabólica (SM) e analisar a melhor resposta entre os treinamentos propostos.

# 1 Capítulo 1. Estresse oxidativo e inflamação nas respostas ao exercício agudo

## 1. 1 Introdução

O dano muscular induzido por exercício tem sido estudado desde o início do século XX, quando Hough (1902) publicou um dos primeiros estudos com o objetivo de elucidar as causas da dor muscular tardia. Apenas nos últimos 30 anos o interesse e a investigação sobre o tema se intensificaram.

Segundo Caldwell et al. (2016), Fouré e Bendaha (2017), o dano muscular induzido por exercício pode ser definido como um conjunto de alterações estruturais e funcionais ocasionadas em músculos estriados esqueléticos, causadas por tensão excessiva em miofibrilas, durante exercícios não habituais e sucedidas por instalação de processo inflamatório local.

Exercícios com ênfase em contrações excêntricas são mais utilizados em estudos que investigam dano muscular induzido por exercício, uma vez que promovem maior quantidade de micro-lesões nas fibras, pelo distanciamento entre origem e inserção musculares. A tensão gerada durante o alongamento das fibras contra uma determinada resistência promove maior quantidade de dano tecidual do que o trabalho concêntrico, por exemplo, em que há aproximação entre origem e inserção das fibras, durante a contração (ALVES et al., 2013; PEAKE et al., 2017).

Os exercícios pliométricos, como os saltos em profundidade, também são utilizados com o objetivo de indução de dano muscular, uma vez que, durante as fases de aterrisagem no solo, o indivíduo realiza a contração excêntrica do músculo quadríceps femoral contra a resistência do próprio corpo. Nos saltos em profundidade, o indivíduo salta de uma plataforma, aterrissa e salta novamente, com a maior velocidade e altura possíveis. (HOWATSON, G. et al, 2012). Em estudo realizado por Howatson G. et al. (2012), um protocolo de 100 saltos em

profundidade foi eficaz na produção de dano muscular significante em indivíduos treinados.

Newham et al. (1983) sugeriram, em estudo pioneiro, que o dano muscular após exercício excêntrico é inicialmente mecânico, em virtude do alongamento das fibras durante a contração. Há dano estrutural em miofibrilas imediatamente após a sessão, com ruptura de sarcômeros, que se acentua entre 24 e 48 horas, envolvendo maior quantidade de fibras. Além do trauma mecânico, o dano muscular caracteriza-se por liberação de enzimas e proteínas musculares (HYLDAHL e HUBAL, 2014).

O dano muscular causado por exercício excêntrico não habitual frequentemente resulta em dor muscular tardia (PEAKE et al., 2017). Os sintomas incluem ainda, perda de força e potência muscular, diminuição de amplitude de movimento, edema, dor e sensibilidade à pressão (JENKINS et al., 2013).

Owens et al. (2018) afirmam que o dano muscular ocasionado por exercício é caracterizado por sintomas que aparecem imediatamente após o treino e podem se perpetuar até aproximadamente 14 dias após a realização do mesmo. Os principais incluem déficits funcionais e dor muscular, que dependem principalmente da intensidade e duração do estímulo, além da susceptibilidade individual ao dano. Embora os mecanismos responsáveis por estes sintomas não estejam completamente compreendidos (JENKINS et al., 2013), sabe-se que inicialmente há dano estrutural em miofilamentos, desorganização da linha Z e prejuízo no mecanismo excitação-contração muscular (CLARKSON et al., 2002; NEWHAM et. al. 1983; PEAKE et al., 2017).

O estudo do dano muscular induzido por exercício em seres humanos pode ser realizado de maneira direta e indireta (PEAKE et al., 2017). As ferramentas utilizadas para acesso direto ao tecido são as biópsias musculares e a ressonância nuclear magnética (RNM), que não são comumente utilizados (CLARKSON e HUBAL, 2002). As biópsias musculares, por serem métodos invasivos e a RNM, por não se entender completamente o que as alterações nas imagens indicam. Além disso, ambos são métodos dispendiosos (CLARKSON e HUBAL, 2002).

Os métodos indiretos de acesso ao dano muscular, apesar de não explicarem os mecanismos pelos quais o mesmo ocorre, são utilizados com mais frequência, por serem mais acessíveis. Alguns exemplos são os déficits funcionais (redução de amplitude de movimento, perda de força e potência muscular), sinais e sintomas clínicos (dor, edema, aumento de sensibilidade à pressão), além da quantificação de proteínas musculares (creatina quinase e mioglobina, por exemplo) e marcadores sistêmicos inflamatórios (TNF-α, IL-1β, IL-6, IL-10) e de estresse oxidativo (peroxidação lipídica, produtos avançados de oxidação proteica, capacidade antioxidante total plasmática) (CLIFFORD et al., 2016; FATOUROS et al., 2010; HOUGHTON e ONAMBELE, 2012; PEAKE et al., 2017).

Estudos sugerem a participação de inflamação asséptica e, consequentemente, estresse oxidativo no processo de resolução e adaptação das fibras após dano muscular induzido por exercício (CLARKSON e HUBAL, 2002; MACINTYRE et al., 1995; PEAKE et al., 2017).

Em estudos sobre o dano muscular induzido por exercício, realizados por meio de biópsias musculares, foram observados, além de desarranjos em miofibrilas e linha Z, ou seja, na ultraestrutura das fibras musculares, alterações na matriz extracelular do tecido (FRIDÉN et al., 1981; NEWHAM et. al. 1983; PEAKE et al., 2017; STAUBER et al., 1990). Segundo Clarkson e Hubal (2002), estas alterações iniciariam uma resposta pró-inflamatória local, que seria importante para o processo de reparação do músculo.

O desarranjo na ultraestrutura muscular seria o primeiro evento no processo de desenvolvimento do dano muscular induzido por exercício e parece estar presente principalmente na linha Z, isto é, nos limites dos sarcômeros, onde estão conectados os filamentos de actina (LENN, et al. 2002).

O segundo evento teria mecanismos não totalmente elucidados e seria dependente deste desarranjo, envolvendo uma resposta pró-inflamatória e pró-oxidativa aguda (LENN, et al. 2002). Há evidências de que, imediatamente após dano estrutural no tecido, neutrófilos presentes no músculo liberem citocinas pró-inflamatórias, incluindo TNF- $\alpha$ (fator de necrose tumoral alfa) e IL-1β (interleucina 1-beta) (HU e YANG, 2018). Além disso, os neutrófilos são apontados como os principais responsáveis pela produção de espécies reativas de oxigênio durante o processo inflamatório agudo, o que exacerbaria o processo de dano, temporariamente (HU e YANG, 2018).

Autores sugerem que nas primeiras horas após a realização do exercício, neutrófilos circulantes infiltram o tecido e atuam com o objetivo de eliminar os detritos celulares e amplificar a resposta pró-inflamatória aguda, por meio da liberação de citocinas pró-inflamatórias (CHAZAUD, 2016; PEAKE et al. 2017). Os eventos pró-inflamatórios resultariam ainda, em aumento da permebilidade vascular pela liberação de histamina e protaglandinas e consequentemente edema localizado. (LENN et al., 2002; HU e YANG, 2018).

Um terceiro evento presente no processo de dano tecidual seria a produção de espécies reativas de oxigênio (ERO), que exacerbaria a dor e o processo de dano tecidual, temporariamente (LENN et al., 2002; HU e YANG, 2018). Após trauma mecânico, neutrófilos infiltrados no músculo iniciam a liberação de ERO (*burst* respiratório), como mecanismo de defesa do tecido. Além disso, as citocinas liberadas por neutrófilos e pelas próprias fibras danificadas ativariam enzimas geradoras de ERO, como xantina oxidase e ciclooxigenase 2 (MICHAILIDIS et al., 2013).

Entre 4 e 24 horas após o dano muscular, macrófagos invadiriam o tecido com o intuito de secretar citocinas pró-inflamatórias, fagocitar o tecido danificado e iniciar a proliferação de mioblastos, o que iniciaria a quarta e última etapa do processo, que resultaria em remodelação do tecido e adaptação no intuito de prevenir novos desarranjos e/ou amenizar a extensão do dano em eventos subsequentes (CHAZAUD, 2016; PEAKE et al. 2017).

Em vista disso, pesquisadores utilizam a quantificação de citocinas pro-inflamatórias e espécies reativas de oxigênio como marcadores indiretos para avaliar a magnitude do dano muscular induzido por exercício e até mesmo como parâmetros de avaliação do efeito da aplicação de terapias de recuperação pós-esforço no manejo da dor muscular e perda de função (CLIFFORD et al., 2016; CORDER et al., 2016; HOUGHTON e ONAMBELE, 2012; LENN et al., 2002). E embora existam algumas evidências sobre a presença de inflamação local em estudos utilizando biópsias musculares após dano muscular induzido por exercício, autores apontam o método da coleta de amostras musculares como um possível viés na pesquisa pelo processo inflamatório, uma vez que a própria incisão pode gerar uma resposta pró-inflamatória aguda (CLARKSON e HUBAL, 2002; MALM, 2002).

Sobre a pesquisa de mediadores sitêmicos, alguns estudos apontam aumento de marcadores pró-inflamatórios e de estresse oxidativo plasmáticos após a realização de exercício agudo de natureza excêntrica, em indivíduos com diferentes níveis de adaptação muscular. (CLIFFORD et al., 2016; FATOUROS et al., 2010; HOUGHTON e ONAMBELE, 2012; TIDBALL, 1995; VIERCK et al., 2000).

Outros estudos apontam para respostas controversas, com défitis funcionais e clínicos que caracterizam o processo de dano muscular, porém, não acompanhados de marcadores sistêmicos de inflamação e estresse oxidativo (DANNECKER et al., 2013; LENN et al., 2002; MALM et al., 2004).

Dannecker et al. (2013), utilizando um protocolo para indução de dano muscular após exercício excêntrico, obtiveram aumento significante de dor muscular, edema, níveis de mioglobina e sensibilidade à pressão, analisada por dolorímetro. E apesar da presença dos sinais indicando presença de dano tecidual, não foi observado pelos autores, aumento dos níveis plasmáticos de interleucina 1-beta (IL-1β), fator de necrose tumoral-alfa (TNF-α) ou óxido nítrico.

Observa-se frequentemente, na literatura, que estratégias de recuperação pós-exercício, tanto nutricionais, quanto relacionadas às ciências do movimento, tem sido pautadas em um consenso de que o dano muscular seria sempre acompanhado por eventos pró-inflamatórios e pró-oxidantes (CLIFFORD et al., 2016; CORDER et al., 2016; FOURÉ, A.; BENDAHA, D., 2017; HOUGHTON e ONAMBELE, 2012; LENN et al., 2002; MALM, 2002), embora já existam evidências que contestam a presença mandatória de inflamação local e/ou liberação de mediadores inflamatórios na circulação sistêmica seguida por dano muscular (DANNECKER et al., 2013; LENN et al., 2002; MALM, 2001; MALM et al., 2004).

Lenn et al. (2002) utilizaram marcadores inflamatórios (TNF-α e IL-6) e de estresse oxidativo (malondialdeído – MDA), para investigar o efeito do óleo de peixe na resolução do dano muscular induzido por exercício. Os autores realizaram um protocolo de 50 contrações excêntricas dos flexores de cotovelo, com alteração apenas de parâmetros funcionais e dor. Não foi observada alteração dos mediadores inflamatórios e oxidativos ao longo do tempo de análise (de 3 a 72 horas após a aplicação do protocolo), portanto os parâmetros utilizados para avaliar a eficácia do óleo de peixe para amenizar os efeitos do dano tecidual foram apenas os déficits funcionais e dor muscular tardia.

Malm et al. (2004) investigaram os efeitos de uma corrida de 45 minutos (*downhill running*) na expressão de marcadores inflamatórios (neutrófilos, monócitos e macrófagos, por exemplo), e citocinas circulantes (IL-6, IL-1β, TNF-α) por meio de amostras de músculo e sangue, respectivamente e não encontraram alterações nestes parâmetros, apenas déficit de função muscular e dor. Os autores concluem que exercícios, mesmo aqueles com grande componente excêntrico, não resultam em inflamação no músculo.

Posta esta divergência, é necessária a utilização de critérios para escolha de marcadores adequados para reprodução da magnitude do dano muscular, especialmente no que diz respeito à utilização de substâncias presentes na circulação sistêmica como preditoras de processo inflamatório localizado causado por exercício.

Obviamente, as controvérsias apontadas pelos estudos supracitados devem-se em grande parte às diferenças metodológicas encontradas em cada protocolo de exercício utilizado, bem como às características intrínsecas de cada população selecionada.

Faz-se necessária alguma cautela na utilização de parâmetros sistêmicos com o objetivo de avaliar o dano muscular induzido por exercício, uma vez que este pode ser influenciado por inúmeros fatores, podendo resultar ou não em processo inflamatório local, seguido por liberação de marcadores pró-inflamatórios e/ou pró-oxidativos na circulação sanguínea.

O estudo dos mecanismos envolvidos no dano muscular induzido por exercício, bem como suas consequências é de extrema importância no âmbito das ciências do exercício e do esporte, tanto no que diz respeito à prescrição de exercícios, quanto em relação ao manejo dos sintomas e limitações funcionais durante a aplicação de terapias de recuperação pós-esforço.

Em vista do exposto, o objetivo do presente estudo é investigar se existe uma relação entre os prejuízos funcionais e clínicos decorrentes do dano muscular induzido por exercício e a liberação de marcadores sistêmicos de inflamação e estresse oxidativo após a realização de exercício pliométrico.

## 1.2 Hipótese

O dano muscular induzido por exercício agudo, que resulta em déficits funcionais, é acompanhado por aumento sistêmico de citocinas pró-inflamatórias e espécies reativas de oxigênio, de maneira tempo-dependente.

## 1.3 Objetivo

Investigar se e por quanto tempo, o dano muscular induzido por exercício agudo é acompanhado pelo aumento de marcadores sistêmicos considerados clássicos para inflamação e estresse oxidativo, mecanismos intrinsicamente ligados ao processo local de dano muscular.

## 1.4 Artigo para publicação

O presente estudo originou um artigo científico, incluído a seguir, intitulado: ***"Systemic inflammatory and oxidative stress responses to plyometric exercise"***, ainda não submetido a periódico (formatação estilo Vancouver).

# Systemic inflammatory and oxidative stress responses after plyometric exercise

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## Abstract

**Background**: after a session of unaccustomed exercise, it is usually observed loss of muscle function and range of motion, soreness and swelling. Other markers, such as proinflammatory cytokines and reactive oxygen species are also utilized to evaluate EIMD as a signal of inflammation. However, there is not a conclusion about the association between the presence of inflammation caused by EIMD and the release of systemic markers of inflammation and oxidative stress after unaccustomed exercise. The aim of the present study was to investigate if there is a relationship among EIMD, presence of systemic inflammatory markers and alterations on systemic redox status. **Methods**: seven healthy young males (age: 25.7 ± 5.7 years; body mass: 76.3 ± 14.4 kg; body height: 176.0 ± 8.0 cm; BMI: 24.76 ± 4.89 kg/m$^2$) performed an acute session of plyometric exercise (5 sets of 20 drop jumps). Blood samples and information such as soreness, pressure pain threshold (PPT), rating of perceived exertion, thigh circumference and countermovement jump (CMJ) were collected before, immediately after, 0.5, 24, 48 and 72 hours post-exercise. Maximal isometric voluntary contraction (MIVC) was analyzed before exercise, 24, 48 and 72 hours post-exercise. **Results**: 100 drop jumps were able to produce significant amount of muscle damage, as it was observed by increased muscle soreness [significant higher at 24 ($p = 0.03$)], 48 ($p = 0.004$) and 72 hours ($p = 0.015$) after exercise, when compared to the baseline], and loss of function [CMJ presented a significant reduction at 48 hours after bout ($p = 0.03$), and MIVC was smaller at 24 ($p = 0.015$) and 48 hours ($p = 0.03$), when compared to the baseline]. . **Conclusions**: in spite of the presence of some classical sigs of local inflammation following an unaccustomed exercise session, systemic levels of proinflammatory cytokines and most of oxidative stress markers were not modified by the eccentric protocol. Therefore, the present study demonstrated that EIMD is not always accompanied by an increase of systemic proinflammatory cytokines or oxidative stress markers. Due to its complexity, the events involved in EIMD should be analyzed individually according to each protocol of exercise before selection of the best parameters to evaluate magnitude of EIMD.


**Keywords:** exercise-induced muscle damage, delayed-onset muscle soreness, inflammation, oxidative stress.

## Introduction

Unaccustomed exercise frequently causes muscle damage, inflammation, leakage of muscle proteins into the circulation and soreness on and several days after, which is called delayed-onset muscle soreness (DOMS), and is frequently considered as a symtom of exercise-induced muscle damage (EIMD)[1-6].

One of the most experimental design used to study the events that mediate the response to damage after exercise is the eccentric exercise (lengthening of a contracting muscle)[4,7]. It has been proposed to cause intracellular fiber swelling, which in turn is associated with the subsequent muscle soreness and stiffness in response to myofibrillar disorganization and consequent inflammation[8]. Thus, the EIMD causes local inflammation, which degenerates and regenerates muscle and surrounding connective tissue[5]. The consecutive events, like neutrophil activation, cell migration and patterns of cytokine production in the muscle itself have been reported in the last years[5,9,10,11,12].

Since the studies in humans suffer from the limitation of direct skeletal muscle assessment, the analysis of blood samples has been used to evaluate inflammation after EIMD[13,14]. Muscle soreness, lower range of motion (RM), swelling and reduced maximal voluntary isometric contraction (MVIC) are some of the most common clinical and functional parameters evaluated in the current literature. In addition, some proinflammatory cytokines, such as interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been widely used as systemic markers of inflammatory process induced by EIMD, in studies involving exercise or exercise recovery[5,14,15,16].

Due to its close relationship with the inflammatory process, oxidative stress has also been associated with EIMS and DOMS investigated in the last years[17]. Although some systemic proinflammatory and oxidative stress markers are extensively used to assess the presence of inflammatory process induced by EIMD and its magnitude[5,14,15,16,18], inflammation is a concept that classically involves 3 of 5 cardinal signs: heat, pain, redness, swelling, and loss of function[19], besides the proinflammatory markers usually evaluated. In the literature, the correlation among systemic increase of proinflammatory and estresse oxidative markers and the functional deficits induced by muscle damage are not frequently evaluated, as soon as their time-dependent relationship.

In this sense, the purpose of this study is to investigate if there is a correlation between the clinical and functional deficits as consequences of eccentric exercise-induced muscle damage and systemic inflammatory and oxidative stress markers after an acute bout of eccentric exercise.

## Materials and Methods

### Participants

Seven insufficiently physically active, healthy young males (age: 25.7 ± 5.7 years; body mass: 76.3 ± 14.4 kg; body height: 176.0 ± 8.0 cm; BMI: 24.76 ± 4.89 kg/m$^2$) volunteered for the present study. The participants were classified as physically active by IPAC (International Physical Activity Questionnaire)[20] and presented no musculoskeletal, bone and joint, or heart or lung diseases, and were not taking any medication. To participate in this study, volunteers could not be involved in any hard exercise or resistance training for at least six months before the exercise bout, take supplements, vitamins, analgesics, anti-inflammatory drugs, or participate in recovery strategies such as massage, stretching, or cryotherapy until the end of the tests. The present study was approved by the Human Ethics Committee of State University of Londrina [No. 1.083.339 – CAAE (Presentation of Certificate for Ethics) No. 0164.0.268.000-09.]. The subjects received and signed their informed consent before starting the tests.

### Experimental design

One week prior to exercise session, the volunteers were submitted to evaluation (body mass, stature and body mass index – BMI) . In this moment, the subjects also executed the maximal isometric voluntary contraction (MIVC) and performed the familiarization of exercise protocol.

Before the exercise session, perceived muscle soreness, perceived exertion, thigh circumference, pressure pain threshold (PPT), countermovement jump (CMJ) and blood samples were collected. Immediately after, the volunteers executed the exercise protocol and were submitted to revaluations of variables collected at baseline; perceived muscle soreness, perceived exertion, thigh circumference, PPT, CMJ and blood samples were repeated at 0,5; 24; 48; and 72 hours after eccentric exercise protocol. MIVC was executed one week before the exercise bout, 24, 48 and 72 hours after the protocol. Table 1 summarizes the moments of analysis and variables involved in the study.

**Exercise protocol**

Volunteers performed a plyometric exercise consisted by 100 drop jumps (5 sets of 20) from a platform with 60 cm of height. After land, they were encouraged to jump vertically with maximum possible effort. They were asked to execute 5 sets of 20 jumps, with 10-seconds interval between each jump and 2 minutes of rest between each set. This protocol previously demonstrated to produce muscle damage in physically active individuals[6].

**Perceived muscle soreness**

Time course of changes in delayed-onset muscle soreness following the drop jumps were determined using a visual analogue scale (VAS) with a 10-mm line. In this scale, the number zero is characterized by “total absence of pain” and the number 10 means “extremely sore”[21]. Participants completed a squat to approximately 90° of knee flexion before standing and immediately marked upon the scale to indicate their level of soreness.

**Thigh circumference**

Thigh circumference, the representative measure of swelling, was measured with an anthropomorphic measuring tape at 3 sites: 10 cm above the upper limit of the patella, halfway between the patella and the inguinal fold, and at the level of the gluteal fold. For all measurements, the tape was held snugly to the limb, without compressing any underlying soft tissues to ensure consistency. The same trained individual performed all measurements and sites were marked with semi-permanent ink to ensure consistent measurements between days[22].

**Pressure pain threshold**

Pressure pain threshold, the representative measure of pain, was investigated in *vastus lateralis* by a pressure algometer (*Wagner Instruments, FPX 50/220*). The measurements were performed in the place corresponding to the thigh circumference assessments and did not exceed 2.55 kgf[21]. The assay was executed by the same trained individual.

**Muscle function**

Loss of muscle function was assessed by the countermovement jump (CMJ) test using a contact mat and a gate unit (Smart Jump™, Fusion Sport, Sumner Park Australia), and MIVC using an isokinetic dynamometer (Biodex Medical Systems™, New York, USA).

For the countermovement jump assessment, participants started in a standing position and were instructed to perform a squat followed by a jump with the hands placed on the hips. The subjects executed 3 attempts with 60 seconds of interval between each one and they were encouraged to perform a maximal effort vertical jump. The highest jump obtained was taken for analysis[23].

For MIVC assay, volunteers performed three 5-s trials of maximal isometric knee extensor torque test, with a 3-min interval and a knee angle of 60° (full extension = 0°). The highest value was used for the analysis. Verbal encouragement was provided at every trial, as suggested by Roschel et al. (2011)[24].

**Blood samples**

Peripheral blood samples were collected after 8 hours of fasting, using 6 mL Na-heparin tubes and centrifuged at 2000 *x g*, for 10 minutes at 4°C**.** Plasma was separated into aliquots and stored at -20° C until the analysis of carbonyl proteins, cytokines and advanced protein oxidation products (AOPP) could be performed. Plasma and leukocytes were carefully removed and erythrocytes were washed three times with NaCl 0.9%. After last wash, erythrocytes were hemolyzed with deionized water for tert-butyl hydroperoxide-initiated chemiluminescence, oxygen uptake and catalase assays.

**Inflammatory cytokines**

TNF-α, IL-6 and IL-10 were quantified by commercial ELISA (Enzyme Linked Immuno Sorbent Assay) kits (eBioscience), according to the manufacturer's instructions. Absorbancies were read in duplicate and the results were expressed in pg/mL of plasma.

**Oxidative stress markers**

Lipid peroxidation was accessed by tert-butyl hydroperoxide (t-BHP)-initiated chemiluminescence (CL) in erythrocytes. The assay was performed in a Turner Designs luminometer TD 20/20 (response range 380-650 nm) according to the assays previously described[25,26] in a dark and temperature-controlled room (30º ± 1°C). The chemiluminesncence reaction was initiated by the addition of tert-butyl to the erythrocyte hemolysate, at final concentration of 3mM, and the resultant luminescence producted in the reaction was registered. Quantitative results were obtained after area under the curve extraction by time-dependent response versus luminescence emition integration.

(t-BHP)-induced oxygen uptake was assessed polarographically with a Clark-type oxygen electrode, at 37°C[27]. In this system, oxygen uptake is directly associated with the susceptibility to lipid peroxidation of erythrocyte membrane elicited by t-BHP[27].

The carbonyl protein content was assayed by ELISA, by means of the assay described by Alamdari et al. (2005)[28]. Carbonyl proteins are a modification caused by exposure of proteins to oxidative stress. The final absorbance was read at 490 nm. The results were calculated using an oxidized bovine serum albumin (BSA) standard curve, and were expressed in nmol of carbonyl proteins/mg of total protein.

Determination of Advanced Oxidation Protein Products (AOPP) levels in plasma samples were performed spectrophotometrically as described by Korkmaz et al. (2013)[29]. Plasma aliquots were diluted 1:5 in PBS to a final volume of 200 µL; 10µL of 1.16 M KI was added to each tube, and 20 µL of acetic acid was added two minutes later. The absorbance was immediately read at 340 nm. AOPP concentrations were expressed in equivalent µmoles of chloramine/mg of proteins.

Catalase activity was determined in hemolyzed erythrocytes, according to the technique described by Aebi (1984)[30]. The assay is based in the hydrogen peroxide decomposition, which is directly related to its absorption at 240 nm. Catalase activity was expressed in absorbance/minute/mg of protein.

**Statistical analysis**

Normality test of the data distribution was performed using Shapiro-Wilk's test. To compare moments of evaluations from parametric data, one-way analysis of variance (ANOVA) for repeated measures followed by Tukey's multiple comparison test was selected. For non-parametric data, Friedman with Dunn's post test was used. The level of significance was set to $P<0.05$.

The analysis of cytokines was performed based on the change among moments of revaluations considering the baseline (delta value), individually. Ordinary one-way ANOVA, followed by Tukey´s multiple comparisons test was used for parametric data and Kruskal-Wallis with Dunn's post test was used for non-parametric distribution.

Data were expressed as mean ± standard deviation (SD) (bar graphs, normal distribution), or median, minimum and maximum values (box plot graphs, non parametric distribution).

## Results

### Clinical and functional parameters

Figure 1 reports the results of functional and clinical parameters analyzed before and immediately after session, 0.5, 24, 48 and 72 hours following exercise. It was observed that the exercise protocol proposed was sufficient to generate significant EIMD. Furthermore, some clinical and functional deficits, as enhanced perceived muscle soreness, diminished CMJ and MIVC, were coincident 48 hours after protocol application.

Muscle soreness was significantly higher at 24 ($p = 0.03$), 48 ($p = 0.004$) and 72 hours ($p = 0.015$) after exercise when compared to the baseline. CMJ presented a significant reduction 48 hours after the bout ($p = 0.03$) and MIVC was smaller at 24 ($p = 0,015$) and 48 hours ($p = 0.03$) in the same comparison. PPT and thigh circumference did not present any significant time effect.

### Inflammatory cytokines

Figure 2 shows  pencentual variation of citokines levelsnormalized by the baseline from each subject. It was not observed any significant alteration (increase or decrease) in the TNF-α, IL-6 or IL-10 analysis.

### Oxidative stress markers

Table 2 contains statistical analysis of oxidative stress markers. It was observed a significant reduction ($p = 0.015$) in the area under CL curve 48 hours after the protocol application (275.2 ± 87.41), when compared to IA moment (357.8 ± 94.74). Values of erythrocytes $O_2$ uptake, CP and catalase did not present any significant difference over time. AOPP, an oxidative stress marker related to inflammatory process, also did not demonstrate any statistical difference.

## Discussion

It is well established that unaccustomed exercise, particularly exercise based on eccentric contractions, causes DOMS[4,31,32]. The symptom varies depending on intensity and duration of the exercise performed, but muscle soreness is common to peak between 1 and 2 days following an unaccustomed session. In the present study, increased DOMS started from 24 hours after session, being most intense muscle soreness observed at 48 hours ($p = 0.004$) after eccentric exercise. In addition, several studies have investigated the functional consequences of DOMS and had demonstrated that muscle soreness causes a prolonged inability of achieving maximal voluntary contractions[4,31]. In the present study, it was noted the presence of significant loss of muscle function.

CMJ and MIVC declined over time and reflected loss of muscle function after exercise protocol in the present study. These data are in agreement with other studies that also utilized MIVC as an indirect marker of muscle damage and loss of function[4,31,32].

There is a consensus in the specific literature that the local inflammatory response to unaccustomed exercise is accompanied by a systemic leakage of proinflammatory and oxidative stress markers[1,3,31,35,36]. Based on this assumption, several studies have been using these markers as parameters to evaluate EIMD magnitude and even the effectiveness of post-exercise recovery methods after EIMD[31,37,38]. Houghton et al. (2012)[39] investigated the effect of eicosapentaenoic acid (EPA) supplementation on DOMS symptoms. The authors proposed a single bout of resistance exercise (3 sets of 10 repetitions of four inferior member exercises at 70% of one-repetition maximum), and it was sufficient to induce significant muscle soreness, the parameter used to assume that some inflammation was induced by the protocol. Besides DOMS, the authors also found loss of function (maximal voluntary contraction) and an increase of IL-6 levels 48 hours following the exercise bout, which was positively correlated with EIMD.

However, some studies have contradictory results regarding the systemic and even local presence of proinflammatory cytokines after acute resistance exercise[37,40]. Lenn et al. (2002)[37] investigated the effects of fish oil and isoflavones on DOMS. The authors induced muscle soreness into the nondominant arm by the performance of 50 maximal effort eccentric contractions at a 90°/s using an isokinetic dynamometer. The protocol succeeded on inducing DOMS and loss of muscle function, but these events were not accompanied by a systemic proinflammatory response. The authors performed analysis of TNF-α, IL-6 and malondialdehyde levels before, immediately after, 3 h, 24 h, 48 h, and 72 h after the exercise bout, in order to access efficiency of fish oil and isoflavones on treatment of DOMS. In the present study, the execution of 100 drop jumps was able to produce significant amount of DOMS and loss of muscle function without any significant time effect regarding the levels of systemic inflammatory markers; TNF-α, IL-6 and AOPP.

Muscular adaptation to physical exercise has been explained by the classical damage–inflammation–repair pathway for many years[1,3,35]. By definition, this process involves: exercise-induced muscle damage; release of chemo-attractive factors; vasodilatation; leukocyte adhesion; neutrophils and macrophages migration, and activation of satellite cells[1]. However, there are some evidences that question this hypothesis[40,41]. Malm et al. (2004)[41] demonstrated that eccentric exercise (45 min of downhill running) did not result in skeletal muscle inflammation 48 h post exercise (as demonstrated by quantitation of macrophages in muscle samples), despite DOMS and increased creatine kinase can eventually be found. Przybyla et al. (2006)[41] showed that after an acute resistance exercise session (three sets of eight repetitions of bilateral leg press, leg curl, and leg extension followed by a fourth set to voluntary failure for each of the three exercises with resistance of 80% 1-RM), the majority of macrophages resident to skeletal muscle display anti-inflammatory characteristics at rest and these numbers increase in response to resistance exercise, and that a relatively small subpopulation dictates the pro-inflammatory response. It can explain the lack of sistemic proinflammatory citokines increase in the present study.

Regarding oxidative stress parameters, the classical damage–inflammation–repair pathway points a secondary damage in the injured muscle by releasing of reactive oxygen species by neutrophils, which can impair cell membranes[1]. Furthermore, exercise can increase the production of free radicals as a result of oxygen consumption[14]. The present protocol did not show significant alterations in systemic levels of AOPP, an oxidative stress marker related to inflammatory process. AOPP are proteins that are damaged by oxygen reactive species, formed primarily by chlorinated oxidants, including hypochloric acid and chloramines, which result from myeloperoxidase activity from neutrophils[29].

## Conclusions

Exercise muscle damage is a complex event that can lead to muscle soreness, loss of function and swelling, depending on intensity, duration and category of exercise. The exercise protocol chosen in the present study was able to promote functional muscle deficits, although it was not mandatorily accompanied by release of systemic proinflammatory cytokines or alterations in redox balance.

## Acknowledgments

The authors are immensely grateful to all the volunteers who kindly participated in this study; to all the members of Laboratory of Pathophysiology and Free Radicals, supervised by Dr. Rubens Cecchini, where the oxidative stress analysis were performed and to Jesus A. Vargas, for the excellent technical support dispensed.

## Author Disclosure Statement

The authors have nothing to disclose. None of the authors had any conflict of interest.

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## Tables and figures

**Table 1** - Distribution of moments of analyses according to the variables of the study

| Variables | basal | IA | 0,5h | 24h | 48h | 72h |
|---|---|---|---|---|---|---|
| Blood samples | X | X | X | X | X | X |
| Perceived muscle soreness | X | X | X | X | X | X |
| Perceived exertion | X | X | X | X | X | X |
| Thigh circumference | X | X | X | X | X | X |
| Pressure pain threshold | X | X | X | X | X | X |
| CMJ | X | X | X | X | X | X |
| MIVC | X | | | X | X | X |

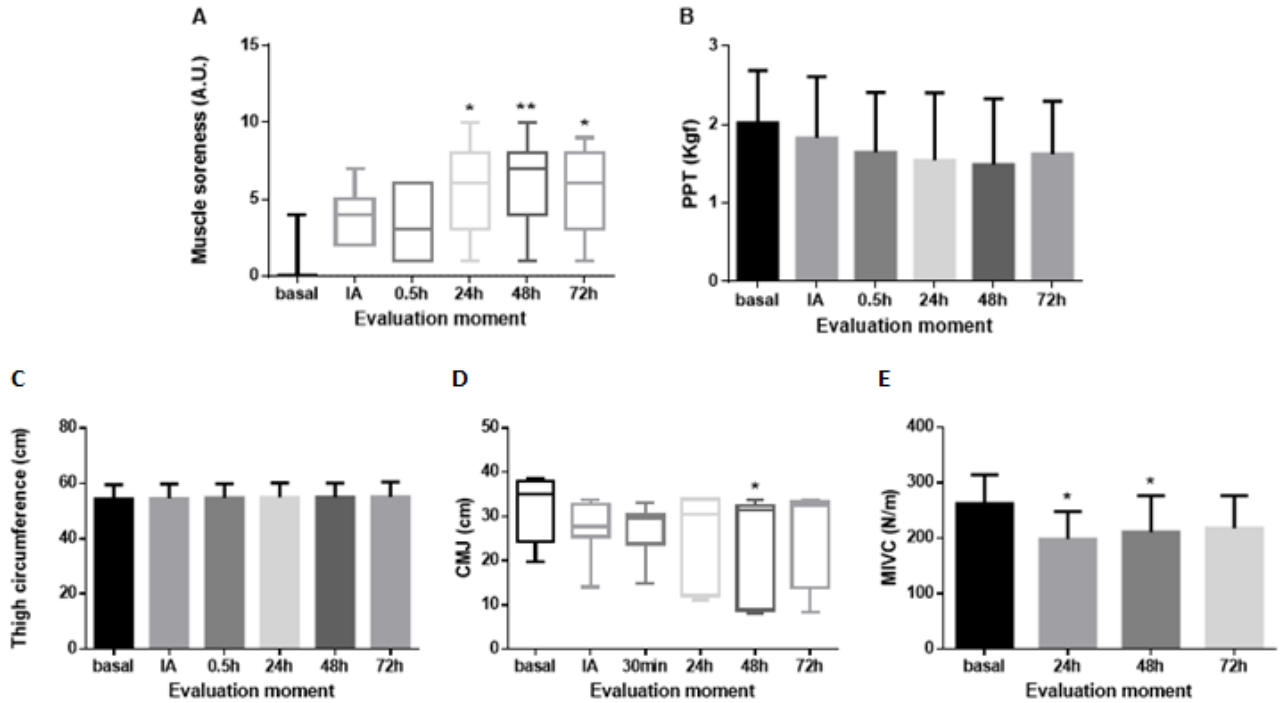
Basal: before protocol; IA: immediately after protocol; 0,5h: 30 minutes after protocol; 24h: 24 hours after protocol; 48h: 48 hours after protocol; 72h: 72 hours after protocol. CMJ: countermovement jump; MIVC: maximal isometric voluntary contraction.

**Table 2** - Estresse oxidative markers

| | **Basal** | **IA** | **0,5h** | **24h** | **48h** | **72h** |
|---|---|---|---|---|---|---|
| **CL** (area under CL curve) | 332.6±126.9 | 357.8±94.74 | 276.9±64.25 | 271.2±70.37 | 275.2±87.41***** | 288.8±84,26 |
| **$O_2$ uptake** (nmol $O_2$/min/mg of protein) | 0.481±0.156 | 0.49±0.156 | 0.513±0.187 | 0.5413±0.164 | 0.4546±0.14 | 0.5013±0.147 |
| **CP** (nmol of carbonyl proteins/mg of protein) | 889.1±180.3 | 936.6±145.9 | 890.0±152.1 | 979.6±224.7 | 924.2±133.2 | 941.6±224.6 |
| **AOPP** (µmol of chloramine/mg of protein) | 1.36 (0.77; 2.92) | 1.271 (0.88; 2.78) | 1.321 (0.62; 3.32) | 1.46 (0.66; 2.61) | 1.11 (0.82; 2.49) | 1.09 (0,86;2,84) |
| **Catalase** (absorbance/minute/mg of protein) | 0.016±0.002 | 0.016±0.001 | 0.017±0.002 | 0.018±0.002 | 0.019±0.004 | 0.018±0.003 |

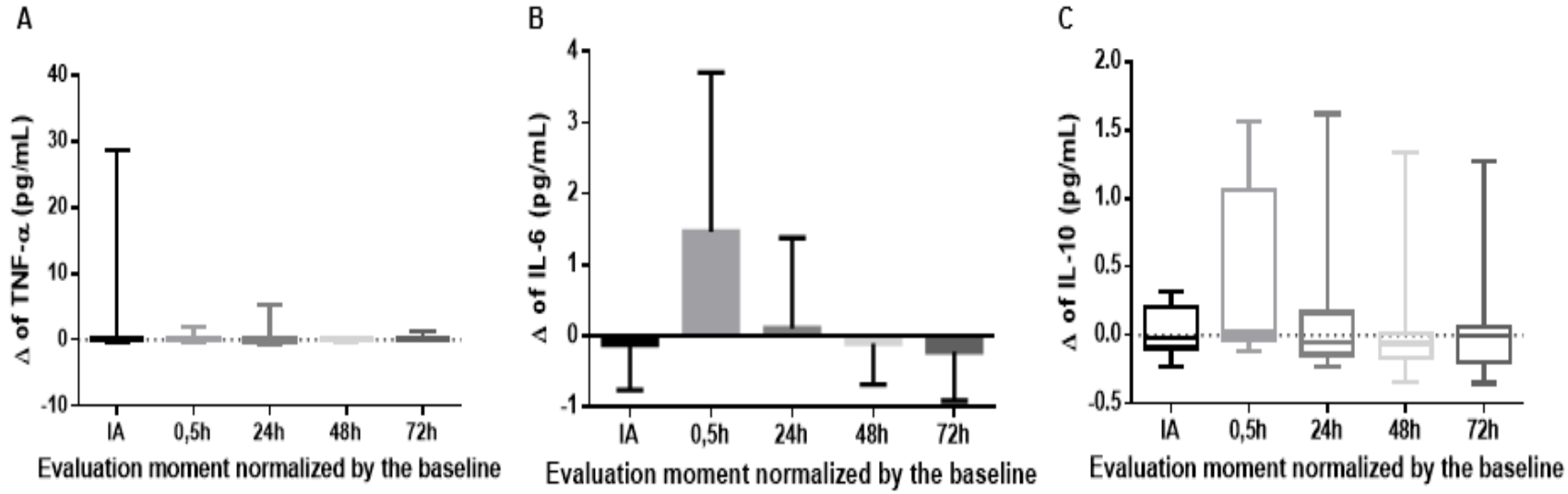
Mean and standard deviation of tert-butyl hydroperoxide-initiated chemiluminescence (CL), erythrocyte oxygen ($O_2$) uptake, carbonyl proteins (CP), catalase and median, minimal and maximal values of advanced oxidation protein products (AOPP) at basal (moment before protocol), IA (immediately after protocol), 0.5h (30 minutes after protocol) 24h (24 hours after protocol), 48h (48 hours after protocol) and 72h (72 hours after protocol).*$p<0.05$: IA vs.48h. IA: immediately after protocol; 0.5h: 30 minutes after protocol; 24h: 24 hours after protocol; 48h: 48 hours after protocol; 72h: 72 hours after protocol.

**Figure 1** - Clinical signs and symptoms of EIMD




**A**: median, interquartile range and minimal and maximal values of muscle soreness (arbitrary units). *p<0.05: in relation to basal; **p<0.01: in relation to basal. **B**: mean and standard deviation of PPT (kgf). **C**: mean and standard deviation of thigh circumference (cm). **D**: media, interquartile range and minimal and maximal values of CMJ. *p<0.05: in relation to basal. **E**: median, interquartile range and minimal and maximal values of MVIC (N/m). *p<0.05: in relation to basal. **A** and **D**: Friedman test. **B**, **C** and **E**: one-way ANOVA of repeated measures. CMJ: countermovement jump, MIVC: maximal isometric voluntary contraction; basal: moment before protocol; IA: immediately after protocol; 0.5h: 30 minutes after protocol; 24h: 24 hours after protocol; 48h: 48 hours after protocol and 72h: 72 hours after protocol. n=7.

**Figure 2** - TNF-α concentration according to evaluation moments




**A**: median (horizontal line), interquartile range (box) and minimal and maximal values (whiskers) of the difference (∆) of TNF-α concentration between each evaluation moment and basal (moment before protocol) level. Kruskal-Wallis followed by Dunn's multiple comparisons test. **B**: Mean (bar) and standard deviation (error bar) of the difference (∆) of IL-6 concentration between each evaluation moment and basal level. One-way ANOVA, followed by Tukey´s multiple comparisons test. **C:** Median (horizontal line), range (box) and minimal and maximal values (whisker) of the difference (∆) of IL-10 concentration between each evaluation moment and basal levels. Kruskal-Wallis followed by Dunn's multiple comparisons test. IA: immediately after protocol; 0.5h: 30 minutes after protocol; 24h: 24 hours after protocol; 48h: 48 hours after protocol; 72h: 72 hours after protocol.

## 1.5 Conclusões

O protocolo escolhido no presente estudo foi capaz de promover uma magnitude de dano muscular que não é acompanhada por alterações significantes na quantidade de citocinas próinflamatórias e marcadores sistêmicos de estresse oxidativo. Conclui-se, a partir do exposto, que o processo inflamatório local gerado por exercício nem sempre pode ser extrapolado para vias sistêmicas, quando considerados marcadores de estresse oxidativo e inflamação, haja vista a complexidade dos eventos envolvidos na liberação dessas substâncias e os vários fatores que podem interferir na magnitude do dano, como duração, intensidade e categoria de exercício, além das características dos praticantes, como idade, sexo e nível de atividade física.

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# 2. Capítulo 2. Estresse oxidativo e inflamação nas respostas ao treinamento resistido em portadores de síndrome metabólica

## 2.1 Introdução

### 2.1.1 Síndrome Metabólica

A Síndrome Metabólica (SM) é considerada, atualmente, o maior problema de saúde pública dos países de cultura ocidental e um dos principais desafios da prática clínica (FRANCISQUETI et. al., 2017). Estimativas da Federação Internacional do Diabetes (FID) apontam que um quarto da população mundial seja portador de SM. Além disso, a doença está associada a complicações cardiovasculares, as principais causas de morte no planeta (FRANCISQUETI et. al., 2017). A SM é definida pela FID e pela Organização Mundial da Saúde (OMS) como a ocorrência de um conjunto de fatores de risco cardiovascular, tais como obesidade abdominal, dislipidemia, hipertensão arterial (HA) e hiperglicemia (ALBERTI et al., 2009; WENG et al., 2012).

O aumento da prevalência da SM nos países desenvolvidos e industrializados está intimamente associado ao aumento da obesidade (GOLBIDI et al., 2012). Nos últimos 30 anos, o estilo de vida sedentário da população e o aumento da disponibilidade de comida levaram a um considerável aumento da prevalência e incidência da obesidade e consequente elevação das taxas de SM (GOLBIDI et al., 2012). O aumento de gordura abdominal está positivamente correlacionado com a ocorrência da SM, sendo um dos principais fatores que compõem a síndrome (PEYROL et al., 2017). A gordura abdominal promove estresse oxidativo e inflamação sistêmica, que são precursores das complicações que envolvem os componentes da SM (DUTHEIL et al., 2012; GOLBIDI et al., 2012; FRANCISQUETI et. al., 2017).

O aumento na geração de espécies reativas de oxigênio (ERO) parece possuir papel central na fisiopatologia da SM (GRATTAGLIANO et al., 2008), estando presente no mecanismo inicial de desenvolvimento da doença, e não apenas como mera consequência da sua instalação (YUBERO-SERRANO et al., 2013). A geração de ERO consiste em um processo fisiológico, decorrente do metabolismo aeróbio e importante na manutenção da homeostase intracelular (PEYROL et al., 2017). No entanto, apesar da existência de mecanismos antioxidantes intracelulares, como as enzimas superóxido dismutase (SOD), catalase e glutationa reduzida (GSH), o excesso da produção de espécies reativas de oxigênio (ERO) é prejudicial à fisiologia celular (PEYROL et al., 2017). Na SM, a inflamação e o estresse oxidativo crônico gerados pelo aumento da gordura visceral são precursores de várias complicações envolvendo seus componentes, como resistência à insulina, hipertensão e hiperlipidemia (DUTHEIL et al., 2012; GOLBIDI et al., 2012; FRANCISQUETI et. al., 2017).

O acúmulo de triglicerídeos e hipertrofia do tecido adiposo visceral leva à irrigação insuficiente das células, falta de oxigênio e consequentemente, necrose dos adipócitos (FRANCISQUETI et. al., 2017). O processo de fagocitose das células mortas leva, além de infiltração de células inflamatórias, à produção de ERO, como mecanismo de defesa (FRANCISQUETI et. al., 2017). Essas espécies reativas, particularmente o peróxido de hidrogênio é convertido pela mieloperoxidase (MPO) em hipoclorito e cloraminas, espécies reativas que podem oxidar proteínas, produzindo os produtos avançados de oxidação de proteínas (AOPP), que nos portadores de SM é 60% maior do que em indivíduos saudáveis (VENTURINI et al., 2015). Além disso, a hipertrofia do tecido adiposo, por si só, aumenta a liberação de adipocinas, como TNF-α, IL-6 e IL-1β, que são citocinas proinflamatórias que inicialmente são liberadas em excesso pelo tecido adiposo e depois atingem a circulação, levando, em longo prazo, a um quadro de inflamação sistêmica de baixo grau (PEYROL et al., 2017).

As próprias ERO também são responsáveis pela exacerbação da inflamação sistêmica, uma vez que são capazes de ativar o fator nuclear-kappa B (NF-κB), um fator transcricional, que sinaliza a cascata do processo inflamatório e também a síntese de diversas proteínas, promovendo a síntese contínua e moderada de espécies reativas e inflamação sistêmica (GACIA-BAILO et al., 2011). Uma das citocinas proinflamatórias secretadas pelo tecido adiposo, o TNF-α, leva ao desenvolvimento de resistência à insulina (RI), pois impede a fosforilação do receptor de insulina na membrana plasmática das células (FRANCISQUETI et. al., 2017). A RI terá como consequência a hiperglicemia e aumento de ácidos graxos livres, que em longo prazo podem levar ao desenvolvimento de diabetes tipo 2 (DM2) e dislipidemia, respectivamente (FRANCISQUETI et. al., 2017).

Conforme o exposto, a obesidade, um dos principais fatores que compõem o conjunto de componentes presentes na SM, é caracterizada pelo excesso de produção de citocinas proinflamatórias e ERO, que são precursores das demais complicações relacionadas à SM (PEYROL et al., 2017). Além disso, a hiperglicemia, o aumento de ácidos graxos livres no plasma e hiperinsulinemia têm sido interligados ao aumento da produção de ERO (GACIA-BAILO et al. 2011), o que se configura como um ciclo vicioso, em que obesidade gera inflamação sistêmica e excesso de produção de radicais livres; estes, por sua vez, induzem às complicações associadas à síndrome e estas perpetuam o quadro de estresse oxidativo e inflamação.

Pressupõe-se, a partir disso, que estratégias que combinem a redução de massa corpórea, aliadas a tratamentos dos demais fatores de risco cardiovascular presentes na SM, controle do quadro de estresse oxidativo crônico e inflamação sistêmica, seriam eficazes no tratamento desta doença.

### 2.1.2 Treinamento resistido na Síndrome Metabólica

A maneira mais eficiente para tratar os componentes da SM parece constituir na redução da ingestão calórica associada à prática de exercício físico (SAKURAI et al., 2017). Um dos principais objetivos dos programas de exercício para portadores de SM, portanto, consiste na redução de massa corporal (SAKURAI et al., 2017). Outro aspecto importante é a inclusão de exercícios resistidos, em função da sarcopenia, isto é, a perda natural e progressiva de força e massa muscular, a partir, aproximadamente, da quinta década de vida (JANSSEN et al., 2000; OGAWA, YAKABE e AKISHITA, 2016).

O exercício crônico reduz a expressão de citocinas proinflamatórias e geração de ERO pelo tecido adiposo, promovendo efeitos anti-inflamatórios, antioxidantes e reduzindo o estresse metabólico em longo prazo, sendo, portanto, coadjuvante no tratamento das complicações associadas à SM (LEMOS et al., 2012; SAKURAI et al., 2017). Exercícios regulares também são considerados um método terapêutico efetivo no controle dos componentes da SM, uma vez que melhorariam o status metabólico e sensibilidade à insulina, reduzindo o risco de desenvolvimento de doenças cardiovasculares (LEMOS et al., 2012).

Exercícios resistidos regulares, além de benéficos no tratamento das comorbidades envolvidas na SM, seriam eficazes na prevenção da perda de força e massa muscular, inerentes ao processo de envelhecimento (DUTHEIL et al., 2012).

Recentemente, novos métodos de treinamento têm sido utilizados, como os exercícios funcionais, definidos como exercícios que desenvolvem, em uma única sessão, diferentes capacidades físicas simultaneamente, como força, resistência, agilidade, propriocepção e controle neuromuscular (NEVES et al., 2017), ao contrário dos exercícios convencionais de musculação, realizados em aparelhos, por exemplo, que isolam o trabalho dos músculos, enfatizando apenas o ganho do trofismo muscular (BERTAZZO, 2010; BIENFAIT, 1999).

Além disso, os exercícios funcionais parecem se mostrar eficientes em relação à melhora no perfil lipídico, quando comparados a outros métodos. Em mulheres pós-menopausa, o treino resistido combinado ao aeróbio parece ser tão eficaz quanto o treinamento funcional na redução de gordura corporal, mas apenas o treinamento funcional seria capaz de reduzir os níveis de LDL-colesterol (NEVES et al., 2017).

Dessa forma, entende-se como pertinente elaborar um método de treinamento, que seja eficaz no tratamento das complicações que compõem a Síndrome Metabólica, levando em consideração a perda de força e massa muscular e demais complicações inerentes ao processo de envelhecimento.

## 2.2 Hipótese

O treinamento funcional apresenta melhores repercussões sobre o perfil oxidativo, inflamatório e sobre o risco cardiovascular em portadores de Síndrome Metabólica, quando comparado ao treinamento convencional de musculação.

## 2.3 Objetivos

### 2.3.1 Objetivo geral

Avaliar os efeitos de dois programas de exercícios resistidos sobre o perfil oxidativo, inflamatório e risco cardiovascular de indivíduos portadores de Síndrome Metabólica.

### 2.3.2 Objetivos específicos

- Verificar se a aplicação de dois protocolos de treinamento resistido interfere nos fatores de risco cardiovascular presentes na SM;
- Quantificar marcadores de estresse oxidativo sistêmico em indivíduos com SM antes e após aplicação de dois protocolos de treinamento resistido.

## 2.4 Artigo para publicação

O presente trabalho originou um artigo científico, incluído a seguir, que intitula-se: **"*Impact of two resistance exercise programs in oxidative status and cardiovascular risk factors of patients with metabolic syndrome*"** e encontra-se submetido ao periódico *Metabolic Syndrome and Related Disorders* [FI (2015) – 2.121; Qualis Medicina II – B1], em formatação própria da revista.

# Impact of two resistance exercise programs in oxidative status and cardiovascular risk factors of patients with metabolic syndrome

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**Running title:** MS oxidative stress under training programs

Funding source: Fundação Araucária, Paraná, Brazil.

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## Abstract

**Background**: metabolic syndrome (MS) is considered a serious condition that comprises a number of cardiovascular risk factors. Physical exercise practicing is one of the best treatment and prevention options, although there is a challenge in prescription of exercises for patients with metabolic syndrome: reducing mass without affecting lean body mass. The purpose of this study was to investigate the impact of two resistance exercise programs on inflammatory and oxidative status and cardiovascular risk factors of volunteers diagnosed with MS. **Methods**: the study analysed 54 adults, both sex, (50.87±5.67 years, 83.14±14.19kg, 166±10,4cm), randomized to exercise groups or to a sedentary group (control). Functional (FT) and conventional training (CT) groups performed 12 weeks of systematized training, 3 times per week and control (CTRL) was instructed to not change habitual activities. Cardiovascular risk factors, muscular strength, inflammatory and stress oxidative markers were analyzed before and after training. **Results**: muscular strength was improved in both training groups when compared to CTRL ($p<0.05$). CT presented an increase of SOD and catalase at the end of training and a significant decrease in tert-butyl hydroperoxide-initiated chemiluminescence ($p<0.05$) after intervention when compared to CTRL. FT presented significantly reduction in systolic blood pressure and increase in catalase activity ($p<0.05$). CTRL presented worsening HDL levels at the end of the period. **Conclusions**: both programs improved muscular strength, antioxidant defense and maintained HDL levels. Therefore, resistance training can be considered an effective long-term therapy for abnormalities that comprise metabolic syndrome.



**Keywords**: metabolic syndrome, resistance training, oxidative stress, inflammation, cardiovascular risk factors.

Trial registration: RBR-8rz4yq (http://www.ensaiosclinicos.gov.br/).

## Introduction

The prevalence of Metabolic Syndrome (MS), associated with risk of cardiovascular disease, has shown alarming results in obese people, and the excess of body fat in this population has shown to provoke subclinical inflammation[1]. Although insulin resistance has been indicated as the main mechanism underlying the development of metabolic syndrome, several studies have suggested that chronic oxidative stress and low-level inflammation are an early event in the pathogenesis of cardiovascular risk factors associated with MS[1-5].

Increased lipid peroxidation and decreased antioxidant defense in patients with visceral obesity, fatty liver and MS were already reported, pointing to a correlation among visceral fat accumulation, MS and systemic oxidative markers[4]. Some authors have suggested that the most efficient strategy to treat MS is to reduce overweight and practice physical exercise with the challenge of reducing fat mass without affecting lean body mass[6-10]. Recently, new training methods have been used, such as functional exercises, defined as exercises that develop different physical capacities in a single session, such as strength, endurance, agility, proprioception and neuromuscular control[11], in contrast to conventional resistance training, that isolate muscle action, emphasizing the trophic gain[9].

In this sense, and considering: (1) the challenge of reducing fat mass without affecting lean body mass in people with MS; (2) the progressive loss of lean mass and strength as age progresses; and (3) the benefits of a functional training program, the present study proposed to investigate two resistance training programs (functional and conventional) in order to establish which program of exercises could benefit patients with metabolic syndrome. Thus, the main goal of the present study was to evaluate the impact of two different supervised resistance training programs on oxidative stress markers, systemic inflammatory status and cardiovascular risk factors in untrained patients previously diagnosed with MS.

## Methods

### Subjects

74 volunteers of both sexes, aged from 40 to 60 years, presenting the characteristics of the MS as defined by the International Diabetes Federation (IDF) criteria in 2005[12], were recruited by a cardiologist. Exclusion criteria included: participation on strength training programs for at least six months previously to the present study. Inclusion criteria: do not smoke or consume alcohol, do not use anti-inflammatory drugs, antioxidant and vitamin supplements, absence of infectious disease or musculoskeletal injuries.

Volunteers were informed about the objectives and procedures of the study and agreed to participate by signing an informed consent. This statement also contained a medical consent to allow the exercise practice. The study received approval from the Human Research Ethics Committee of the State University of Londrina (5231).

**Study design**

Prior to the data collection, one week was dedicated exclusively to the one repetition maximum (1RM) test and familiarization of the participants with the equipment and exercise protocol. Table 1 displays the evolution of the training loads and repetitions.

A computer-generated random list was used for allocation. The volunteers were randomly assigned into three groups: conventional or functional training groups (12-week periodized training program, 3 times per week) and a control group (CTRL). CTRL did not perform the exercise training program, but only the collection of information and blood samples. Figure 1 displays the flowchart of the distribution of the volunteers in each group and losses throughout the study, due to withdraw and hemolysis of some blood samples. The number of blood samples analyzed were showed in each figure legend.

**Training programs**

Training sessions were supervised by physical education professionals and consisted of 8 exercises (5 for upper body and 3 for lower body). Upper body and lower limb exercises were the same in both training programs, functional training (FT) and conventional training (CT). For the type of training considered functional (FT), upper body exercises were adapted with inclined boards and unstable surfaces, providing the work out of different muscle groups simultaneously.

For CT, upper body exercises were composed by bench press, behind neck lat pulldown, triceps and biceps pulley and upright row. Participants from FT performed bench press and behind neck lat pulldown with the inclined board in the supine position to provide contraction of anterior chain. For triceps and biceps pulley, the inclined board was used in the prone position, providing contraction of the entire posterior chain. An unstable surface was used for upright row exercise, providing contraction of the lower limbs and constant proprioception. The lower limb exercises were the same for both training programs and were consisted of leg press, knee extension and leg curl. Table 1 shows the progression loads over 12 weeks. The weeks 4 and 9 were recuperative (the participants did not realize any exercise). Participants from CTRL were instructed to keep their normal activities. All evaluations were performed just before or just after interventions.

**Muscular strength**

Muscular strength was accessed by 1RM test, that started with weights between 30-50% of body mass for lower limbs and 10-20% for upper limbs. Increments of 20-30% for lower limbs and 5% for upper limbs were determined based on the subject's perception until reaching the maximum load, in which the subject could execute the movement without mechanical failure. No more than five attempts were allowed for the determination of maximum load, otherwise the test was considered invalid and the subject must repeat the procedure in another day[13].

**Blood samples and cardiovascular risk factors**

Blood samples were collected after 12 hours of fasting for triglycerides, fasting plasma glucose and HDL-cholesterol analysis, assayed in a clinical laboratory. Samples were collected in the day before the first session and in the day after the last session. For oxidative stress analysis, blood samples were collected before execution of the first exercise session of the program, and before the beginning of last session. CTRL was submitted to the same period of blood collection. Blood pressure was accessed after 15 minutes resting, in sitting position, and waist circumference was measured with the subject in orthostatic position. The same licensed professional collected blood pressure and waist circumference measurements. Body weight and height were accessed in a standard digital scale (Tanita BC 554, Ironman/Inner Scanner, Illinois, USA) and a portable stadiometer, respectively.

**Oxidative stress markers**

Lipid peroxidation was accessed by tert-butyl hydroperoxide-initiated chemiluminescence (CL) in plasma. The assay was performed in a Turner Designs luminometer TD 20/20 (response range 380-650 nm) according to the assays previously described[14,15] in a dark room and controlled temperature (30º ± 1°C). The chemiluminescence reaction was initiated by the addition of tert-butyl at final concentration of 3mM, and the resultant luminescence producted in the reaction was registered. Quantitative results were obtained after area under the curve integration. Results were expressed in percentage difference of the arbitrary units (area under CL curve).

Total thiols reflect total redox status from plasma and were quantified in plasma, using a spectrophometric method based on the reaction of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB; Sigma-Aldrich™, St. Louis, MO, USA) with sulfhydryl groups to yield a colored product at 412nm[16]. Results were calculated using a calibration curve provided by known concentrations of GSH (Sigma-Aldrich™, St. Louis, MO, USA), and the results were expressed in µmol GSH/mL of plasma.

Superoxide dismutase activity was assayed in erythrocyte hemolysate based on the technique described by Marklund & Marklund (1974)[17]. The test is determined by inhibition of pyrogallol oxidation in aqueous solution, which results in appearance of yellow color at 420nm. Results were expressed as USOD/mg of protein. Catalase activity was determined in erythrocyte hemolysate, according to Aebi (1984)[18]. The assay is based in the hydrogen peroxide decomposition, which is directly related to its absorption at 240 nm. Results were expressed in absorbance/minute/mg of protein.

The total radical-trapping antioxidant parameter (TRAP) was quantified in plasma according to Repetto *et al*. (1996)[19]. This technique evaluates total antioxidants levels in a determined sample. We used 2,2′-azo-bis (2-amidinopropane) (ABAP) as a potent free radical generator that degrades itself to emit photons in a process that is amplified by the addition of luminol and can be quantified by a luminometer. The photon emission profile was measured in a Turner Designs luminometer TD 20/20, with sensitivity of 5 readings/second. Results were expressed in equivalent µmoles of Trolox/mL plasma.

Determination of advanced protein oxidation products (AOPP) levels in plasma was performed spectrophotometrically as described by Korkmaz *et al*. (2013)[20]. Plasma was diluted 1:5 in PBS to a final volume of 200 µL and AOPP concentrations were expressed in equivalent µmoles of chloramine/mL plasma.

**Statistical analyses**

A two-tailed hypothesis test was performed, with a 5% level of significance and 80% test power. A sample size of 21 participants per group was then stipulated[21]. Shapiro-Wilk was used to verify normality of data distribution. For comparisons of moments before and after training of each group, paired t test was used for parametric data and Wilcoxon test was used for non parametric data, when appropriate.

To compare data between groups, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was utilized for parametric data. For non-parametric data, Kruskal-Wallis with Dunn's post test was used. The level of significance was set to $p \leq 0.05$[22]. Data were expressed as mean ± standard deviation (SD) (bar graphs, normal distribution), or median, minimum and maximum values mean (box plot graphs, no normal distribution). Percentage change of parameters analyzed, between first and last session were calculated and transformed in percentage of increase or decrease (Δ%), individually. Positive values mean that the parameter increased after 12 weeks, and negative values that absolute value in that parameter decreased after 12 weeks of training.

**Results**

Comparative analysis between groups presented any significant difference regarding anthropometric variables and age, as demonstrated in table 2. Muscular strength revealed that both CT and FT increased strength after 12 weeks, when pre-training and post-training moments were compared (data not shown). Table 3 shows the results of 1RM test before/after CT and FT in comparison to CTRL. Both training programs improved in part muscle strength, when compared to CTRL. Bench press, behind neck lat pulldown and upright row, did not demonstrate any significant difference in all comparisons.

As showed in Table 4, CTRL presented decrease in HDL-cholesterol levels ($p<0.05$) at the end of the period of analysis (before training: 44.45 ± 9.9 mg/dL; after training: 42 ± 9.22 mg/dL). Additionally, FT promoted reduction of systolic blood pressure ($p<0.05$) after 12 weeks [before training: 123.3mmHg (103.3 mmHg; 163.3 mmHg); after training: 116.7 mmHg (90 mmHg; 160 mmHg)]. All the other parameters evaluated showed no statistical difference.

**Oxidative stress markers**

The analysis of tert-butyl hydroperoxide-initiated chemiluminescence revealed decline in Δ% of plasma lipid peroxidation in CT when compared to CTRL. After 12 weeks, CT demonstrated 4.2x less lipid peroxidation (when median was considered, in arbitrary units, $p<0.05$) than CTRL group. FT showed 1.9x of decreasing in the same comparison ($p>0.05$). The analysis of plasma redox status by means of total thiols (Panis et al., 2012) showed significant decrease in Δ% CT ($p=0.0002$) and FT ($p=0.0016$), when compared to CTRL. No significant difference was detected between trained groups (Figure 2B).

When erythrocyte enzymatic activity was evaluated, SOD activity from CT group demonstrated to have significant positive increase on Δ% (p= 0.0072) at the end of 12 weeks period, when compared to CTRL (Figure 3A). Although the magnitude was smaller than SOD, the percentage change of catalase activity significantly increased in both CT (p=0.0044) and FT (p= 0.013), as showed in Figure 3B. No significant differences were observed in AOPP plasma levels and plasma total antioxidant capacity (Figure 4A and B).

**Discussion**

It is well established that resistance training induces differential benefits, such as increase muscle strength and improve metabolic profile[23,24]. Here, we demonstrated that 12 weeks of resistance training, independently of modality (functional or conventional) could increase muscle strength of patients with MS. The protocols of resistance training found in literature vary greatly in duration and intensity, most of all improving strength[24,25]; which seems particularly important to this population due to the progressive loss of lean body mass frequently observed[7].

At the end of training period, CT presented higher decline in lipid peroxidation levels compared to CTRL. This is an important finding, as chronic oxidative stress is an important mechanism of pathogenesis of MS[4]. Available evidences indicate that elevated systemic oxidative stress is closely associated with MS and exercise has been implicated as an effective strategy to treat and prevent metabolic and cardiovascular abnormalities related to MS[6,26]. According to Holvoet (2008)[27], lipid peroxidation correlates with low levels of HDL in MS, which is in accordance with the results showed here.

While CTRL showed practically unaltered SOD activity after the period analyzed, CT showed increased SOD and catalase activity. Meantime, FT presented increase only in catalase activity, in less intensity. Among antioxidant defenses, enzymes are the most responsive elements. It is well know that different types of systematic training are able to induce muscle mitochondrial biogenesis, and, consequently superoxide generation and antioxidant induction[28]. SOD activity is directly related to superoxide generation, which dismutation generates $H_2O_2$. This molecule is a potential ROS generator and is converted to $H_2O$ and $O_2$ by means of catalase action[29]. Here, although SOD activity showed some increase in FT group in the same magnitude of that considered significant in catalase activity, the difference was not considered significant. Carteri *et al.* (2015)[30] demonstrated that an acute session of resistance exercise significant increased lipid peroxidation compared to baseline levels in postmenopausal women. According to the authors, a single bout of exercise in an untrained individual increases the production of ROS in different tissues. Pro-oxidant stimuli is necessary to promote chronic adaptations to the antioxidant defenses induced by exercise, as also observed in the present study.

Increased oxidant production promoted by exercise causes rapid transient reduction in muscle protein thiol content, followed by increases in the activities of superoxide dismutase and catalase and in content of heat shock proteins[31]. It can be considered as a signal of positive adaptation. Total thiol levels of patients that did not practice any type of training showed significant increase at the end of 12 weeks. It seems that both training types were able to avoid this increasing, promoting decreased Δ% in time, and consequently, less alterations on redox balance. Moreover, in aspects related to protein oxidation, AOPP are formed primarily by chlorinated oxidants, including hypochloric acid and chloramines, which result from myeloperoxidase activity. Levels of AOPP were found to be significantly higher (by nearly 60%) in MS patients than in controls[32]. Here, we had no control healthy group to compare, but the 12 weeks training programs proposed were not capable to change protein oxidation related to inflammatory status in patients with MS.

Low HDL is a strong biomarker of cardiovascular disease, and oxidative stress. HDL dysfunction is considered the second most important determinant of metabolic syndrome after waist circumference[1]. It has a sort of described important roles, including the ability to reduce superoxide production, antioxidant and anti-inflammatory functions[33]. Here, participants of CTRL showed decreased levels of HDL at the end of 12 weeks. Raised systemic inflammation and chronic oxidative stress are associated to plasma triglyceride-rich lipoproteins and oxidized lipoprotein phospholipids that underlie cardiovascular risk[34]. These disturbances are further increased by the reduction of anti-inflammatory, anti-oxidative, and atheroprotective properties of HDL[1]. In addition, it is well established that exercise (in general terms) can improve lipid profile and increase atheroprotective properties of HDL[11,21,25,] although better effects were already observed in periods longer than 12 weeks[11].

Besides the effects related above, 12 weeks of functional training improved systolic blood pressure. In line with this, Lemes *et al*. (2016)[8] showed in their meta-analysis that only systolic blood pressure was significantly reduced following resistance training. Tibana *et al*. (2013)[24] also demonstrated reduced systolic blood pressure after eight weeks of resistance training, confirming strength training as a good strategy in the management of cardiovascular risk. Here, in aspects related to oxidative stress, resistance training was also proved to promote systemic protection of oxidative (and also exercise-related metabolic) stress.

## Conclusions

The present study demonstrated for the first time that different protocols of strength training promoted diverse adaptations in oxidative stress. After 12 weeks of training, CT program was able to improve redox status, decrease systemic lipid peroxidation, and induce antioxidant enzymatic activity in patients with metabolic syndrome. FT could decrease systolic blood pressure, redox unbalance and induce catalase activity. Both training protocols were capable of prevent HDL decreasing, reinforcing the fact that sedentary lifestyle is the worse option to these patients. This can be considered an important finding, considering chronic oxidative stress an important mechanism involved in pathophysiology of MS. However, it was not possible to conclude if one method is better than the other one. The results of the present study suggests resistance training as an effective long-term therapy in the treatment of comorbidities that comprise metabolic syndrome.

**Acknowledgments**

We are grateful to Studio Salus (Presidente Prudente, São Paulo, Brazil) for providing space and structure for training programs. We would like to thank all the volunteers who kindly participated in this study, *Fundação Araucária* (Paraná, Brazil, Grant #: 977/2013) and *Fundação de Amparo à Pesquisa do Estado de São Paulo* (São Paulo, Brazil) (Grant #: 2013/10857-6) for financial support.

**Author Disclosure Statement**

The authors have nothing to disclose. None of the authors had any conflict of interest.

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## Tables and figures

**Table 1**. Evolution of training, according to volume and intensity.

| Week | Sets x repetitions | Intensity (1RM) |
|---|---|---|
| **1/2/3** | 2x 12/16/20 | 30 to 40% |
| **5** | 1x 16/12/9 | 40% - 50% - 60% |
| **6** | 1x 12/9/6 | 50% - 60% - 70% |
| **7** | 1x 10/8/6 | 60% - 70% - 80% |
| **8** | 1x 8/6/4 | 70% - 80% - 90% |
| **10** | 1x 6/4/2/4 /6 | 80% - 90% - 100% - 90% - 80% |
| **11** | 1x 6/4/2/2/4/6 | 80% - 90% - 100% - 100% - 90% - 80% |
| **12** | 1x 6/4/2/2/2/4/6 | 80% - 90% - 100% - 100% - 100% - 90% - 80% |

1RM: one repetition maximum test.

**Table 2**. Mean and standard deviation of anthropometric variables, age and sex, according to group.

| Group | Body mass (kg) | BMI (kg/m$^2$) | Height (cm) | Age (years) | Sex |
|---|---|---|---|---|---|
| CTRLb | 77.16±15.5 | 27.32±3.96 | 167.5±9.47 | 49.6±5.87 | 14 men / 6 women |
| CTRLf | 77.85±16.03 | 27.5±3.99 | | | |
| CTb | 87.84±14.57 | 32.05±4.09 | 165.3±10.63 | 51.44±4.98 | 12 men / 6 women |
| CTf | 88.28±14.77 | 32.2±4.06 | | | |
| FTb | 85.32±9.34 | 31.72±3.8 | 164.4±11.45 | 51.81±6.18 | 10 men / 6 women |
| FTf | 85.19±9.46 | 31.75±4.45 | | | |

CT: conventional training n; FT: functional training; CTRL: control group; b: before training; f: after period of training. BMI: body mass index. CTRL: n=20; CT: n=18; FT: n=16.

**Table 3.** Mean and standard deviation of percentage change (final-initial; Δ%) of one maximum repetition test according to exercise and group analyzed.

| Exercise | CTRL | CT | FT |
|---|---|---|---|
| Biceps pulley | 6.56±18.30 | 21.64±16.38* | 30.46±12.71*** |
| Triceps pulley | 6.36±22.28 | 54.74±46.85*** | 44.19±24.46** |
| Bench press | 27.2±19.83 | 32.97±17.67 | 21.69±28.43 |
| Behind neck lat pulldown | 16.32±21.75 | 29.16±46.12 | 37.95±41.98 |
| Upright row | 22.39±33.36 | 16.21±18.13 | 21.08±16.8 |
| Leg extension | 14.87±26.65 | 40.33±20.04** | 36.86±14.79* |
| Leg curl | -0.24±14.68 | 33.07±11.39*** | 43.64±25.68*** |
| Leg press | 6.16±17.55 | 30.96±22.9*** | 37.64±17.30*** |

One-way ANOVA followed by Tukey's multiple comparison test. CT: conventional training; FT: functional training; CTRL: control group. CTRL: n=20; CT: n=18; FT: n=16. ***p<0,0001 in comparison to Control. **p<0,001 in comparison to Control. *p<0,01 in comparison to Control.

**Table 4.** Cardiovascular risk factors according to group and evaluated moment.

| **Group/ moment** | **Fasting plasma glucose** (mg/dL) | **Triglycerides** (mg/dL) | **LDL** (mg/dL) | **HDL** (mg/dL) | **SBP** (mmHg) |
|---|---|---|---|---|---|
| **CTRLb** | 87,5 (77;207) | 139 (66;375) | 115 (77;204) | 44,45±9,9 | 116,7 (93,3;136,7) |
| **CTRLf** | 95 (82;142) | 142 (56;466) | 133 (77;201) | **42±9,22*** | 111,7 (90;153,3) |
| **CTb** | 105,5 (63;206) | 153 (97;559) | 120,5 (36;180) | 42,9±11,4 | 113,3 (96,7;140) |
| **CTf** | 112,5 (89;268) | 182 (79;426) | 138,5 (82;158) | 39,3±8,2 | 116,7 (96,7;150) |
| **FTb** | 93,50 (63;211) | 166 (95;647) | 90 (38;194) | 38,6±11,2 | 123,3 (103,3;163,3) |
| **FTf** | 96 (87;256) | 169 (80;525) | 103 (51;195) | 37,2±9,6 | **116,7 (90;160)**[#] |

Median, minimum and maximum values of fasting plasma glucose, triglycerides, LDL (low density lipoprotein) and SBP (systolic blood pressure) - Wilcoxon matched-pairs signed rank test. Mean and standard deviation of HDL (high density lipoprotein) - paired t test. CT: conventional training; FT: functional training; CTRL: control group; b: before training; f: after period of training. CTRL: n=20; CT: n=18; FT: n=16. *p<0.05 in comparison to CTRLb. #p<0,05 in comparison to Fb.

**Figure 1**. Distribution of the participants in each group and losses throughout the study.

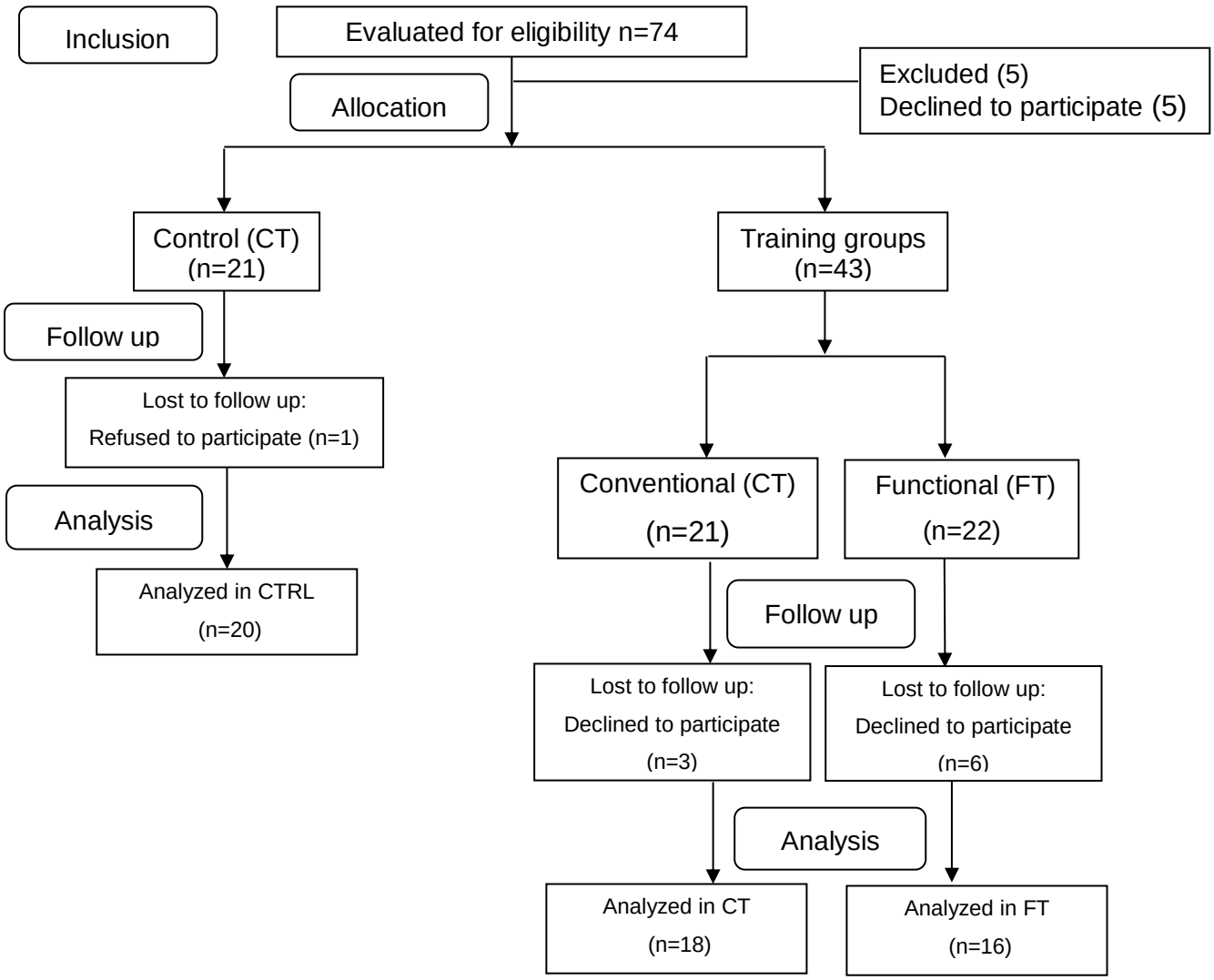
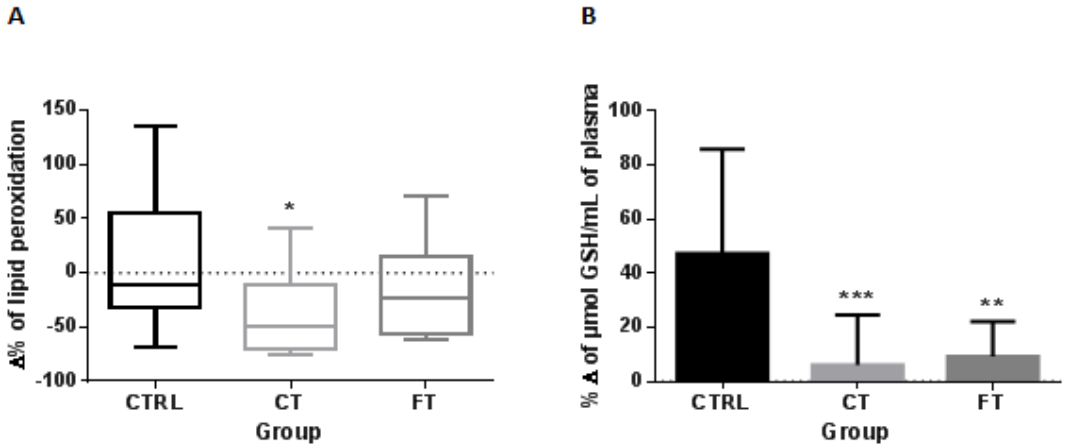
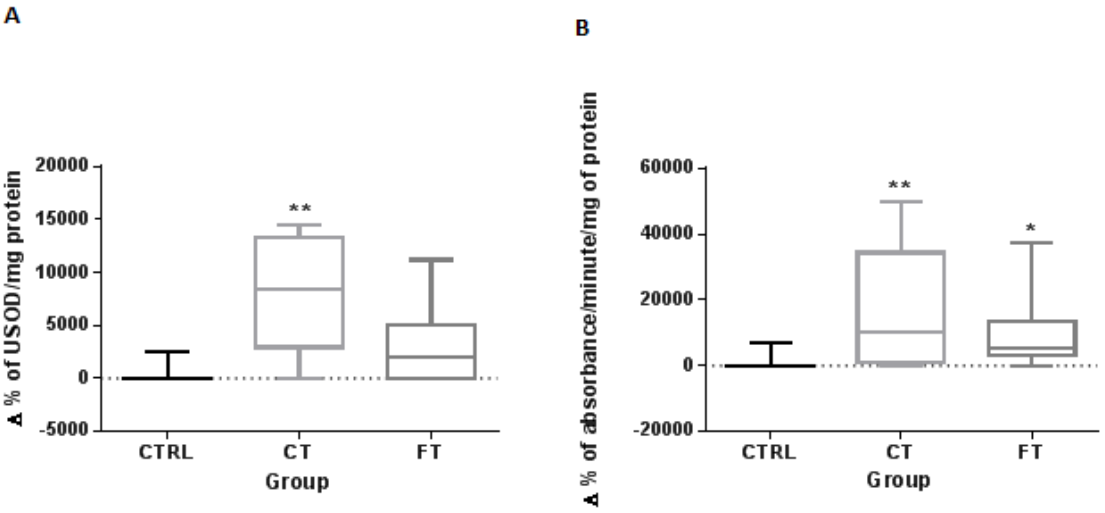


**Figure 2.** Values of lipid peroxidation evaluated by percentage change on tert-butyl hydroperoxide-initiated chemiluminescence (A) and plasma total thiols (B).



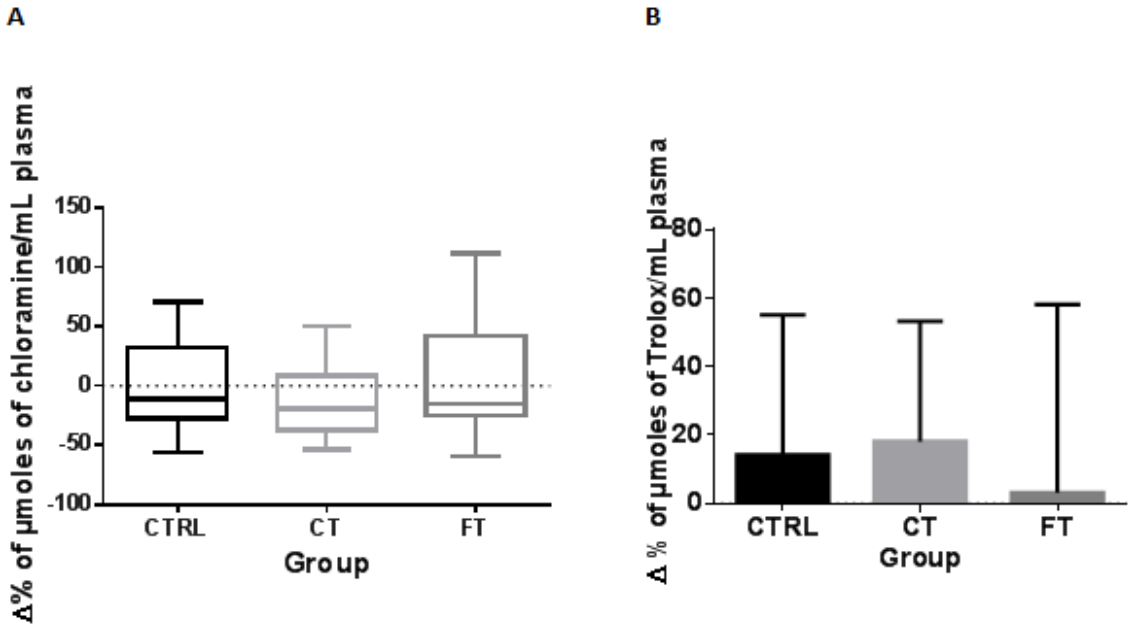

**A**: Kruskal-Wallis followed by Dunn's multiple comparisons test. Median (central point), interquartile range (box) and minimal and maximal values (whiskers). CTRL: n=15; CT; n=13; FT: n=11. *p<0.05: CT vs. CTRL. **B**: One-way ANOVA, followed by Tukey´s multiple comparisons test. Mean and standard deviation. ***p<0.0005: CT vs. CTRL; **p<0.0005: FT vs. CTRL. CTRL: n=15; CT; n=13; FT: n=14. CTRL: control group; CT: conventional training. FT: functional training.

**Figure 3.** Median, minimum and maximum values of percentage change of superoxide dismutase and catalase activity in erythrocytes, according to groups.




Kruskal-Wallis followed by Dunn's multiple comparisons test. Median range (box) and minimal and maximal values (whiskers). **A:** (CTRL: n=15; CT: n=6; FT: n=10); **p<0.005: CT vs. CTRL. **B**: (CTRL: n=12; CT; n=8; FT: n=10); **p<0.005: CT vs. CTRL, *p<0.05: FT vs. CTRL. CTRL: control group; CT: conventional group, FT: functional group.

**Figure 4.** Percentage change of advanced oxidation protein products and plasma total antioxidant capacity, according to group.




Percentage change of advanced oxidation protein products (**A**) and plasma total antioxidant capacity (**B**). **A**: Kruskal-Wallis followed by Dunn's multiple comparisons test. Median range (box) and minimal and maximal values (whiskers), (n=14). **B**: One-way ANOVA, followed by Tukey´s multiple comparisons test. CTRL: n=16; CT; n=8; FT: n=10. CTRL: control group; CT: conventional group, FT: functional group.

## 2.5 Conclusões

O presente estudo demonstrou, pela primeira vez, que diferentes protocolos de treinamento de força promovem adaptações em relação ao estresse oxidativo e metabólico, em portadores de SM. Após 12 semanas, o treinamento convencional melhorou o perfil oxidativo dos participantes, diminuindo a lipoperoxidação sistêmica, induzindo aumento na defesa antioxidante enzimática. O treino funcional foi capaz de diminuir a pressão sistólica e o desbalanço redox presente em indivíduos destreinados, promovendo aumento da atividade da enzima catalase. O grupo que não sofreu intervenção alguma por 12 semanas apresentou piora na taxa de HDL ao final do período de treinamento, reforçando o fato de que o sedentarismo é a pior opção para portadores da doença. Estes são achados relevantes, considerando o fato de que o estresse oxidativo é um mecanismo importante envolvido na fisiopatologia da SM. Assim, os resultados do presente estudo apontam o treino de força, realizado em longo prazo, por meio de supervisão profissional, como terapia eficiente no tratamento da SM.

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