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EFEITO DO TREINAMENTO MUSCULAR VENTILATÓRIO COMBINADO À LASERTERAPIA SOBRE O ESTRESSE OXIDATIVO DE RATOS COM DIABETES MELLITUS TIPO 2

Santa Maria, RS
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Dissertação apresentada ao curso de Pós-Graduação em Reabilitação Funcional, da Universidade Federal de Santa Maria (UFSM, RS) como requisito parcial para obtenção do título de **Mestre em Reabilitação Funcional.**

Orientadora: Profª. Drª. Maria Elaine Trevisan
Coorientador: Prof. Dr. Rodrigo Boemo Jaenisch

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Aprovado em 09 de setembro de 2021

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RESUMO

EFEITOS DO TREINAMENTO MUSCULAR VENTILATÓRIO COMBINADO À LASERTERAPIA SOBRE O ESTRESSE OXIDATIVO DE RATOS COM DIABETES MELLITUS TIPO 2

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Introdução: O Diabetes mellitus (DM) é considerado como uma das principais doenças crônicas não transmissíveis na atualidade. Entre os principais tipos, o mais predominante é o DM tipo 2 (DM2) o qual está relacionado com o estresse oxidativo, o aumento de citocinas pró-inflamatórias e a redução de citocinas anti-inflamatórias. O treinamento muscular ventilatório (TMV) e a laserterapia de baixa intensidade (LBI) são ferramentas não farmacológicas amplamente relatadas na literatura promovendo vários benefícios em diversas populações, porém, pouco se sabe sobre os efeitos da combinação dessas duas terapias sobre o estresse oxidativo em animais com DM2 induzida por dieta hipercalórica e estreptozotocina. **Objetivo:** Avaliar os efeitos da terapia combinada (CB) de LBI e TMV sobre o estresse oxidativo em ratos com DM2. Método: Pesquisa experimental com a utilização de ratos Wistar machos, alocados em um dos grupos experimentais descritos abaixo, perfazendo 8 animais por grupo: Grupo 1 - animais controle sem DM2 sedentários (C-*Sham*), Grupo 2 - animais sem DM2 e CB (CB-*Sham*), Grupo 3 - animais com DM2 sedentários (C-DM) e Grupo 4 - animais com DM2 e CB (CB-DM). O DM2 foi induzido por meio de uma dieta hiperlipídica e baixa dose de estreptozotocina (35 mg/kg) enquanto os grupos *Sham* receberam dieta comercial padrão. O protocolo de TMV foi aplicado por 30min/dia, 5 dias/semana, durante 6 semanas. A LBI foi aplicada em dois pontos no músculo gastrocnêmio direito, 5 dias/semana, durante 6 semanas, dose de 21 J/cm e comprimento de onda de 660nm. Vinte e quatro horas após o último dia de intervenção os animais foram eutanasiados e amostras de sangue e tecidos (coração, diafragma, fígado, gastrocnêmio direito, pulmões e rins) foram coletados, pesados e homogeneizados para posteriores análises. **Resultados:** O protocolo combinado reduziu o estresse oxidativo no diafragma de ratos diabéticos (aumento de DCF-RS), no gastrocnêmio o protocolo combinado reduziu o estresse oxidativo no grupo não diabético (redução de TBARS) entretanto, houve aumento do estresse oxidativo no gastrocnêmio de ratos diabéticos que receberam o protocolo combinado comparado aos demais grupos (aumento de DCF-RS). No plasma houve redução do estresse oxidativo em ratos diabéticos (redução de TBARS). O protocolo combinado aumentou a atividade antioxidante no coração, pulmão, rim e músculos no grupo diabetes (aumento de SH) e no coração, pulmão e diafragma (aumento da SOD). Os dados foram analisados usando a software estatístico GraphPad Prism. Para verificar a normalidade dos dados foi utilizado o teste Kolmogorov-Smirnov. As variáveis de mais de duas medidas foram comparadas por ANOVA de duas vias para medidas repetidas seguidas de post hoc de Bonferroni. As variáveis contínuas foram apresentadas na forma de média ± desvio padrão (DP). Considerou-se um nível de significância $p<0,05$ para todos os testes. **Conclusão:** o protocolo combinado foi eficaz para reduzir o estresse oxidativo além de aumentar a atividade antioxidante em músculos, órgãos e plasma de animais com DM2.

Palavras-chave: Diabetes Mellitus. Terapia com Luz de Baixa Intensidade. Músculos Respiratórios. Estresse Oxidativo. Inflamação.

ABSTRACT

EFFECTS OF VENTILATORY MUSCLE TRAINING COMBINED WITH LASER THERAPY ON OXIDATIVE STRESS IN RATS WITH MELLITUS DIABETES TYPE 2

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Introduction: Diabetes mellitus (DM) is considered one of the main non-communicable chronic diseases today. Among the main types, the most predominant is type 2 DM (DM2) which is related to oxidative stress, the increase in pro-inflammatory cytokines and the reduction in anti-inflammatory cytokines. Ventilatory muscle training (MVT) and low-intensity laser therapy (LLL) are non-pharmacological tools widely reported in the literature, promoting several benefits in different populations, however, little is known about the effects of the combination of these two therapies on oxidative stress in animals with DM2 induced by hypercaloric diet and streptozotocin. **Objective:** to evaluate the effects of combined therapy (CB) of LLLT and TMV on oxidative stress in rats with DM2. **Method:** Experimental research using male Wistar rats, allocated in one of the experimental groups below, totaling 8 animals per group: Group 1 - sedentary control animals without DM2 (C-Sham), Group 2 - animals without DM2 and CB (CB- Sham), Group 3 - animals with DM2 sedentary (C-DM) and Group 4 - animals with DM2 and CB (CB-DM). T2DM was induced by means of a high-fat diet and low dose streptozotocin (35 mg/kg) while the Sham groups received standard commercial diet. The TMV protocol was sold for 30min/day, 5 days/week for 6 weeks. LLL was applied in two points in the right gastrocnemius muscle, 5 days/week, for 6 weeks, at a dose of 21 J/cm and wavelength of 660nm. Twenty-four hours after the last day of intervention, the animals were euthanized and blood and tissues (heart, diaphragm, liver, right gastrocnemius, lungs and kidneys) were collected, weighed and homogenized for further analysis. **Results:** The combined protocol reduced the oxidative stress in the diaphragm of diabetic rats (increase of DCF-RS), in the gastrocnemius the combined protocol reduced the oxidative stress in the non-diabetic group (reduction of TBARS) however, there was an increase in oxidative stress in the gastrocnemius of diabetic rats that received the combined protocol compared to the other groups (increase in DCF-RS). There was no reduction in plasma oxidative stress in diabetic rats (reduction of TBARS). The combined protocol increased antioxidant activity in heart, lung, kidney and muscle in the diabetes group (increase in SH) and in heart, lung and diaphragm (increase in SOD). Data were compensated using GraphPad Prism statistical software. To verify the normality of the data used in the Kolmogorov-Smirnov test. Variables of more than two measures were compared by two-way ANOVA for repeated measures followed by Bonferroni post hoc. Continuous variables were detected as mean ± standard deviation (SD). Consider a significance level of $p<0.05$ for all tests. **Conclusion:** the combined protocol was effective in reducing oxidative stress in addition to increasing antioxidant activity in muscles, organs and plasma of animals with DM2.

Keywords: Diabetes Mellitus. Low Intensity Light Therapy. Respiratory Muscles. Oxidative stress. Inflammation.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ATP	Trifosfato de adenosina
CAT	Catalase
CEUA	Comissão de Ética no Uso de Animais
COBEA	Colégio Brasileiro de Experimentação Animal
DCF	Diclorofluoresceína Oxidada
DM	Diabetes Mellitus
DM1	Diabetes Mellitus tipo 1
DM2	Diabetes Mellitus tipo 2
DMSO	Dimetilsulfóxido
DNA	Ácido Desoxirribonucleico
DPOC	Doença Pulmonar Obstrutiva Crônica
EDTA	Ácido Etilenodiamino Tetra-Acético
EROs	Espécies Reativas de Oxigênio
GAP	Gabinete de Projetos
GPx	Glutationa Peroxidase
GSH	Glutationa Reduzida
IC	Insuficiência Cardíaca
IL-6	Interleucina 6
LAFEX	Laboratório de Fisiologia Experimental
LBI	Laserterapia de Baixa Intensidade
MDA	Malonaldeído
MPO	Mieloperoxidase
MTT	Metil Tetrazólio
NO	Óxido Nítrico
NPSH	*Non-protein thiol*
PImáx	Pressão Inspiratória Máxima
-SH	Grupo Sulfidrila
SOD	Superóxido Dismutase
STZ	Estreptozotocina
TBA	*Thiobarbituric acid*
TBARS	*Thiobarbituric acid reactive substances*
TCA	Ácido Tricloroacético
TFK	Tampão K-fosfato
TMV	Treinamento Muscular Ventilatório
UFSM	Universidade Federal de Santa Maria

SUMÁRIO

1 INTRODUÇÃO

O Diabetes Mellitus (DM) é considerado um importante e crescente problema de saúde pública global, com estimativa para 2045 de 700 milhões de pessoas com DM (INTERNATIONAL DIABETES FEDERATION, 2019). É considerada uma doença multifatorial e está intimamente associada ao estilo de vida (INTERNATIONAL DIABETES FEDERATION, 2019; SANTOS et al., 2014).

O DM pode ser classificado em DM do tipo 1 (DM1), DM do tipo 2 (DM2), diabetes gestacional e outras causas (AMERICAN DIABETES ASSOCIATION, 2021), sendo o DM2 o responsável por cerca de 90% de todos os casos (BRASIL, 2019; INTERNATIONAL DIABETES FEDERATION, 2015). O DM2 ocorre pela combinação do aumento da resistência periférica à insulina e a secreção inadequada, em graus variáveis, da mesma pelas células-beta pancreáticas desencadeando a hiperglicemia (MOTTA, 2005).

As altas taxas de glicemia estão relacionadas com o aumento de citocinas pró-inflamatórias (MALIK et al., 2018) e a enzima mieloperoxidase (MPO) o que contribui para o aumento de células inflamatórias (SHIU et al., 2014). Para além disso, observa-se redução nos níveis de citocinas anti-inflamatórias em pacientes com DM2 (GUPTA et al., 2017). Concomitantemente, há um aumento do estresse oxidativo o que se acredita, ser o principal fator fisiopatológico (RAINS; JAIN, 2011).

Estudos têm evidenciado que o estresse oxidativo está intimamente associado à prevalência de DM2 (ASMAT et al., 2016; BURGOS-MORÓN et al., 2019; OGUNTIBEJU, 2019). Estudos experimentais e de revisão sistemática indicam aumento significativo dos níveis de biomarcadores do estresse oxidativo (AMARAL et al., 2018; DOS SANTOS et al., 2017; NANKAR et al., 2020) e além disso, redução da atividade de enzimas antioxidantes (DOS SANTOS et al., 2017; OGUNTIBEJU, 2019).

Alterações em nível microvasculares e macrovasculares como a redução da atividade e biodisponibilidade de óxido nítrico (TABIT et al., 2010), redução da força e da massa muscular periférica também são observadas em pacientes com DM2. Van Eetvelde et al. (2018) observaram redução da pressão inspiratória máxima (PImáx), em DM com e sem neuropatia periférica, quando comparados com controles normoglicêmicos. Anteriormente, Leenders et al. (2013) haviam observado redução da massa magra da perna e da massa muscular esquelética apendicular em idosos com DM2, em comparação com controles normoglicêmicos.

Com o interesse em estudos para melhorar as condições de pessoas com DM, surgem

novas ferramentas terapêuticas como o treinamento muscular ventilatório (TMV) e a laserterapia de baixa intensidade (LBI). O TMV consiste em uma terapêutica não farmacológica que demonstrou reduzir a sensação de dispneia (LANGER et al., 2018), aumentar a capacidade respiratória, diminuir a fadiga dos músculos respiratórios (ARCHIZA et al., 2018), aumentar a capacidade funcional (DE MEDEIROS et al., 2017) e reduzir os níveis de marcadores inflamatórios em humanos (FIGUEIREDO et al., 2018). Ainda, aumenta a força muscular inspiratória e a espessura do músculo diafragma em humanos com DM2 (KAMINSKI et al., 2015). Estudo de Corrêa et al. (2015) demonstrou que pacientes com DM que realizaram exercício de TMV a 60% da PImáx demonstraram redução aguda dos níveis de glicose, imediatamente após uma sessão de 10 minutos se assemelhando às sessões de 40 minutos de exercício aeróbico.

Outra forma de intervenção não medicamentosa que vem sendo utilizada é a terapia por LBI. Esse recurso terapêutico pode modificar o metabolismo celular por estimular a atividade das enzimas anti-inflamatórias, reduzir o estresse oxidativo e aumentar as citocinas pró-inflamatórias (FRIGERO, et al. 2018; SILVA et al., 2017). Estudos realizados em modelo animal (DENADAI, et al. 2017) e em humanos (LENIFA, et al., 2018) com DM apresentam efeitos positivos da LBI de luz vermelha (660 nm) no reparo de feridas cutâneas que induz granulação mais rápida, contração da ferida e reepitelização. Além disso, tendem a diminuir o estresse oxidativo do músculo gastrocnêmio após exercício de alta intensidade avaliados pelos níveis de substâncias reativas ao ácido tiobarbitúrico do inglês *Thiobarbituric acid reactive substances* (TBARS). Também se mostrou capaz de aumentar os níveis de atividade de enzimas antioxidantes como superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx), demonstrando ser eficaz na redução do estresse oxidativo de ratos com DM (FRIGERO, et al., 2018).

Porém, estudos com terapêutica combinada com o propósito de verificar os efeitos sobre o estresse oxidativo em humanos com DM2 ainda são escassos, justificando-se a realização primeiramente em animais com DM2 induzido. Frente ao exposto, a pergunta de pesquisa foi: Quais os efeitos da aplicação do TMV combinado à LBI sobre o estresse oxidativo de ratos com DM2.

O presente estudo foi aprovado pela Comissão de ética no uso de animais (ANEXO A), registrado no gabinete de projetos institucional (ANEXO B) e desenvolvido no Laboratório de Fisiologia Experimental (APÊNDICE B) e é apresentado em forma de artigo científico, conforme as normas da revista Lasers in Medical Science (ANEXO C).

2 REVISÃO DE LITERATURA

2.1 DIABETES MELLITUS E ESTRESSE OXIDATIVO

O DM é considerado como uma das principais doenças crônicas não transmissíveis onde o pâncreas não produz insulina suficiente ou o corpo não consegue utilizá-la de maneira eficaz (BRASIL, 2019). De acordo com a Sociedade Brasileira de Diabetes (2017), existiam no Brasil mais de 13 milhões de pessoas entre 20 e 79 anos de idade vivendo com a doença em 2015, o que representa 6,9% da população nacional, podendo chegar a 23,3 milhões em 2040.

O DM é classificado em dois tipos principais, ou seja, DM tipo 1 e DM tipo 2. No DM1 há deficiência de insulina devido à destruição autoimune de células beta do pâncreas por clones autorreativos de linfócitos citotóxicos (HOBER; SANE, 2010) sendo responsável por 5 a 10% de todos os casos (SOCIEDADE BRASILEIRA DE DIABETES, 2017). O DM2, responsável por até 90% de todos os casos, ocorre pela combinação de dois fatores: resistência periférica à insulina e secreção inadequada de insulina pelas células-beta pancreáticas que, devido à falência dessas, desencadeia hiperglicemia (INTERNATIONAL DIABETES FEDERATION, 2019).

Acredita-se que a hiperglicemia é um fator causal do estresse oxidativo no DM2, o qual surge durante o desenvolvimento da doença e demonstra ser o principal fator patogênico (RAINS-JAIN, 2011; REHMAN-AKASH, 2017; LUC et al., 2019). Estudos realizados recentemente associam a hiperglicemia com aumento dos níveis de biomarcadores do estresse oxidativo (AMARAL et al., 2018; DOS SANTOS et al., 2017), redução da atividade de enzimas antioxidantes (DOS SANTOS et al., 2017; OGUNTIBEJU, 2019), aumento de mediadores pró-inflamatórias (ARATANI, 2018) e redução dos níveis de citocinas anti-inflamatórias em pacientes com DM2 (GUPTA et al., 2017).

O estresse oxidativo propriamente dito é caracterizado pelo desequilíbrio entre o sistema oxidante, o qual predomina, com produção de radicais livres e capacidade do organismo em neutralizá-los através do sistema antioxidante (SCHAFER; BUETTNER, 2001). Tal processo pode resultar em prejuízo para o organismo como danos ao DNA (HALLIWELL, 1994; HERBET et al., 2017).

2.2 MARCADORES OXIDANTES

Dentre as análises de dano oxidativo destaca-se a dosagem do malonaldeído (MDA) a partir do ácido tiobarbitúrico do inglês *Thiobarbituric acid* (TBA) uma das mais utilizadas e consideradas como biomarcador de estresse oxidativo de maior relevância (BARBOSA et al., 2010).

O MDA é um dos produtos finais da peroxidação lipídica das membranas celulares. Após sua formação, o MDA reage com o TBA e resulta na formação de complexos de coloração rosa a vermelho os quais possuem um coeficiente de absorção máxima de 532 nanometros (nm) denominados, então, de substâncias reativas ao ácido tiobarbitúrico (KNIGHT et al., 1988). Dessa forma, a análise da formação do TBARS pode ser aplicada como índice de comprometimento lipídico oriundo do dano pelo estresse oxidativo (PUNTEL et al., 2007; TSIKAS, 2017).

Outro tipo de avaliação do dano oxidativo é pela verificação dos níveis de espécies reativas à diclorofluoresceína (DCF). Os DCF são amplamente utilizados como técnicas de avaliação pro-fluorescentes para o estresse oxidativo. Embora exijam que um catalisador seja oxidado pelo peróxido de hidrogênio e reaja indiscriminadamente com radicais oxidantes e o produto fluorescente (DCF), é um potencial fotossensibilizador da geração de superóxido (WRONA;WARDMAN, 2006). Esta avaliação foi inicialmente desenvolvida na década de 1960 para a quantificação de espécies reativas de oxigênio (EROs) através do peróxido de hidrogênio (BRANDT;KESTON, 1965), sendo uma técnica comprovada pela literatura como de alta simplicidade e reprodutibilidade (WARDMAN, 2007); BARTOSZ, 2006) e muito utilizada em pesquisas tanto com animais como em humanos para avaliar as EROs no DM (WANG et al., 2019; JIANG et al., 2019).

2.3 MARCADORES DE VIABILIDADE CELULAR E ANTIOXIDANTE

Os níveis de redução de metil tetrazólio (MTT) é um marcador de viabilidade celular amplamente utilizado na literatura. A redução do MTT depende da atividade da família de enzimas localizadas principalmente nas mitocôndrias (BERNAS;DOBRUCKI, 2002) que transferem elétrons para o MTT. Muitos estudos utilizam o ensaio de redução de MTT como um indicador de viabilidade celular e marcador antioxidante (FURTADO et al., 2018; MARTINS et al., 2016). Células viáveis com metabolismo ativo convertem o MTT em um produto de coloração púrpura. Quando as células morrem, perdem a capacidade de converter

o MTT no produto com coloração, portanto a formação de cores serve como um marcador útil e conveniente para avaliar as células viáveis (RISS et al., 2016).

Como marcadores do sistema antioxidante, podemos destacar os níveis de tiol não protéico (NPSH)**.** Os tióis são considerados antioxidantes importantes, pois abrangem em sua estrutura um grupo sulfidrila (SH) e são facilmente oxidados, ou seja, abrem mão do átomo de hidrogênio para formar ligações estáveis. A glutationa reduzida (GSH) é um exemplo de um tiol de baixo peso molecular (CANTIN; BÉGIN, 1991; MEISTER; ANDERSON, 1983) a qual reage por reação direta com o xenobiótico ou via GPx para culminar na formação da glutationa dissulfeto, não-enzimatica (HUBER; ALMEIDA, 2008; JACOBSON et al., 1990).

Os NPSH são conhecidos como todos os tióis de baixo peso molecular. A GSH representa cerca de 90% do NPSH intracelular e os demais 10% são instituídos de outros pequenos aminoácidos tiólicos, como cisteína e metionina (JACOBSON et al., 1990). Resultados de estudos clássicos referentes ao processo de dano oxidativo demonstram que baixos níveis de SH são indicativos de presença de estresse oxidativo (MULIER et al., 1998).

Além disso, a superóxido dismutase (SOD) é considerada uma enzima antioxidante presente no organismo com a importante função de quebrar as moléculas de oxigênio potencialmente prejudiciais transformando-as em compostos menos tóxicos (TIWARI et al., 2013). Esta enzima atua contra a lesão celular induzida por espécies reativas de oxigênio (EROs) catalisando o superóxido convertendo a dismutação do radical anion superóxido em oxigênio molecular, água e peróxido de hidrogênio, sendo assim, é considerada como um mecanismo de defesa de primeira linha contra as EROs (REHMAN; AKASH, 2017). O radical ânion superóxido é um produto do metabolismo oxidativo muito prejudicial e desempenha papel crucial na patogênese do DM2 (FUKAI; USHIO-FUKAI, 2011).

2.4 TERAPIAS NÃO FARMACOLÓGICAS

Decorrentes da doença outras complicações também são observadas, assim como no estudo experimental de Oyenihi e colaboradores (2019), no qual estudaram o dano morfológico no músculo gastrocnêmio, em ratos com DM2 induzido. Dentre os resultados, destacam-se descontinuidade entre as fibras musculares esqueléticas, redução da espessura da fibra muscular em 16%, diminuição do número de fibras musculares e aumento do espaço do tecido conjuntivo no grupo DM2 quando comparado ao grupo controle sem DM2.

Evidências têm mostrado que a força muscular inspiratória pode estar reduzida em

humanos com DM2 e presença de neuropatia diabética comparados a indivíduos sem DM2, observando que há predominância de fibras musculares tipo 2 e redução da rede de capilares nessa população (GROEN et al., 2014).

O DM é uma doença complexa e requer cuidados médicos contínuos com estratégias multifatoriais com o objetivo de reduzir os riscos e manter o controle glicêmico. A educação em saúde e o suporte contínuo ao paciente são de extrema relevância para prevenir complicações e reduzir o risco das mesmas a longo prazo. Do mesmo modo, existem evidências que apoiam uma série de intervenções que podem trazer benefícios para essa população (AMERICAN DIABETES ASSOCIATION, 2019).

Evidências consolidadas na literatura apontam terapias não medicamentosas como, por exemplo, o exercício físico tanto aeróbico quanto de resistência, como primordiais em populações com DM. O exercício promove incremento da capacidade oxidativa além do remodelamento neuromuscular. Ambas as modalidades de treinamento têm evidências na melhora da sensibilidade à insulina e redução do risco cardiovascular (ZANUSO et al., 2017). Nos últimos anos tem-se intensificado os estudos abordando novas terapias e dentre elas podemos destacar a LBI. Trata-se de um recurso que utiliza fótons através da emissão de irradiação (laser) proporcionando alterações biológicas (SILVA et al., 2017).

A irradiação da luz é absorvida por moléculas fotorreceptoras do tecido alvo que possuam afinidade com determinado comprimento de onda. Essa absorção acontece devido a captação da energia luminosa pelos elétrons partindo para um estado excitatório de energia, a qual é utilizada pelas células para realizar suas funções metabólicas. A irradiação pode ser visível com luz monocromática em azul e vermelho sendo os efeitos fotobiológicos da estimulação dependentes do comprimento de onda, dose e intensidade da luz (KARU, 1989).

A laserterapia de baixa intensidade (LBI) tem sido aplicado isoladamente e associado à outras intervenções em diversas patologias proporcionando benefícios na redução da dor (GLAZOV et al., 2016), redução dos níveis totais de colesterol (ABDEL-WAHHAB et al., 2018), melhorando a cicatrização de feridas diabéticas, reduzindo os níveis de glicose, reduzindo o estresse oxidativo e citocinas inflamatórias em experimentação animal com ratos com DM2 (AHMED et al., 2018; DOS SANTOS et al., 2017). Isso porque o LBI tem a capacidade de aumentar o metabolismo celular, potencializa a regeneração das células teciduais (BASSO et al., 2018), promove neovascularização (MOON et al., 2018) e ativa a produção de ATP (JÚNIOR et al., 2004).

Um estudo avaliou o incremento da LBI em dois pontos do músculo gastrocnêmio, 5 vezes por semana, durante 8 semanas à um programa de exercícios de natação em relação aos

efeitos na área dos adipócitos, na atividade da enzima citrato sintase (CS) e na análise morfológica muscular de ratos com e sem alimentação hipercalórica. Como resultados, o uso combinado das terapias aumentou a atividade da enzima CS e diminuiu a área adipocitária branca epididimal, retroperitoneal e visceral em ratos obesos, melhorando os efeitos do exercício (AQUINO et al., 2015). Além disso, em ratos com insuficiência cardíaca (IC) a LBI quatro vezes por semana durante 8 semanas associada ao treinamento de resistência foi capaz de melhorar a captação de oxigênio e a tolerância ao exercício em comparação com o grupo IC que realizou exercício, mas sem irradiação (HENTSCHKE et al., 2017).

Dados da literatura mostram que o DM pode causar redução da força e resistência muscular respiratória (VAN EETVELDE et al., 2018; FUSO et al., 2012), causando perda de fibras musculares que afetam as propriedades contráteis e leva à fadiga da musculatura diafragmática (MANTILLA; SIECK, 2013).

Em experimentação animal, a carga alinear é frequentemente utilizada para o treinamento muscular ventilatório (TMV), na qual, a respiração se dá através de uma válvula unidirecional oferecendo aumento progressivo da resistência através de orifícios, cada vez menores, por onde é realizada a inspiração (BISSCHOP et al., 1997; JAENISCH et al., 2011).

Essa terapia tem demonstrado importante impacto no desempenho físico global, possivelmente pela atenuação do metaborreflexo dos músculos inspiratórios e por melhorar o suprimento de sangue e oxigênio aos músculos dos membros periféricos (ARCHIZA et al., 2018; BAILEY et al., 2010). Sua aplicação pode estar presente em várias condições patológicas, pois tende a reduzir a sensação de dispneia (LANGER et al., 2018), aumenta a capacidade respiratória, diminui a fadiga dos músculos respiratórios (ARCHIZA et al., 2018) e aumenta a capacidade funcional (DE MEDEIROS et al., 2017).

Surgem evidências de que essa terapia também tem efeito na redução da variabilidade da glicose nessa população (CORRÊA et al., 2015) e é capaz de reduzir os danos ao ácido desoxirribonucleico (DNA) em ratos com IC (JAENISCH et al., 2018). Além disso, redução dos níveis de (MDA) e aumento de óxido nítrico (NO) plasmático em humanos com DPOC moderada após treinamento de 6 semanas, evidenciando uma redução do estresse oxidativo sistêmico (LEELARUNGRAYUB et al., 2017).

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar os efeitos do treinamento muscular ventilatório associado à laserterapia de baixa intensidade aplicada no músculo gastrocnêmio, sobre o estresse oxidativo em ratos com diabetes mellitus induzido por dieta hipercalórica e estreptozotocina.

3.2 Objetivos Específicos

- Verificar o impacto do protocolo combinado sobre o estresse oxidativo sistêmico e local no diafragma e gastrocnêmio, em ratos com e sem DM2.
- Verificar o impacto do protocolo combinado sobre a atividade antioxidante sistêmica e local no diafragma, gastrocnêmio, rins, coração e pulmões em ratos com e sem DM2.

4 ARTIGO

Effects of ventilatory muscle training combined with laser therapy on oxidative stress in rats with mellitus diabetes type 2

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Declarations

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Compliance with ethical standards

Research involving human and/or animal participants: Study approved by the Ethics and Animal Welfare Committee of UFSM under number 9241020620.

Abstract

Objective: To evaluate the effects of Ventilatory Muscle Training (VMT) combined with Low-level laser therapy (LLLT) on oxidative stress in rats with streptozotocin-induced diabetes mellitus (DM2). **Method:** 32 male Wistar rats were randomly allocated into 4 experimental groups and 31 completed the study: G1 - control without sedentary DM2 (C-*Sham*; n = 7); G2 - without DM2 and with VMT + LLLT (CB-*Sham*; n = 8); G3 - sedentary DM2 (C-DM; n = 8); G4 - DM2 and with VMT + LLLT (CB-DM; n = 8). Protocols performed for 6 weeks being: VMT (30min / day, 5 days / week); LLLT applied in two points in the gastrocnemius muscle (5 days/week, 21 J/cm2, 36 seconds in each irradiated point). Blood and tissue samples were collected for further analysis of antioxidant activity and oxidative stress. **Results:** The combined protocol demonstrated lower levels of DCF-RS in the diaphragm of diabetic rats, higher levels in the gastrocnemius of the diabetic group and lower levels of TBARS in the gastrocnemius of non-diabetics. In plasma, oxidative stress levels in diabetics were lower. SH levels were higher in heart, lung, kidney and muscle and SOD activity in heart, lung and diaphragm in the diabetes group. Conclusion: The combined protocol promoted lower levels of oxidative stress in addition to higher levels of antioxidant activity in muscles, organs and plasma of animals with DM2.

Keywords: Diabetes Mellitus. Low Intensity Light Therapy. Respiratory Muscles. Oxidative stress. Inflammation.

Introduction

Diabetes Mellitus (DM) is characterized as a metabolic disorder with persistent hyperglycemia, due to a deficiency in insulin production or its action [1]. DM can be classified into two main types: type 1 DM (DM1) and type 2 DM (DM2) [2], with DM2 being responsible for about 90% of all cases, generating high health costs [3].

DM2 occurs by the combination of increased peripheral resistance to insulin and inadequate secretion, in varying degrees of it by pancreatic beta-cells, triggering hyperglycemia [4]. It is considered an important and growing global public health problem that is closely associated with lifestyle [3, 5].

High blood glucose levels are related to an increase in inflammatory cells [6]. Furthermore, there is a reduction in the levels of anti-inflammatory cytokines [7] and antioxidant defenses [8, 9], concomitantly, there is an increase in oxidative stress, which is believed to be the main pathophysiological factor [9, 10].

In addition to pharmacological therapies, in order to improve the living conditions of this population, new therapies such as ventilatory muscle training (VMT) and low-level laser therapy (LLL) have been the object of studies. VMT is a non-pharmacological therapy that has been effective in reducing the levels of inflammatory markers in humans [11] and acutely reducing glucose levels [12]. LLLT has also been effective in reducing oxidative stress and pro-inflammatory cytokines [13, 14], in addition to being able to increase levels of antioxidant enzyme activity and reduce oxidative stress [13].

Due to its action in reducing oxidative stress, new studies regarding the combination of VMT and LLLT and its potential treatment for DM2 play an important role in the field of health sciences. However, studies with combined therapy with the purpose of verifying the effects on oxidative stress in humans with DM2 are still scarce, justifying their performance primarily in animals with induced DM1. Given the above, the aim of this study was to investigate the effect of the combined protocol on oxidative stress in rats with DM2.

Materials and methods

Animals

Thirty-two male Wistar rats, 7-weeks-old (200-250g body weight) were obtained from the Central Animal Laboratory of the Federal University of Santa Maria (UFSM) and randomly housed in a group of 3 animals in polypropylene cages (41 x 33 x 16 cm) in different experimental groups. The animals were kept in a room with controlled temperature and humidity (22±2ºC; 50 to 60%, respectively), with air exhaustion and a 12-hour “light-dark” cycle and with water and ad libitum feeding. They were treated in accordance with the ethical

principles of animal experimentation developed by the Brazilian College of Animal Experimentation (COBEA). Study approved by the Ethics and Animal Welfare Committee of UFSM under the number 9241020620.

DM2 induction

The animals were fed with a high-energy density diet composed of 70% standard commercial feed, 15% sucrose, 10% lard and 5% egg yolk powder [15] for an initial period of four weeks. Sham group received standard feed. After this period, a single dose of streptozotocin (STZ, 35 mg/kg; Sigma Aldrich, St. Louis, MO, USA) was administered – intraperitoneally, dissolved in vehicle (0.01 M sodium citrate solution, pH=4 ,5) with a volume of 1ml/kg [16, 17] while in the Sham group only the vehicle was applied. The groups received their respective diets for another four weeks and then the training protocols were started. After a 12-hour fast, blood was collected from the tail vein and blood glucose was measured using a manual glucometer (G TECH FREE Lite, Infopia Co., Ltd., South Korea). These measurements were taken after the acclimation period (14 days); 1 day before and 7 days after STZ application and one day before euthanasia [18]. Animals with blood glucose greater than or equal to 200 mg/dL were considered diabetic. The diets persued as described above until the end of the experiment and body weight was measured weekly [19].

Experimental draw

The animals were randomly divided into 4 groups of 8 animals: control group without DM2 sedentary (C-Sham), group without DM2 that received VMT combined with LLLT (CB-Sham), control group with DM2 sedentary (C-DM), group with DM2 who received VMT combined with LLLT (CB-DM).

Intervention protocols

After 8 weeks of induction of the DM2 experimental model, the animals which received the combined intervention were initially submitted to the VMT protocol and, subsequently, to LLLT according to the adapted protocol from Aquino [21].

The VMT protocol comprehended 30 min/day, 5 days/week, for 6 weeks. Training progress was achieved by increasing load resistance, reducing the internal diameter of the orifice through which the animal breathed. During the first week of training, the orifice of the inspiratory port was fixed in an internal diameter of 0.8 mm for adaptation and was reduced by 0.1 mm daily, on the second day the diameter was 0.7 mm and so on. At the end of the first week, the diameter was reduced to 0.3 mm (maximum strength). The inspiratory load imposed on the trained animals is equivalent to a resistance of 0.7 cmH2O/ml/s at a flow rate of 5 ml/s (with an internal diameter of 0.8 mm) and a resistance of 18.4 cmH2O/ml/s at a flow rate of 5 ml/s (with an internal diameter of 0.3 mm [22, 23].

In the LBI protocol, the animals were submitted to the continuous wave diode InGaAlP type LBI (model Endophoton-LLT-0107; KLD Biosistemas Equipamentos Eletrônicos Ltda, São Paulo, Brazil) with output power of 20mW and wavelength of 660nm (red visible). The stitch size was 0.035cm2, a dose of 21 J/cm², for a period of 36 seconds in each stitch and continuous frequency. The LLLT was irradiated into the skin at an angle of 90°, at two points in the right gastrocnemius muscle, these being medial and lateral, approximately 3cm from the beginning of the paw, for 5 days/week, for 6 weeks, according to the adapted protocol [21].

Sample calculation

The sample size calculation was estimated to obtain a significance level (alpha) of 5% ($p<0.05$) and power (beta) of 80%. The sample was estimated at 8 animals per group, based on the study by Frigero et al. (13), based on the primary outcome of the present study (TBARS).

Tissue Preparation

At the end of treatment, the animals fasted overnight, with free access to water, and then were anesthetized with isoflurane (4%) [20]. Blood was collected by cardiac puncture, completing euthanasia and stored in tubes with anticoagulant ethylenediaminetetracetate (EDTA). After euthanasia, the heart, lung, kidney, diaphragm and gastrocnemius muscles were removed. Organ homogenates were prepared for analysis of oxidative stress and antioxidant capacity. The muscles were stored in a freezer at -80ºC for further analysis described above.

Heart, lungs and kidneys organs were removed, weighed and homogenized (UltraTurrax, Staufen, Germany) in phosphate buffer (1:4 heart, 1:5 lung, 1:10 kidney). The diaphragm and gastrocnemius muscles were removed, weighed and homogenized in NaCl (0.9%) (10mL/1g of tissue). After homogenization, the samples of organs and muscles were centrifuged at 3000 rpm for 10 minutes (SPIN MAX 80-2B, Didactics SP, SP) in order to obtain a low-speed supernatant fraction (S1), which was used for different biochemical assays [24].

Oxidizing Markers

TBARS levels were determined as an index of peroxidation according to the method described by Ohkawa et al.[25]. 200 µL aliquots of organs and muscles, in addition to plasma (500µL) were added to the color reaction, the readings were analyzed at 532 nm. TBARS levels were measured using MDA standard curve and corrected by the protein content.

DCF levels were determined by reduced dichlorofluorescein (DCFH-RS) and were quantified according to the modified Perez-Severian protocol [26]. Aliquots of homogenate from the samples in addition to plasma (50µL) were added to a medium containing Tris-HCl buffer (10mM; pH 7.4; 243µL) and dichloro-dihydro-fluorescein diacetate (1µM; 2µL). Then, the medium was incubated in the dark for 1 hour until verification of fluorescence (excitation at 488nm and emission at 525nm; both slit widths used were at 1.5nm). DCFH-RS levels were determined using a DCF standard curve and the results were corrected by milligram of protein.

Antioxidant Status Markers

NPSH levels of organs, muscles and plasma were determined in homogenates and the sample was precipitated with Trichloroacetic acid (TCA 5%) and subsequently centrifuged in eppendorf at 4000 rpm for 10 minutes in microtubes (SPIN MAX 80-2B, Didática SP, SP). 134µL of the supernatant fraction was added to a reaction medium containing K-phosphate buffer (0.25 mM TFK, pH=7.4; 100µL), distilled water and 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) (1mM; 2µL) in luminescence microplates. Spectrophotometric measurements were made at 412 nm through a plate reader. The results were calculated in relation to a standard curve constructed with reduced glutathione (GSH) and also corrected for protein content [27].

To determine SOD activity, sample homogenates and plasma (100µL) were added to a medium containing ethylenediamine tetraacetic acid (2mM EDTA) and bicarbonate buffer (NaHCO3/ Na2CO3 50 mM, pH 10.3). Epinephrine (4mM; 150µL) was added at the time of plate reading to initiate the kinetic activity of SOD for 5 minutes, being verified spectrophotometrically at 480 nm. SOD enzyme activity was expressed in units of enzyme activity per milligram of protein [28].

For analysis of MTT levels, the organ and muscle homogenates, in addition to the plasma, were incubated with 27 µl of sample at 30ºC for 60min in eppendorf. Subsequently, 270 µl of dimethyl sulfoxide (DMSO) were added to extract colored components, later it was transferred to luminescence microplates and through a plate reader, obtaining measurements at 570 nm. The results were expressed as a percentage of the control values [29].

Protein quantification

Protein content was measured according to the method described by Lowry et al., (1951) [30] using bovine plasma albumin as a standard. Samples were pipetted in a microplate and comassie, right after, in a microplate reader (Spectramax® i3x Multi-mode Microplate Reader) measurements were taken at 595 nm.

Statistical analysis

Data were analyzed using GraphPad Prism 5 statistical software (GraphPad Software Inc., San Diego, CA, USA). To verify the normality of the data, the Kolmogorov-Smirnov test was used. Variables from more than two measures were compared by two-way ANOVA for repeated measures followed by Bonferroni post hoc. Continuous variables were presented as median ± standard deviation (SD). A significance level of $p<0.05$ was considered for all tests.

Results

The experimental groups were C-Sham (n= 8), CB-Sham) (n= 8), C-DM (n= 7) and CB-DM (n= 8) and one animal from the C-DM group died.

Animal weight was homogeneous between groups at different times of analysis. When comparing final and initial weight, there was an increase in all groups ($p<0.05$). There was an increase in the C-Sham group when compared to the other groups ($p<0.05$) and an increase in the CB-Sham group when compared to the C-DM and CB-DM groups ($p<0.05$).

Regarding blood glucose, diabetic animals had increased blood glucose after the induction by STZ when compared to the beginning and remained with hyperglycemia until the end of the experiment. Furthermore, in the C-DM and CB-DM groups, blood glucose was higher when compared to the C-Sham and CB-Sham groups ($p<0.05$) (Table 1).

Oxidative Markers

Regarding plasma TBARS levels, values were higher in C-DM compared to C-Sham ($p<0.05$) and in CB-DM compared to C-Sham ($p<0.05$) and CB-Sham ($p<0.05$) and lower in CB-DM compared to C-DM ($p<0.05$) indicating that the therapy is able to reduce oxidative stress (Fig 1). In the gastrocnemius, TBARS levels were lower in the CB-Sham group compared to the C-Sham group ($p<0.05$) indicating that the effect of the combined intervention is effective in reducing oxidative stress. However, in the CB-DM group, the values were higher compared to the CB-Sham group ($p<0.05$) (Table 2).

In gastrocnemius, there were lower levels of DCF-RS in C-DM compared to C-Sham ($p<0.05$) and CB-Sham. In addition, the levels of DCF-RS were higher in the CB-DM group compared to C-Sham ($p<0.05$) and CB-Sham and CB-DM compared to C-DM ($p<0.05$) demonstrating that in this case, the combined intervention increased oxidative stress in the gastrocnemius of rats with DM (Table 2). In the diaphragm, the levels of DCF-RS were higher in the C-DM group compared to the C-Sham and the CB-Sham group ($p<0.05$) and in the CB-DM group compared to the CB-Sham ($p<0.05$). Furthermore, in the CB-DM group there were lower levels of DCF-RS compared to C-DM ($p<0.05$) suggesting that the combined intervention reduces the oxidative stress levels in the diaphragm in rats with DM (Fig 2).

Antioxidant Activity Markers

The lungs of rats in the C-DM group had a lower level of MTT compared to the C-Sham group ($p<0.001$). The combined protocol maintained lower levels of MTT in the lungs of rats with DM compared to Sham rats that also received the combined protocol ($p<0.05$).

No significant results were found for MTT levels in the heart, diaphragm and plasma (Table 3).

In the CB-DM group compared to CB-Sham ($p<0.001$) and C-Sham ($p<0.05$) there was a reduction in SH levels in the hearts of these animals, the same result observed in the CB-Sham group compared to C-Sham ($p<0.001$), however, higher HS levels were observed in the CB-DM group compared to the C-DM group ($p<0.001$) in the same organ.

In the lung SH levels were reduced in C-DM compared to C-Sham ($p<0.001$) and CB-DM compared to CB-Sham ($p<0.001$) and C-Sham ($p<0.05$), there were higher levels in CB-Sham compared to C-Sham ($p<0.001$). Higher HS levels were observed in CB-Sham compared to C-Sham ($p<0.05$), CB-DM compared to C-DM ($p<0.001$) and CB-Sham compared to C-DM ($p<0 .05$) in the kidney of the animals.

SH levels were lower in the diaphragm in C-DM compared to C-Sham ($p<0.001$), CB-Sham compared to C-Sham ($p<0.01$) and higher in CB-DM compared to C-DM ($p<0.001$). There were higher levels of SH in CB-DM compared to CB-Sham ($p<0.001$) and C-Sham ($p<0.05$) and CB-DM compared to C-DM ($p<0.001$) in gastrocnemius.

In plasma, there was a reduction in SH levels in CB-DM compared to CB-Sham ($p<0.001$) and CB-DM compared to C-DM ($p<0.001$) (Table 3). In the CB-Sham compared to C-Sham ($p<0.001$) and C-DM ($p<0.05$) groups, in addition to CB-DM compared to C-DM ($p<0.001$), SOD levels were higher in the heart. In the lung, there were lower SOD levels in CB-DM compared to CB-Sham ($p<0.05$) and higher levels in CB-Sham compared to C-Sham ($p<0.001$) and CB-DM compared to C-DM ($p <0.01$).

In the kidney, there were higher values in SOD activity levels in CB-Sham compared to C-Sham ($p<0.001$) and C-DM ($p<0.05$) and reduction in CB-DM compared to CB-Sham ($p< 0.001$). SOD activity levels had higher values in CB-Sham compared to all groups ($p<0.05$), in CB-DM higher levels were observed in relation to C-Sham and C-DM in the diaphragm.

In gastrocnemius, SOD levels were lower in C-DM compared to C-Sham ($p<0.001$) and CB-Sham ($p<0.05$) and CB-DM compared to CB-Sham ($p<0.001$) and C- Sham ($p<0.05$).

In plasma, SOD levels were lower in C-DM compared to C-Sham ($p<0.01$), CB-DM compared to CB-Sham ($p<0.001$) and higher in CB-Sham compared to C-Sham ($p<0.05$) and C-DM ($p<0.05$) (Table 3).

Discussion

Due to the scarcity of research involving this theme and the lack of previous studies with similar methodology in DM2, many of the findings of the present study were confronted with studies conducted on other outcomes and with isolated protocols.

The results of this study indicate that there was an increase in the body weight of animals in the C-Sham group at the end of the experiment compared to the initial weight in all groups. The efficacy of the diabetes induction protocol was demonstrated by the increase in blood glucose after STZ induction, which was maintained until the end of the experiment, in the C-DM and CB-DM groups [16, 17, 23]. However, the combined protocol was not effective in reducing glucose levels at the end of the experiment, agreeing with the result of the study in animals with DM2 [23], which verified the effect of VMT on sympathetic activity for 6 weeks in diabetic rats and opposing the study [12] which verified a reduction in blood glucose in humans with DM2 after a VMT protocol.

The results of this study demonstrated that the combined protocol showed lower values in the oxidative stress variables in the gastrocnemius muscle in the non-diabetic group compared to the control group, evidencing the beneficial effects of the protocol in healthy animals. Additionally, in plasma and diaphragm, there were lower levels of oxidative stress in the diabetic group, demonstrating its benefits at the systemic and local levels, respectively.

Our results corroborate a recent study [31] in which after a 6-week VMT protocol there were lower levels of bioactivity of reactive oxygen species and higher of bioavalilability of NO evaluated in healthy adult subjects. In rats with HF, a VMT protocol has been reducing the DNA damage analyzed in the diaphragm, suggesting a reduction in oxidative activity after training [32].

Regarding the LLLT study [13], it demonstrated that a single laser application in the gastrocnemius muscle promoted lower TBARS levels and higher antioxidant activity after high-intensity exercise training in rats with DM1. However, our results showed higher values of oxidative stress in the gastrocnemius of the diabetic group after the combined protocol.

Previous studies have identified changes in DM2 at the micro and macrovascular levels such as reduced activity and bioavailability of nitric oxide [35] and reduced strength and peripheral muscle mass, in addition to respiratory dysfunctions [36]. In healthy individuals, the VMT for 6 weeks was able to increase the inspiratory force and blood flow of the peripheral muscles, reducing the attenuation of the metaboreflex [37]. The increase in blood flow promotes the release of nitric oxide (NO) which has the function of smooth muscle relaxation and vasodilation [38]. The NO produced induces the response of antioxidants, including SOD, which has a protective action against oxidants [40]. LLLT also has the ability to increase cellular metabolism and promote neovascularization [34], other studies have reported beneficial effects of LLLT on mitochondria, such as increased mitochondrial membrane potential that protects against oxidative damage [38] that added to VMT

demonstrated improvement in oxidative stress variables in the present study, justifying our results, although blood flow was not evaluated.

However, the combined protocol increased oxidative stress levels in the diabetic group in the gastrocnemius muscle, but these values were also higher in the antioxidant activity identified by the increase in SH levels. Previous studies have shown that muscle contraction as a result of physical exercise increases the production of ROS and promotes oxidative stress [40, 41]. However, the increase in oxidative stress in this case may be beneficial because the exercise-induced ROS production promotes a physiological adaptation in skeletal muscles such as mitochondrial biogenesis and antioxidant enzyme synthesis, being a signaling pathway [40] agreeing with our results.

In our study, we also found lower values for antioxidant capacity in the lung, heart, diaphragm, gastrocnemius and plasma of diabetic animals in the different analyses. However, the combined protocol was able to elevate the levels of antioxidant activity observed by the increase of SH in the heart, lung, kidney and muscles and higher levels of SOD in the heart, lung and diaphragm. Furthermore, it was also beneficial to healthy animals compared to controls, which had higher levels of SH in lung and kidney, lung, kidney and diaphragm due to increased levels of SOD activity.

Our results agree with those of the previous study [13], which showed higher levels of antioxidant markers such as catalase, SOD and glutathioneperoxidase in the gastrocnemius muscle of diabetic animals that received a single irradiation of the LLLT before performing high-intensity exercise on a treadmill. Regarding the effects of VMT on the oxidative profile, a study [41] compared a group that performed aerobic and resistance exercise to a group that additionally associated VMT, observing an increase in plasma antioxidant levels in the group that associated VMT with other exercises.

Conclusion

In conclusion, this study provides an important contribution to the understanding of the effects of a protocol combining low-intensity laser therapy and ventilatory muscle training in rats with streptozotocin-induced Diabetes Mellitus. The positive effect was demonstrated by lower values of oxidative stress and higher values of local and systemic antioxidant activity in muscles, organs and plasma of rats with DM2. These results seem to indicate that this combination of non-pharmacological therapies are effective and safe in attenuating and oxidative state caused by DM2 and justify further studies, including in humans.

Some suggestions for future studies can be cited, such as: performing the metaboreflex that could contribute to more robust results; the comparison of the combined protocol with the isolated interventions, which could elucidate whether the effects of the combined therapy are added or not to the isolated ones.

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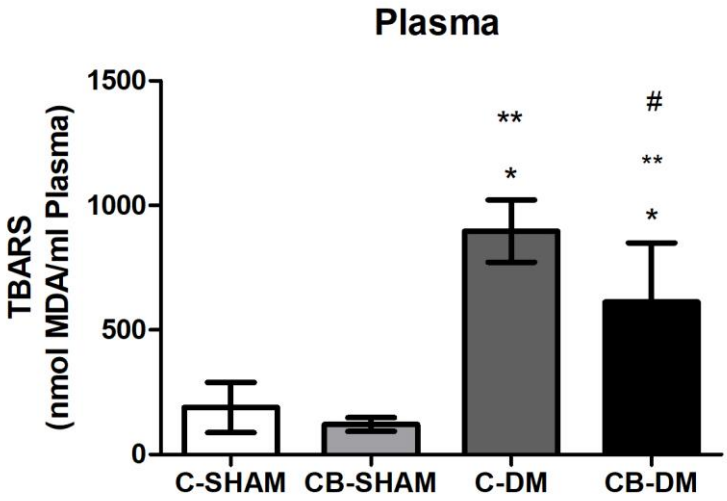
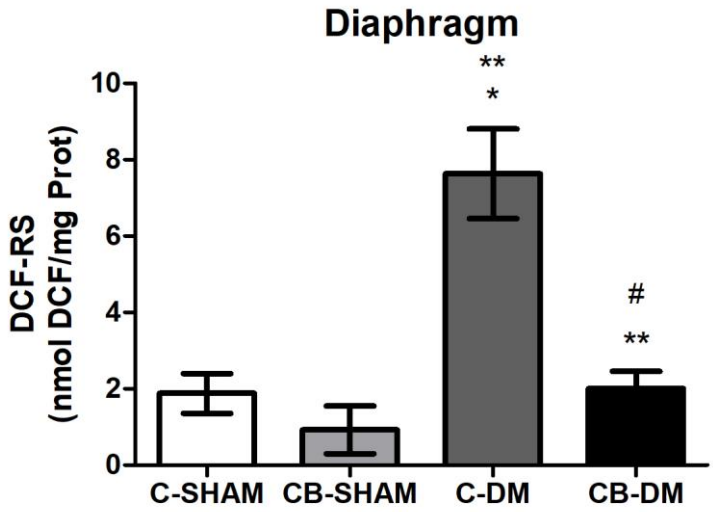


Values in mean ± standard deviation. The groups were compared by two-way ANOVA with post hoc Bonferroni. Analysis of variance (ANOVA); control group (C-Sham, n=8); Combined group (CB-Sham, n=8); diabetic control group (C-DM, n=7); Combined diabetic group (CB-DM, n=8).
* p values <0.05 compared to C-*Sham*
** p values <0.05 compared to CB-Sham
# p values <0.05 compared to C-DM

Fig 1 - Oxidizing activity levels in the plasma of the studied groups



Values in mean ± standard deviation. The groups were compared by two-way ANOVA with post hoc Bonferroni. Analysis of variance (ANOVA); control group (C-Sham, n=8); Combined group (CB-Sham, n=8); diabetic control group (C-DM, n=7); Combined diabetic group (CB-DM, n=8).
* p values <0.05 compared to C-*Sham*
** p values <0.05 compared to CB-Sham
# p values <0.05 compared to C-DM

Fig 2 - Oxidizing activity levels in the diaphragm of the studied groups

Table 1 Body weight and blood glucose

Body weight and blood glucose					
Groups	Starting weight (g)	Last weight (g)	Initial blood glucose (mg/dL)	Post STZ blood glucose and vehicle (mg/dL)	Last blood glucose (mg/dL)
C-*Sham*	266±18	486±23*[c,b,d]	125±7	113±12	124±21
CB-*Sham*	251±17	413±31*[c,d]	137±21	127±11	127±20
C-DM	248±17	362±45*	139±16	407±70*[a,b]	428±44*[a,b]
CB-DM	247±16	372±22*	161±26	444±15*[a,b]	427±92*[a,b]

Values in mean ± standard deviation. The groups were compared by two-way ANOVA with post hoc Bonferroni. Analysis of variance (ANOVA); control group (C-Sham, n=8); Combined group (CB-Sham, n=8); diabetic control group (C-DM, n=7); Combined diabetic group (CB-DM, n=8).

* P-values <0.05 comparing initial and final values

[a] P-values<0.05 compared to the group C-*Sham*

[b] P-values<0.05 compared to the group CB-*Sham*

[c] P -alues<0.05 compared to the group C-DM

[d] P-values<0.05 compared to the group CB-DM

Table 2 Oxidizing activity markers in gastrocnemius

	TBARS	**DCF-RS**
Groups	Gastrocnemius (nmol/µmol MDA/Prot)	Gastrocnemius (nmol DCF/mg Prot)
C-*Sham*	111.75±29.04	0.81±0.18
CB-*Sham*	48.53±12.10[a]	0.75±0.08
C-DM	126.29±65.66	0.20±0.05[a.b.d]
CB-DM	216.07±25.61[b]	3.63±0.45[a.b]

Values in mean ± standard deviation. The groups were compared by two-way ANOVA with post hoc Bonferroni. Analysis of variance (ANOVA); control group (C-Sham, n=8); Combined group (CB-Sham, n=8); diabetic control group (C-DM, n=7); Combined diabetic group (CB-DM, n=8); Thiobarbituric Acid Reactive Substances (TBARS); Dichofluorescein (DCFH-RS).

[a] P-values<0.05 compared to the group C-*Sham*

[b] P-values<0.05 compared to the group CB-*Sham*

[c] P -alues<0.05 compared to the group C-DM

[d] P-values<0.05 compared to the group CB-DM

Table 3 Antioxidant Activity Markers

MTT

Groups	Heart (% do controle)	Diaphragm (% do controle)	Lung (% do controle)	Plasma (% do controle)
C-*Sham*	115.90±39.63	100.17±9.95	155.54±27.12	104.50±8.25
CB -*Sham*	118.76±14.13	100.00±10.19	128.70±18.13	111.36±15.50
C-DM	102.76±26.45	108.84±8.10	93.22±2.86[a]	109.18±5.00
CB -DM	123.99±53.53	100.20±9.61	99.44±13.42[b]	96.17±5.74

SH

Groups	Heart (nmol SH/mg prot.)	Diaphragm (nmol SH/mg prot.)	Gastrocnemius (nmol SH/mg prot.)	Lung (nmol SH/mg prot.)	Kidney (nmol SH/mg prot.)	Plasma (nmol SH/mg plasma)
C-*Sham*	136.40±6.36	48.17±1.24	42.47±14.05	74.62±9.37	24.92±0.01	13.09±2.96
CB -*Sham*	85.11±7.00[a]	33.83±6.21[a]	32.75±6.54	109.62±4.87[a]	40.94±5.67[a]	16.94±2.98
C-DM	15.83±5.87[a,d]	20.01±4.94[a.d]	35.72±1.76[d]	44.50±17.32[a]	13.48±1.83[b.d]	13.18±4.52[d]
CB -DM	34.81±8.81[a,b]	39.62±5.70	1174.42±361.95 [a,b]	37.91±11.17[a.b]	43.65±15.33	4.20±2.32[b]

SOD

Groups	Heart (U/mg)	Diaphragm (U/mg)	Gastrocnemius (U/mg)	Lung (U/mg)	Kidney (U/mg)	Plasma (U/mg)
C-*Sham*	0.0017±0.00	0.0007±0.00	0.0309±0.01	0.0006±0.00	0.0014±0.00	0.0346±0.01
CB -*Sham*	0.0043±0.00[a]	0.0057±0.00[a.c.d]	0.0418±0.01	0.0063±0.00[a]	0.0120±0.00[a]	0.0466±0.01[a]
C-DM	0.0007±0.00[a.b.d]	0.0007±0.00	0.0017±0.00[a.b]	0.0016±0.00[d]	0.0006±0.00[b]	0.0015±0.00[a.b]
CB -DM	0.0039±0.00	0.0022±0.00[a.c]	0.0027±0.00[a.b]	0.0040±0.00[b]	0.0019±0.00[b]	0.0027±0.00[b]

Values in mean ± standard deviation. The groups were compared by two-way ANOVA with post hoc Bonferroni. Analysis of variance (ANOVA); control group (C-Sham, n=8); Combined group (CB-Sham, n=8); diabetic control group (C-DM, n=7); Combined diabetic group (CB-DM, n=8); Methyl Tetrazolium (MTT); Sulfidryl Group (SH); Superoxide Dismutase (SOD).

[a] P-values<0.05 compared to the group C-*Sham*

[b] P-values<0.05 compared to the group CB-*Sham*

[c] P -alues<0.05 compared to the group C-DM

[d] P-values<0.05 compared to the group CB-DM

5 CONCLUSÃO

O presente estudo é o primeiro a avaliar os efeitos da combinação do TMV e LBI sobre o estresse oxidativo de ratos com DM2. Os objetivos deste trabalho foram totalmente atingidos.

Em conclusão, o presente estudo sugere que o protocolo combinado foi eficaz na redução do estresse oxidativo além de aumentar a atividade antioxidante em músculos, órgãos e plasma de animais com DM2.

Dessa forma, os resultados demonstrados neste trabalho elucidam o potencial terapêutico da combinação dos protocolos e podem ser novas ferramentas a serem estudadas e utilizadas no DM2.

Para estudos futuros sugere-se a realização de análises referente ao metaborreflexo que poderia corroborar com os achados deste estudo; a comparação do protocolo combinado com protocolos de intervenções isoladas, o que poderia elucidar se os efeitos da terapêutica combinada se somam ou não às isoladas.

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6 ANEXOS

ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA NO USO DE ANIMAIS (CEUA)

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "EFEITO DO TREINAMENTO MUSCULAR VENTILATÓRIO COMBINADO À LASERTERAPIA SOBRE O ESTRESSE OXIDATIVO E O PERFIL INFLAMATÓRIO DE RATOS COM DIABETES MELLITUS TIPO II", protocolada sob o CEUA nº 9241020620 (ID 003122), sob a responsabilidade de **Maria Elaine Trevisan** *e equipe; Nubia Gonzatti; Rodrigo Boemo Jaenisch; Liliane de Freitas Bauermann; Camille Gaube Guex; Larissa da Silva Tonetto; Carlos Cassiano Figueiró da Silva; Nandiny Paula Cavalli* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 04/08/2020.

We certify that the proposal "EFFECT OF VENTILATORY MUSCLE TRAINING COMBINED TO LASERTHERAPY ON OXIDATIVE STRESS AND INFLAMMATORY PROFILE IN RATS WITH TYPE II DIABETES MELLITUS", utilizing 36 Heterogenics rats (36 males), protocol number CEUA 9241020620 (ID 003122), under the responsibility of **Maria Elaine Trevisan** *and team; Nubia Gonzatti; Rodrigo Boemo Jaenisch; Liliane de Freitas Bauermann; Camille Gaube Guex; Larissa da Silva Tonetto; Carlos Cassiano Figueiró da Silva; Nandiny Paula Cavalli* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 08/04/2020.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 09/2020 a 09/2021 Área: Departamento de Fisioterapia E Reabilitação

Origem:	Biotério Central UFSM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	7 a 8 semanas	N:	36
Linhagem:	Wistar			Peso:	220 a 250 g		

Local do experimento: LABORATÓRIO DE FISIOLOGIA EXPERIMENTAL (LAFEX), PRÉDIO 21, UFSM.

Santa Maria, 03 de fevereiro de 2021

Profa. Dra. Patrícia Severo do Nascimento
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Saulo Tadeu Lemos Pinto Filho
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

ANEXO B – REGISTRO NO GABINETE DE PROJETOS (GAP)

UNIVERSIDADE FEDERAL DE SANTA MARIA - UFSM

PROJETO NA ÍNTEGRA

Data/Hora: 08/10/2020 19:10
Autenticação: 3D17.D6EB.AB7B.BD0A.3D48.2079.9F37.AB52
Consulte em http://www.ufsm.br/autenticacao

Título: EFEITO DO TREINAMENTO MUSCULAR VENTILATÓRIO COMBINADO À LASERTERAPIA SOBRE O ESTRESSE OXIDATIVO E O PERFIL INFLAMATÓRIO DE RATOS COM DIABETES MELLITUS TIPO II

Número: 054301 **Classificação**: Pesquisa **Registrado em**: 27/05/2020

Situação: Em andamento **Início**: 01/07/2020 **Término**: 30/06/2021

Avaliação: Avaliado **Última avaliação**:

Fundação: Não necessita contratar fundação **Número na fundação**: *Não se aplica*

Supervisor financeiro: *Não se aplica*

Proteção do conhecimento: Projeto não gera conhecimento passível de proteção

Tipo de evento: Não se aplica **Carga Horária**: *Não se aplica*

Alunos matriculados: *Não se aplica*
Alunos concluintes: *Não se aplica*

Palavras-chave: Diabetes, Exercício, Laser

Resumo: O diabetes mellitus tipo II (DM II) é considerada uma das principais doenças crônicas não transmissíveis. Ocorre pela combinação do aumento da resistência periférica à insulina e a secreção inadequada de células-beta pancreáticas.Aexposição de altas concentrações de glicose, como no DM II, está relacionada aoestresse oxidativo,ao aumento de citocinas pró-inflamatórias e a diminuição de citocinas anti-inflamatórias, o que contribui para as complicações locais e/ou sistêmicas. Otreinamento muscular ventilatório (TMV) e a laserterapiade baixa intensidade (LBI), quando utilizados de forma isolada, promovem efeitos benéficos em pacientes com DM II. Entretanto, até o nosso conhecimento, nenhum estudo pré-clínico foi desenvolvido utilizando de forma combinada o TMV e a LBI em ratos com DM II, com a finalidade de esclarecer os mecanismos fisiológicos dessas ferramentas terapêuticas no modelo experimental animal.Assim, o presente projeto utilizará ratos Wistar machos, alocados para um dos 4 grupos experimentais descritos abaixo, perfazendo um n=8 animais por grupo:Grupo 1 - animais sem DM II sedentários, Grupo 2 - animais com DM II sedentários,Grupo 3- animais sem DM II e TMV combinado ao LBI 21J, Grupo 4- animais com DM II e TMV combinado ao LBI 21J.Os grupos 3 e 4, que realizarão o protocolo combinado, iniciarão como TMV e, logo após o LBI. O protocolo de TMV será realizado pelo período de 30min/dia, 5 dias/semana, durante 6 semanas.A LBI será aplicada por meio de duas doses irradiadas em dois pontos no músculo gastrocnêmio direito, pelo mesmo período de 5 dias/semana, durante 6 semanas, após o protocolo de TMV.A hipótese inicial é que o protocolo combinado (TMV + LBI) demonstre se é capaz de diminuir os marcadores de estresse oxidativo e melhorar o perfil inflamatório em ratos com DM II.

Objetivos: OBJETIVO GERAL: Avaliar os efeitos do TMV combinado ao LBI sobre o estresse oxidativo e o perfil inflamatório em ratos com DM II. OBJETIVOS ESPECÍFICOS: Verificar o impacto da terapêutica combinada sobre o estresse oxidativo e atividade antioxidante sistêmico e local no diafragma, gastrocnêmio, rins, fígado, coração e pulmões em ratos com DM II. - Avaliar o impacto da terapêutica combinada sobre o perfil inflamatório sistêmico e local no diafragma, gastrocnêmio, rins, fígado, coração e pulmões em ratos com DM II. - Avaliar o impacto da terapêutica combinada sobre parâmetros hematológicos em ratos com DM II. - Comparar grupos com DM II. O projeto será desenvolvido junto ao Laboratório de Fisiologia Experimental (LAFEX) da UFSM.

Justificativa: Diante do exposto percebe-se que, a utilização de terapias combinadas, em diversas situações patológicas, promove benefícios ainda mais significativos quando comparadas a terapias isoladas. O nosso grupo de pesquisa já realizou alguns estudos no modelo experimental, em ratos, com diferentes patologias, utilizando o TMV (JAENISCH et al., 2011; JAENISCH et al., 2017a; JAENISCH et al., 2017b; JAENISCH et al., 2018), o exercício aeróbio (EA) (BARCELOS et al., 2017), o exercício resistido (ER) (ALVES et al., 2017), estimulação elétrica funcional (FES) (RUCATTI et al., 2015) e a LBI (HENTSCHKEet al.,2012), entretanto não verificamos o efeito de terapias combinadas. A ideia de elucidarmos os efeitos positivos, com o tratamento de terapias combinadas, ainda geram dúvidase necessitam ser investigadas, principalmente sobre os aspectos fisiopatológicos da DM II no modelo experimental.

Resultados esperados: Espera-se que o TMV combinado ao LBI reduza o estresse oxidativo e modifique o perfil inflamatório a nível sistêmico e local no diafragma, gastrocnêmio, rins, fígado, coração e pulmões em ratos com DM II. Além de melhorar os parâmetros hematológicos em ratos com DM II.

PARTICIPANTES

MATRÍCULA	NOME	VÍNCULO	FUNÇÃO	C.H.*	INÍCIO	TÉRMINO
201660457	CAMILLE GAUBE GUEX	Aluno de Pós-graduação	Colaborador	2	01/07/2020	03/10/2020
201870544	CARLOS CASSIANO FIGUEIRÓ DA SILVA	Aluno de Pós-graduação	Participante	2	01/07/2020	30/06/2021
2313176	JAIME SARDÁ ARAMBURÚ JUNIOR	Técnico-Administrativo em Educação	Colaborador	1	07/10/2020	30/06/2021
201870534	LARISSA DA SILVA TONETTO	Aluno de Pós-graduação	Participante	2	01/07/2020	30/06/2021
2227178	LILIANE DE FREITAS BAUERMANN	Docente	Colaborador	2	01/07/2020	30/06/2021
378922	MARIA ELAINE TREVISAN	Docente	Orientador	2	01/07/2020	30/06/2021
229830	NANDINY PAULA CAVALLI	Externo	Participante	2	01/07/2020	30/06/2021
201870535	NUBIA GONZATTI	Aluno de Pós-graduação	Executor	10	01/07/2020	30/06/2021
2395822	RODRIGO BOEMO JAENISCH	Docente	Co-orientador	2	01/07/2020	30/06/2021

* carga horária semanal

UNIDADES VINCULADAS

UNIDADE	FUNÇÃO	VALOR	INÍCIO	TÉRMINO
04.00.00.00.0.0 - CENTRO DE CIÊNCIAS DA SAÚDE	Responsável		01/07/2020	30/06/2021
04.10.27.00.0.0 - PROGRAMA DE PÓS-GRADUAÇÃO EM REABILITAÇÃO FUNCIONAL	Promotor		01/07/2020	30/06/2021

CLASSIFICAÇÕES

TIPO DE CLASSIFICAÇÃO	CLASSIFICAÇÃO
Classificação CNPq	4.08.00.00-8 - FISIOTERAPIA E TERAPIA OCUPACIONAL
Linha de pesquisa	02.06.00 - FISIOTERAPIA
Quanto ao tipo de projeto de pesquisa	2.05 - Projeto de Pesquisa e Ensino

ANEXO C – NORMAS DA REVISTA LASERS IN MEDICAL SCIENCE

Instructions for Authors

Types of papers

- Original Article – limited to 4000 words, 45 references, no more than 5 figures
- Review Article – limited to 5000 words, 50 references, no more than 5 figures
- Brief Report - limited to 2000 words, 25 references, no more than 4 figures - Case Reports will not be accepted!
- Letter to the Editor – up to 600 words

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit manuscript" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Editorial Procedure

Double-blind peer review

This journal follows a double-blind reviewing procedure. Authors are therefore requested to submit:

A blinded manuscript without any author names and affiliations in the text or on the title page. Self-identifying citations and references in the article text should be avoided.

A separate title page, containing title, all author names, affiliations, and the contact information of the corresponding author. Any acknowledgements, disclosures, or funding information should also be included on this page.

Title page

Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide a structured abstract of 150 to 250 words which should be divided into the following sections:

- Purpose (stating the main purposes and research question)
- Methods
- Results
- Conclusion

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by "retrospectively registered"

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for all articles, including articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 190 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Scientific style

Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.

Units and abbreviations

- Please adhere to internationally agreed standards such as those adopted by the commission of the International Union of Pure and Applied Physics (IUPAP) or defined by the International Organization of Standardization (ISO). Metric SI units should be used throughout except where non-SI units are more common [e.g. litre (l) for volume].

- Abbreviations (not standardized) should be defined at first mention in the abstract and again in the main body of the text and used consistently thereafter.

Drugs

- When drugs are mentioned, the international (generic) name should be used. The proprietary name, chemical composition, and manufacturer should be stated in full in Materials and methods.

References

Citation

Reference citations in the text should be identified by numbers in square brackets. Some examples:

1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The entries in the list should be numbered consecutively.

If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

- Journal article
  Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI
  Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

- Book
  South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter
  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document
  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation
  Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN.org LTWA

If you are unsure, please use the full journal title.

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer's LaTeX macro package.

Tables

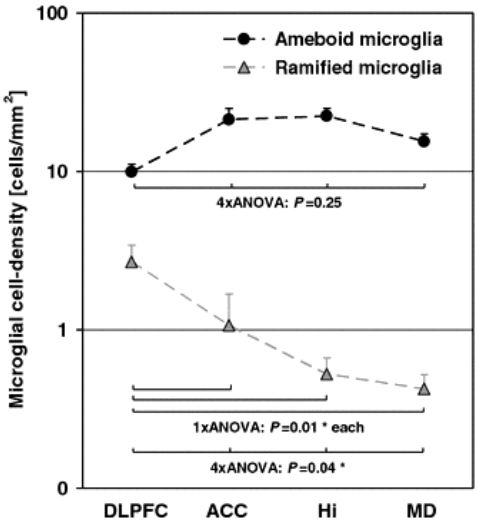
- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

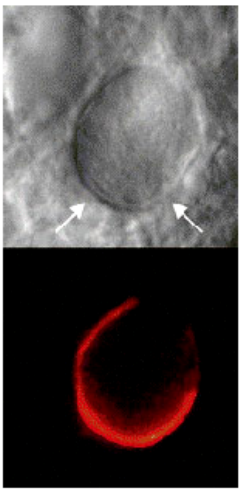
- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art



- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

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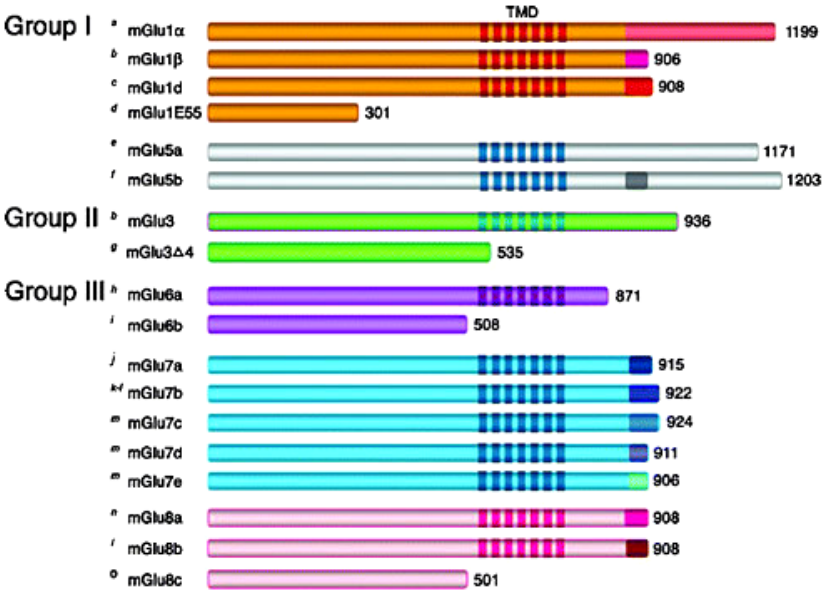

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Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

• Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

Consent to Participate

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For

manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

Consent to Publish

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

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List of Repositories

Research Data Policy

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Datasets that are assigned digital object identifiers (DOIs) by a data repository may be cited in the reference list. Data citations should include the minimum information recommended by DataCite: authors, title, publisher (repository name), identifier.

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7 APÊNDICES

APÊNDICE A - TERMO DE CONSENTIMENTO DO DEPARTAMENTO DE FISIOLOGIA E FARMACOLOGIA

TERMO DE CONSENTIMENTO DO DEPARTAMENTO DE FISIOLOGIA E FARMACOLOGIA

Eu, William Schoenau, abaixo assinado, responsável pelo Departamento de Fisiologia e Farmacologia da Universidade Federal de Santa Maria (UFSM), autorizo a realização do estudo intitulado "EFEITOS DO TREINAMENTO MUSCULAR VENTILATÓRIO E DA LASERTERAPIA SOBRE O PERFIL INFLAMATÓRIO E O ESTRESSE OXIDATIVO EM RATOS COM DIABETES MELLITUS TIPO II " a ser conduzido pelos pesquisadores Camille Gaube Guex, Carlos Cassiano Figueiró da Silva, Jhulie Anne Pinheiro Kemerich, Larissa da Silva Tonetto, Maria Elaine Trevisan, Nandiny Paula Cavalli, Nubia Gonzatti e Vanessa Ortiz de Andrade, orientado pelo professor Rodrigo Boemo Jaenisch e contando com colaboração da professora Liliane de Freitas Bauermann.

Fui informado, pelo responsável do estudo sobre as características e objetivos da pesquisa bem como das atividades que serão realizadas na instituição a qual represento.

Esta instituição está ciente de suas responsabilidades como instituição coparticipante do presente projeto de pesquisa e seu compromisso no resguardo da segurança e bem-estar dos sujeitos nela recrutados, dispondo de infraestrutura necessária para garantia de tal bem-estar.

Assinatura e carimbo
Prof. WILLIAM SCHOENAU
Chefe do Departamento de Fisiologia
e Farmacologia
CCS/UFSM
Santa Maria, 30 de abril de 2018

APÊNDICE B - TERMO DE CONSENTIMENTO DO LABORATÓRIO DE FISIOLOGIA EXPERIMENTAL

APÊNDICE B - TERMO DE CONSENTIMENTO DO LABORATÓRIO DE FISIOLOGIA EXPERIMENTAL

Eu, Liliane de Freitas Bauermann , abaixo assinado, responsável pelo Laboratório de Fisiologia Experimental da Universidade Federal de Santa Maria (UFSM), autorizo a realização do estudo intitulado "EFEITOS DO TREINAMENTO MUSCULAR VENTILATÓRIO E DA LASERTERAPIA SOBRE O PERFIL INFLAMATÓRIO E O ESTRESSE OXIDATIVO EM RATOS COM DIABETES MELLITUS TIPO II " a ser conduzido pelos pesquisadores Camille Gaube Guex, Carlos Cassiano Figueiró da Silva, Jhulie Anne Pinheiro Kemerich, Larissa da Silva Tonetto, Maria Elaine Trevisan, Nandiny Paula Cavalli, Nubia Gonzatti e Vanessa Ortiz de Andrade, orientado pelo professor Rodrigo Boemo Jaenisch.

Fui informado, pelo responsável do estudo sobre as caracteristicas e objetivos da pesquisa bem como das atividades que serão realizadas na instituição a qual represento.

Esta instituição está ciente de suas responsabilidades como instituição coparticipante do presente projeto de pesquisa e seu compromisso no resguardo da segurança e bem-estar dos sujeitos nela recrutados, dispondo de infraestrutura necessária para garantia de tal bem-estar.

Liliane de F. Bauermann

Assinatura e carimbo

Liliane de F. Bauermann
CRB: 17045 - 03D
MEC: LP 02218/89
UFSM - MAT 2227178

Santa Maria, 30 de Abril de 2019.

APÊNDICE C - TERMO DE COMPROMISSO

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

SantaMaria,15 de 03 de 2019

TERMO DE COMPROMISSO

Eu, Rodrigo Boemo Jaenisch, CPF 00935046089, responsável pelo projeto intitulado: "EFEITOS DO TREINAMENTO MUSCULAR VENTILATÓRIO E DA LASERTERAPIA SOBRE O PERFIL INFLAMATÓRIO E O ESTRESSE OXIDATIVO EM RATOS COM DIABETES MELLITUS TIPO II", declaro que:

a) li o disposto na Lei n 11.794, de 8 de outubro de 2008, e nas demais normas aplicáveis à utilização de animais em ensino e/ou pesquisa, especialmente as Resoluções Normativas do Conselho Nacional de Controle de Experimentação Animal - CONCEA;

b) este estudo não é desnecessariamente duplicativo, possuindo mérito científico e a equipe participante deste projeto/aula foi treinada e é competente para executar os procedimentos descritos neste protocolo;

c) não existe método substitutivo que possa ser utilizado como uma alternativa ao projeto.

Responsável: Rodrigo BoemoJaenisch

15/03/2019 Assinatura:Data:

Executor: Jhulie Anne Pinheiro Kemerich

Assinatura: Data:15/03/2019

Jhulie Kemerich

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Horáriodeatendimento:das8:30às11:30e14:00às16:30hs:email:ceua.ufsm@gmail.com

APÊNDICE D - TERMO DE RESPONSABILIDADE

Universidade Federal de Santa Maria
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

TERMO DE RESPONSABILIDADE

Mediante este termo eu, Rodrigo Boemo Jaenisch, pesquisador da UFSM, e coordenador do projeto submetido à CEUA, comprometo-me em providenciar as autorizações necessárias ao desenvolvimento do projeto, tais como IBAMA, ICMBio, CTNBio CNPq, CGEN, FUNAI e Polícia Federal, quando for o caso, bem como verificar as condições de biossegurança necessárias.

Santa Maria, 10 de novembro de 2018.