UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

MARCELO MENDONÇA MOTA

EFEITOS AGUDOS DE DIFERENTES INTENSIDADES DE EXERCÍCIO RESISTIDO SOBRE OS AJUSTES VASCULARES EM ARTÉRIA MESENTÉRICA DE RATOS

ARACAJU
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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Sergipe como requisito parcial à obtenção do grau de Doutor em Ciências da Saúde.

Orientador: Prof. Dr. Márcio Roberto V. Santos

Co-orientador: Prof. Dr. Rogério Brandão Wichi

ARACAJU

2014

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DA SAÚDE
UNIVERSIDADE FEDERAL DE SERGIPE

Mota, Marcelo Mendonça
M917e Efeitos agudos de diferentes intensidades de exercício resistido sobre os ajustes vasculares em artéria mesentérica de ratos / Marcelo Mendonça Mota; orientador Márcio Roberto Viana Santos, co-orientador Rogério Brandão Wichi. – Aracaju, 2014.
93f. : il.

Tese (Doutorado em Ciências da Saúde - Núcleo de Pós-Graduação em Medicina), Pró-Reitoria de Pós-Graduação e Pesquisa, Universidade Federal de Sergipe, 2014.

1. Exercícios físicos - Aspectos da saúde. 2. Exercício resistido. 3. Óxido nítrico. 4. Educação física. I. Santos, Márcio Roberto Viana, orient. II. Wichi, Rogério Brandão, co-orient. III. Título

CDU 613.71
796

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PARECER

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AGRADECIMENTOS

Ao único Deus, criador da Terra e de todo o ser vivente, quem dá a sabedoria e o conhecimento e tem o domínio sobre todas as coisas;

À minha esposa Daniela, amor da minha vida, companhia que constrói, que ensina, que motiva e que proporciona os melhores momentos da minha vida. A você todo o meu amor hoje e sempre;

Aos meus filhos Moana e Mauê, que refizeram o meu conceito de amor e doação e me desperta a cada momento para o melhor dessa vida tão fugaz;

Aos meus pais por sempre me despertar o interesse pelo conhecimento e pelo apoio incondicional;

Ao meu amigo Tharciano pelo socorro nos momentos difíceis, pelas palavras de sabedoria e de incentivo, pela companhia e disposição;

Ao meu querido e admirável orientador Márcio, sempre pronto a acreditar, motivar, simplificar, ensinar, apoiar e confiar. Obrigado por toda atenção, paciência e carinho presentes desde o primeiro momento;

Ao meu co-orientador Rogério pelos valorosos ensinamentos e por me proporcionar um aprimoramento da minha redação científica;

Aos professores Lucindo, Adriano, Leonardo, Eduardo Seixas, Sandra Lauton pelos ricos momentos de aprendizagem, pelos valiosos conselhos e pela grande atenção científica e burocrática;

À Universidade Federal de Sergipe e ao Núcleo de Pós-Graduação em Medicina/UFS pela oportunidade e suporte à qualificação docente.

Aos colegas do LAFAC, LAPENE, LAPEC e LBC em especial os que ajudaram na construção deste material.

"A ciência nunca resolve um problema sem criar pelo menos outros dez" (George Bernard Shaw).

EFEITOS AGUDOS DE DIFERENTES INTENSIDADES DE EXERCÍCIO RESISTIDO SOBRE OS AJUSTES VASCULARES EM ARTÉRIA MESENTÉRICA SUPERIOR DE RATOS. MOTA, Marcelo Mendonça. Universidade Federal de Sergipe, Aracaju, 2014.

RESUMO

A relação direta entre o exercício resistido e a saúde vascular é certa, mas o complexo conjunto de vias metabólicas, hemodinâmicas e os efeitos desta modalidade de exercício físico sobre os tecidos cardiovasculares ainda é bastante controverso. Embora seja consenso na literatura que o óxido nítrico (NO) contribui para o controle do fluxo sanguíneo durante o exercício resistido, ainda não estão completamente compreendidos os eventos de sinalização na vasculatura que medeiam à liberação deste agente vasoativo. Neste sentido, o objetivo do presente estudo foi avaliar os efeitos agudos de diferentes intensidades de exercício resistido sobre os ajustes vasculares em artéria mesentérica superior de ratos saudáveis. A tese é composta por dois capítulos, constituídos de dois artigos originais. O primeiro artigo, "Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats", no qual avaliou os efeitos agudos de uma sessão de exercício resistido sobre as ações vasculares da insulina em artéria mesentérica de ratos. Neste primeiro estudo foi demonstrado que uma sessão de exercício resistido aumentou o vasorelaxamento via PI3K/eNOS. Este aumento, se deve em parte a uma maior produção de NO, associado a um aumento da participação dos canais para K^+ e da Na^+/K^+-ATPase. O segundo artigo, "Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals" demonstrou que uma sessão de exercício resistido moderado e/ou vigoroso melhora o relaxamento dependente do endotélio induzido por insulina devido a um aumento nos níveis de fosforilação da eNOS ^{ser1177} e, consequente, incremento da produção endotelial de NO em animais saudáveis. A partir destes achados é possível sugerir que o exercício resistido é capaz de promover ajustes vasculares importantes que atuam diretamente no favorecimento do melhor controle do tônus vascular. Além disso, a magnitude destes benéficos ajustes vasculares estão fortemente relacionados ao aumento da intensidade do exercício resistido a partir da intensidade de 50% de 1 RM.

Palavras-Chave: Exercício resistido; óxido nítrico; eNOS; reatividade vascular.

ACUTE EFFECTS OF DIFFERENT INTENSITIES OF RESISTANCE EXERCISE ON VASCULAR ADJUSTMENTS IN THE SUPERIOR MESENTERIC ARTERY OF RATS. MOTA, Marcelo Mendonça. Federal University of Sergipe, Aracaju, 2014.

ABSTRACT

The direct relationship between resistance exercise and vascular health is certain, but the complex set of metabolic and hemodynamic pathways and the effects of this type of exercise on cardiovascular tissues are still controversial. Although consensus that nitric oxide (NO) contributes to the control of blood flow during resistance exercise, are not yet fully understood the events signaling in the vasculature that mediate the release of this vasoactive agent. In this sense, the objective of the present study was to evaluate the acute effects of different intensities of resistance exercise on vascular adjustments in the superior mesenteric artery of healthy rats. The thesis comprises two chapters, consisting of two original articles. The first article, "Resistance exercise enhances insulin mesenteric artery acutely-induced relaxation in healthy rats", which assessed the acute effects of an exercise session held on vascular actions of insulin in rat mesenteric artery. In this first study, it was observed that a resistance exercise session increased the vasorelaxation via PI3K/eNOS. Such increase is due in part to an increased production of NO, associated with an increase in the participation of channels for K^+ and Na^+/K^+-ATPase. The second article, "Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals", demonstrated that a session of vigorous exercise moderately weathered and/or improves endothelium-dependent relaxation induced by insulin due to an increase in the levels of phosphorylation of $eNOS^{ser1177}$ and, consequently, increased endothelial production of NO healthy animals. From these findings it is possible to suggest that resistance exercise promotes vascular adjustments that work in favor of better control of vascular tone. In addition, the magnitude of these beneficial vascular adjustments is strongly related to increased resistance exercise intensity from the intensity of 50% of 1 RM.

Keywords: Resistance exercise, nitric oxide; eNOS, vascular reactivity.

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Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals:

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LISTA DE ABREVIATURAS

ACh- Acetilcolina

Akt- Proteína quinase B (PKB)

AMPK- 5' Proteína quinase ativada por AMP

ANOVA- Análise de variânca

AUC - Área sobre a curva

c-Src- Proteínas Proto-Oncogênicas

DAF-FM- 4-amino-5-metilamino-2',7'-difluorofluoresceína diacetato

dAUC- Diferença da area sobre a curva

eNOS- Óxido nítrico sintase endotelial

ET-1- Endotelina-1

FEN - Fenilefrina

HIF-1- Fator 1 Induzível por Hipóxia

HSP- Proteínas de choque térmico

IR- Receptor de insulina

K^+- Potássio

K^+_{ATP} - Canal para potássio sensível ao ATP

K_{Ca}^{2+}- Canal para potássio sensível ao cálcio

L-NAME- NG-nitro-L-arginina-metil-éster

MAPK- Proteína quinase ativada por mitógeno

Na^+- Sódio

Na^+/K^+-ATPase- Bomba de sódio-potássio

NO- Óxido nítrico

PDK- Proteína quinase dependente de fosfoinositídeo

PI3K- Fosfatidilinositol 3-quinase

PKB- Proteína quinase B

PKC- Proteína quinase C

RM- Repetição máxima

PKC- Protein kinase C

Rmax- Resposta máxima

SDS-PAGE- Dodecyl sulfato de sódio poliacrilamida

SEM- Erro padrão da média

TEA- Tetraetilamônio

u.a.- Unidades arbitrárias

SUMÁRIO

1. INTRODUÇÃO

Numerosas evidências demonstram que o exercício físico, especialmente o aeróbio, promove uma melhora na função vascular em humanos e animais (LI et al., 2009; LINKE, ERBS, HAMBRECHT, 2008; MAIORANA et al., 2000; VONA et al., 2004; VONA et al., 2009; ZHANG et al., 2009). Os mecanismos envolvidos nesta melhoria abrangem uma variedade de ajustes moleculares e celulares incluindo o aumento da vasodilatação dependente do endotélio, um aumento na expressão e/ou atividade da óxido nítrico sintase (eNOS) e de enzimas antioxidantes (DE MORAES et al., 2008; LI et al., 2009). Grande parte dos estudos que avaliam os efeitos do exercício físico sobre a vasodilatação dependente do endotélio utilizou como agente indutor do relaxamento vascular a acetilcolina (ACh), um agonista muscarínico (DELP; LAUGHLIN, 1997; LAUGHLIN et al., 2001; WOODMAN; PRICE; LAUGHLIN, 2005).

A ACh promove o relaxamento vascular através do aumento na produção endotelial de óxido nítrico (NO) por vias de ativação que dependem do aumento da concentração intracelular de cálcio na célula endotelial (FURCHGOTT; VANHOUTTE, 1989). O aumento na produção endotelial de NO também pode ser causado pela insulina através de outra cascata enzimática acionada independentemente do aumento da concentração intracelular de cálcio (MONTAGNANI et al., 2001; MUNIYAPPA; QUON, 2007). O relaxamento arterial mediado pela insulina exerce um papel importante no controle do tônus vascular, uma vez que a redução da resposta vascular a este hormônio está diretamente relacionada ao surgimento da disfunção endotelial, hipertensão arterial e resistência à ação da insulina (MUNIYAPPA; SOWERS, 2013). Em artérias aortas isoladas de ratos obesos, foi observada uma significativa redução dos relaxamentos vasculares induzidos por ACh e por insulina, evidenciando que as

diferentes vias de sinalização desencadeadas por estes dois agentes vasoativos estão prejudicadas (ZECCHIN et al., 2007)

Dados na literatura indicam que tanto o exercício aeróbio, quanto o exercício resistido têm um papel importante no aumento da resposta vasodilatadora em diferentes artérias devido uma maior produção endotelial de NO (FONTES et al., 2014; HARRIS et al., 2010; WANG et al., 2010). A explicação mais satisfatória para a otimização da função vascular induzida por estas duas modalidades de exercício físico seria através do aumento do estresse de cisalhamento (*shear stress*). O *shear stress* gera um aumento do fluxo sanguíneo, que por sua vez ativa a via de sinalização da fosfatidilinositol 3-quinase/proteína quinase B (PI3K/Akt) levando a um aumento da atividade enzimática da óxido nítrico sintase através do mecanismo de fosforilação do aminoácido serina na posição 1177 ($eNOS^{ser1177}$) (CHAUDHURI; DANDONA; FONSECA, 2012; HIGASHI; YOSHIZUMI, 2004). O aumento nos níveis de fosforilação da $eNOS^{ser1177}$ induzido pela insulina via PI3K/Akt promove um incremento na produção endotelial de óxido nítrico (NO) e consequente vasodilatação (SALT, 2013; WANG et al., 2010).

Zhang et al. (2009) demonstraram que uma sessão de exercício aeróbio promove melhora na vasodilatação induzida por insulina devido um aumento nos níveis de fosforilação da $eNOS^{ser1177}$ e consequente aumento na produção endotelial de NO. Em outro estudo, utilizando o modelo de exercício resistido, Faria et al. (2010) demonstraram que uma sessão de exercício resistido realizada na intensidade de 50% do valor obtido no teste de 1RM aumentou a vasodilatação dependente de NO na artéria caudal, promovendo uma redução na pressão arterial em ratos espontaneamente hipertensos.

Embora esteja estabelecida na literatura a contribuição do NO para o controle do fluxo sanguíneo durante o exercício físico, os eventos de sinalização na vasculatura que medeiam à

liberação endotelial deste agente vasoativo ainda não estão completamente compreendidos (MCALLISTER; PRICE, 2010). Além disso, não se sabe ao certo a partir de qual intensidade o exercício resistido consegue promover ajustes vasculares benéficos em animais saudáveis. Dados recentes do nosso laboratório demonstraram que uma sessão de exercício resistido realizado na intensidade de 70% do valor obtido no teste de 1RM promoveu um aumento na participação do NO no relaxamento induzido por insulina em artéria mesentérica de ratos saudáveis (FONTES et al., 2014). Porém, diferentes diretrizes sugerem a inclusão de exercício resistido de leve (30% de 1RM) a moderada (50% de 1RM) intensidade como ferramenta não farmacológica para tratamento de doenças cardiovasculares e metabólicas (AHA, 2000; ADA, 2010). Frente a isso, decidimos investigar se intensidades menores de exercício resistido também promovem o mesmo benefício que o exercício resistido realizado a uma intensidade maior (70% de 1RM). O esclarecimento dos efeitos vasculares provenientes da realização do exercício resistido de menor intensidade reforça a possibilidade da utilização dessa modalidade e intensidade de exercício no tratamento coadjuvante de condições patológicas que envolvam a redução da biodisponibilidade de NO, como por exemplo, o diabetes mellitus e a hipertensão arterial. Assim, o objetivo do presente estudo foi avaliar os efeitos agudos de diferentes intensidades de exercício resistido sobre as ações vasculares da insulina em artéria mesentérica de ratos.

Dentro deste contexto, foram elaborados dois artigos originais que se encontram no capítulo de resultados da presente Tese. O primeiro artigo, “Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats”, avaliou os efeitos agudos de uma sessão de exercício resistido sobre as ações vasculares da insulina em artéria mesentérica de ratos. Neste primeiro estudo foi demonstrado que uma sessão de exercício resistido aumentou o vasorelaxamento via PI3K/eNOS. Este aumento, se deve em parte a uma maior produção de NO, associado a um aumento da participação dos canais para K^+ e da

Na^+/K^+-ATPase. O segundo artigo, “Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals” teve como objetivo verificar os efeitos agudos de diferentes intensidades de exercício resistido sobre a vasodilatação dependente do endotélio, o nível de fosforilação da $eNOS^{ser1177}$ e a produção endotelial de NO em artéria mesentérica superior de ratos saudáveis. Neste estudo foi demonstrado que uma sessão de exercício resistido moderado e/ou vigoroso melhora o relaxamento dependente do endotélio induzido por insulina devido a um aumento nos níveis de fosforilação da $eNOS^{ser1177}$ e, consequente, incremento da produção endotelial de NO em animais saudáveis. A partir destes achados foi possível sugerir que o exercício resistido é capaz de promover ajustes vasculares importantes que atuam diretamente no favorecimento do melhor controle do tônus vascular. Além disso, a magnitude destes benéficos ajustes vasculares estão fortemente relacionados ao aumento da intensidade do exercício resistido a partir da intensidade de 50% de 1 RM.

2. OBJETIVOS

2.1. OBJETIVO GERAL

- Avaliar os efeitos agudos de diferentes intensidades de exercício resistido sobre as ações vasculares da insulina em artéria mesentérica de ratos.

2.1. OBJETIVOS ESPECÍFICOS

- Caracterizar as alterações promovidas por diferentes intensidades de exercício resistido na via de sinalização IR/PI3K/eNOS em artéria mesentérica de ratos;
- Estimar a participação do NO nos vasorelaxamentos;
- Estimar a participação dos canais para K^+ nos efeitos vasculares da insulina;
- Estimar a participação da Na^+/K^+-ATPase nos vasorelaxamentos;
- Estimar a participação da ET-1 sobre os efeitos vasculares da insulina;

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3.2. Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals.

Artigo escrito para:

European Journal of Applied Physiology

Fator de Impacto - 2012: 2.660

ISSN: 1439-6327 (electronic version)

Editor-Chefe: H. Westerblad; K.R. Westerterp.

Original Article

Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals

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Abstract The aim of the present study was evaluate the acute effects of different intensities of resistance exercise over the endothelium-dependent vasodilatation, the eNOSser1177 phosphorylation level and the endothelial production of NO in superior mesenteric artery of healthy rats. Groups: control (Ct), resistance exercise in the intensities of 30% (Ex30%), 50% (Ex50%) and 70% (Ex70%) of the maximal load established by the maximal repetition test (1RM). Exercise protocol: 15 sets of 10 repetitions. The maximal response of the relaxation induced by insulin was not altered in the animals of the Ex30% group when compared to the Ct group. However, the animals of the Ex50% and Ex70% groups presented an increase in this response ($p<0.001$) (11.1 ± 0.6% and 16.7 ± 0.8%, respectively) when compared to the group (Ct 6.5 ± 0.6%). The eNOSser1177 phosphorylation levels showed an increase in Ex50% and Ex70% groups when compared to the Ct, 1.6-fold ($p<0.01$) and 3.3-fold ($p<0.001$) groups, respectively. In the endothelial production of NO, it was observed that the Ex30% group did not show alteration in the NO production when compared to the Ct group. On the other hand, the animals exercised in the Ex50% and Ex70% groups showed increase in the NO synthesis when compared to the animals in the Ct ($p<0.001$) group. Our results suggest that the magnitude of these vascular endothelium adjustments is strongly related to the increase of the resistance exercise intensity from the intensity of 50% of 1 RM.

Keywords Resistance exercise · eNOS phosphorylation · Nitric oxide · Vascular reactivity.

List of abbreviations

(ACh) Acetylcholine chloride

(Akt) Protein kinase B

AMPK- 5' adenosine monophosphate-activated protein kinase

(c-Src) Proto-oncogene c-Src

(DAF-FM) 4,-amino-5 methylamino-2',7'-diaminofluorescein diacetate

($eNOS^{ser1177}$) Nitric oxide synthase through the phosphorylation mechanism of the serine amino acid in the 1177 position

(HIF-1) Hypoxia-inducible factor-1

(HSP) Heat shock protein

(L-NAME) $L\text{-}N^{G}$-Nitroarginine methyl ester (L-NAME)

(Na^{+}/K^{+}-ATPase) Sodium-potassium adenosine triphosphatase

(NO) Nitric oxide

(Phe) L- Phenylephrine

(PI3K) Phosphatidylinositol 3 kinase

(PKC) Protein kinase C

(RM) maximal repetition

(Rmax) Maximal response

(SDS-PAGE) Sodium dodecyl sulphate polyacrylamide gel electrophoresis

(SEM) Standard error of the mean

(u.a.) Arbitrary unit

Introduction

Studies have demonstrated that insulin plays a major role in the control of the vascular tonus (Richey 2013). Insulin binds to its receptor in the endothelial cells activating the phosphatidylinositol 3 kinase /protein kinase B pathway (PI3K/Akt), what leads to an increase in the enzymatic activity of the endothelial nitric oxide synthase through the phosphorylation mechanism of the serine amino acid in the 1177 position ($eNOS^{ser1177}$) (Chaudhuri et al. 2012). The phosphorylation of this serine is necessary for the eNOS maximal activation (Wang et al. 2010). In addition, the increase in the $eNOS^{ser1177}$ phosphorylation levels induced by insulin via PI3K/Akt promotes an increase in the endothelial production of nitric oxide (NO) and a consequent vasodilation (Salt 2013). Several studies have showed that the physical exercise plays a major role in the increase of the vasodilatator response in different arteries due to a higher endothelial production of NO (Harris et al. 2010; Wang et al. 2010; Fontes et al. 2014). The most satisfactory explanation for the improvement induced by the exercise in the vascular function would be through the shear stress, causing an increase in blood flow and, thus, stimulating the PI3K/eNOS signaling pathway (Higashi et al. 2004).

Zhang et al. (2009) showed that a session of aerobic exercise promotes an improvement in the relaxation induced by insulin due to an increase in the $eNOS^{ser1177}$ phosphorylation levels and consequently an increase in the endothelial production of NO. In another study using the resistance exercise model, Faria et al. (2010) showed that a session of resistance exercise conducted in the intensity of 50% of the value obtained in the 1RM test increased the NO-dependent relaxation in the caudal artery, promoting a reduction in the arterial pressure in spontaneously hypertensive rats.

Even though the contribution of NO in the blood flow control during the exercise is established in literature, the signaling events in the vasculature that mediates the endothelial release of this vasoactive agent are still not completely understood (Symons et al. 1999). Furthermore, it is not really known from which intensity on the resistance exercise can promote beneficial vascular adjustments in healthy animals (Kemi et al. 2005). Recent data from our laboratory have showed that a session of resistance exercise performed in the intensity of 70% of the value obtained in the 1RM test promoted an increase in the participation of NO in the relaxation induced by insulin in mesenteric arteries of healthy rats (Fontes et al. 2014). Nonetheless, different guidelines suggest the inclusion of light resistance exercise (30% of 1RM) to moderate (50% of 1RM) intensity as a non-pharmacological tool for the treatment of cardiovascular and metabolic diseases (AHA 2000, ADA 2010). Based on that, we decided to investigate whether lower (30% of 1RM) or higher (70 % of 1RM) intensities of

resistance exercise would promote intensity-dependent beneficial effects in the vascular endothelium. The understanding of the vascular effects stemming from the performance of lower-intensity resistance exercise reinforces the possibility for the use of such modality and intensity of exercise in the auxiliary treatment of pathological conditions that involve the reduction in the NO bioavailability, such as diabetes mellitus and arterial hypertension. Henceforth, the aim of this study was to verify the effects of different intensities of resistance exercise (30%, 50% and 70% of 1 RM) over the endothelium-dependent vasodilatation, the eNOS^{ser1177} phosphorylation level and the endothelial production of NO in mesenteric artery of healthy rats.

Material and methods

Animals

Three-month-old male Wistar rats were obtained from the Central Animal Facility of the Federal University of Sergipe. Rats were kept in collective cages (5 animals/cage), in a temperature-controlled room (22 ± 2°C) with 12 h light/12 h dark cycle, and received commercial rodent chow (Labina Purina®) and filtered water *ad libitum*, with free access to food and water. The rats were randomized into four groups: control (Ct, n = 16), resistance exercise intensity of 30% 1RM (Ex30%, n = 16), resistance exercise intensity of 50% 1RM (Ex50%, n = 16) and resistance exercise intensity of 70% 1RM (Ex70%, n = 16). All procedures described in this study are in agreement with the Brazilian Society of Laboratory Animal Sciences and were approved by the Ethics Committee on Animal Research of the Federal University of Sergipe, Brazil. The control animals were kept in their boxes without exposure to exercise, whereas animals in the other groups underwent a session of resistance exercise in their respective intensities.

Resistance exercise protocol

Animals were exercised following a model described by Tamaki et al. (1992). Rats in the Ex30%, Ex50% and Ex70% groups were wearing a canvas jacket to be able to regulate the twisting and flexion of their torsos, and were fixed by a holder in a standing position on their hinder limbs (Tamaki et al. 1992; Barauna et al. 2005; Pinter et al. 2008). Electrical stimulation (20 V, 0.3 s duration, at 3 s intervals) was applied to the tail of the rat through a surface electrode. The animals underwent three days of familiarization: they were placed in the

device in the exercise apparatus starting position and were kept this way for 5 min in order to reduce the stress caused to the animal by the equipment and handling. After the familiarization period, the animals performed the test of a maximum repetition (1RM), which consisted of determining the maximum weight lifted by each rat in the exercise apparatus. The 1RM test is used to assess maximal muscle strength in humans and animals (ACSM, 2011; Barauna et al. 2005). After 2 days, the animals were subjected to the exercise protocol (15 sets with 10 repetitions with a 180 s resting period between each set). The animals exercised in intensity of 30% 1RM: Ex30% group, 50% of 1RM: group Ex50% and 70% of 1RM: group Ex70%.

Vascular reactivity studies

Immediately after exercise, the animals were sacrificed. The superior mesenteric artery was removed, stripped from connective and fatty tissues and sectioned into rings (1–2 mm). Rings were suspended from fine stainless steel hooks, connected to a force transducer (Letica, Model TRI210; Barcelona, Spain) with cotton threads in organ baths containing 10 mL of Tyrode's solution (composition in mM: NaCl 158.3, KCl 4.0, $CaCl_2$ 2.0, $NaHCO_3$ 10.0, $C_6H_{12}O_6$ 5.6, $MgCl_2$ 1.05 and NaH_2PO_4 0.42). This solution was continually gassed with carbogen (95% O_2 and 5% CO_2) and maintained at 37 °C under a resting tension of 0.75 g for 60 min (stabilization period). During this time, the nutrient solution was changed every 15 min to prevent the interference of metabolites (Altura and Altura, 1970). Isometric tension was recorded through the force transducer (TRI210, Letica, Barcelona, Spain) coupled to an amplifier-recorder (BD-01, AVS, SP, Brazil).

The functionality of the endothelium was assessed by the ability of acetylcholine (1 μM) to induce more than 75% relaxation of phenylephrine (1 μM)-induced pre-contraction (Furchgott and Zawadzki, 1980). After that, changes in vascular reactivity were assessed by obtaining concentration-response curves for insulin (10^{-13}–10^{-6} M; Human Regular Insulin, Novo Nordisk, Bagsvaerd, Denmark). These same curves were obtained after incubation for 30 min of the following inhibitor: L-N^G-Nitroarginine methyl ester (L-NAME), which was used to evaluate the role of NO (inhibitor of nitric oxide synthase).

Western Blot Analysis

Western blot was performed as previously described (Capettini et al. 2011). Superior mesenteric arteries were homogenized in lysis buffer (in mmol/L: 150 NaCl, 50 Tris·HCl, 5 EDTA. 2 Na and 1 $MgCl_2$, pH 8.0) containing 1% Triton X-100, 1% NP-40, 1% sodium deoxycholate, 0.5% SDS enriched with 100 mM DTT, 10 mM $Na_4P_2O_7$, 10 mM Na_3VO_4 and 10 mM NaF, 100 mM PMSF and 100 mM protease inhibitors cocktail (Sigma FAST, Sigma, St. Louis, MO). Homogenates were cleared by centrifugation at 13,000×g for 15 min at 4ºC and protein content was quantified according to Lowry assay (Lowry et al., 1951) with BSA as a standard for comparison. Proteins (50 µg) were denatured and separated on 10% of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), followed by an electronic transfer onto a 0.45 µm nitrocellulose membrane (Millipore, Billerica, MA) and blocked at room temperature with 5% non-fat milk in Tris-buffered saline plus 0.1% Tween 20 before incubation with rabbit polyclonal anti-eNOS or goat polyclonal anti-peNOS^{Ser1177} (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) at 4ºC overnight. The primary antibody binding was detected with anti-rabbit and anti-goat secondary antibodies linked to horseradish peroxidase (dilution 1:5000, Sigma-Aldrich, St. Louis, MO) for 2 h at room temperature and visualized by means of chemiluminescence reaction (Luminata strong™ - Western HRP substrate, Merck-Millipore, Darmstad, Germany). Blots were then stripped by washing in stripping solution (ReBlot Plus Strong, Merck-Millipore, Darmstad, Germany) and reprobed with antibodies. Afterwards, the membrane was exposed to a film, and bands were analyzed through densitometry in ImageJ software (NIH).

Measurement of NO production

NO production in mesenteric artery ring was determined by using a fluorescent cell permeable dye for NO, DAF-FM (4,-amino-5 methylamino-2',7'-diaminofluorescein diacetate, Molecular Probes), as previously described (De Angelis et al. 2004). In order to detect NO, freshly aorta was incubated loaded at 37ºC with 10 µmol/L of the probe for 40 min, 20 min after the onset of the probing, some rings were stimulated with 10 nmol/L regular human insulin (Novo Nordisk, Bagsvaerd, Denmark) for 20 min and then washed for 40 min with Tyrode´s solution. The mesenteric artery ring was immersed in medium for cryosectioning and cut into 20 µm thickness (Leica CM 1850 cryostat, Leica Instruments, Germany). After this time, the fluorescence generated by NO was recorded using a fluorescence microscopy (IX2-ICB, Olympus®, USA). Analyses of images were performed in ImageJ software (NIH).

Statistical analysis

The values were expressed as mean ± standard error of the mean (SEM). One-way variance analysis (ANOVA) followed by the Bonferroni post hoc test were performed to assess the significance of the differences among the means. The proteic expression results of the phosphorylated eNOS, obtained through the western blot technique, were normalized by the total expression result of the eNOS protein and the fluorescence microscopy images were analyzed according to the intensity of the fluorescence per area, both represented in arbitrary unit (u.a.), mean ± SEM. Pearson´s correlation was used to determine the association between the intensities of the resistance exercise and the variables investigated in the study (maximal response, eNOS phosphorylation levels and the endothelial production of NO). The values were considered statistically significant when $p<0.05$. For all those procedures and design of the charts, we used the statistical software GraphPad Prism version 5.1 (GraphPad Software, San Diego, CA, USA).

Results

Effect of insulin over the endothelium-dependent relaxation

As shown in Table 1, insulin induced the concentration-dependent relaxation rings isolated from superior mesenteric arteries with intact endothelium in all groups. The Rmax of the relaxations induced by insulin was not altered in the animals of the Ex30% group when compared to the Ct group (7.2 ± 0.7% and 6.5 ± 0.6%, respectively). Nonetheless, when the exercise was performed with intensity of 50 and 70%, an increase ($p<0.001$) was observed in the Rmax of insulin-induced relaxation, 11.1 ± 0.6% and 16.7 ± 0.8%, respectively, when compared to the Ct group (6.5 ± 0.6%). Similar results, yet amplified, were found in the vasodilatator response of the Ex70% group (16.7 ± 0.8%), which was increased in relation to the Ex30% (7.2 ± 0.7%; $p<0.001$) and Ex50% (11.1 ± 0.6%; $p<0.001$) groups. Pearson´s analysis revealed that there is a positive correlation between the resistance exercise intensity and the insulin-induced vascular relaxation ($r = 0.91$, $p = 0.04$).

Effect of insulin over the endothelium-dependent relaxation in the presence of L-NAME

In order to assess the contribution of the NOS in the vascular relaxation, concentration-response curves for insulin were made, which were shown to be significantly reduced in the Ct group ($p<0.001$) in the presence

of L-NAME (Table 1). There was also the identification, in all groups exercised, of a significant reduction in the vasodilatator response. Interestingly, the Ex70% group showed a strong dependence on NOS for the occurrence of the vascular dilatation, identified by the significant reversion of ($p<0.001$) the curve when compared to the other groups.

eNOS enzymatic activity

Having identified the participation of NOS in the insulin-induced relaxation in animals undergoing different intensities of exercise, we assessed the enzymatic activation of eNOS through the phosphorylation of the serine residue 1177. As demonstrated in figure 1, the animals of the Ex30% group did not show differences in the phosphorylation of the $eNOS^{ser1177}$ vasodilatator enzyme when compared to the Ct group. The Ex50% and Ex70% groups presented a significant increase in the $eNOS^{ser1177}$ phosphorylation levels when compared to the Ct group, 1.6-fold ($p<0.01$) and 3.3-fold ($p<0.001$), respectively. Among the animals exercised, the Ex50% group presented an increase ($p<0.05$) of 1-fold in the $eNOS^{ser1177}$ enzymatic activity in relation to the Ex30% group. Similarly, the Ex70% group potentialized the $eNOS^{ser1177}$ phosphorylation in 2.2-fold ($p<0.001$) in the Ex30% group, and 0.6-fold ($p<0.05$) when compared to the Ex50% group. Pearson´s analysis revealed a strong positive correlation between the eNOS phosphorylation and the resistance exercise intensity ($r = 0.93$, $p = 0.03$).

NO production

In order to assess whether the exercise intensities improve the NO endothelial biosynthesis, its intracellular production was measured through the DAF-FM. As shown in figure 2A, in basal condition, it was observed that the Ex30% group did not present alteration in the NO production when compared to the Ct group. In agreement to the findings of the $eNOS^{ser1177}$ phosphorylation, the animals exercised of the Ex50% and Ex70% groups presented an increase in the NO synthesis when compared to the animals of the Ct group, 30% and 90% ($p<0.001$) , respectively. Among the groups exercised, the animals of the Ex50% group presented an increase of 18% ($p<0.001$) in the endothelial production of NO when compared to the Ex30% group. Strengthening such hypothesis, there was also the finding of significant increases in the production of NO in the Ex70% group when compared to the Ex30% and Ex50% groups, 73% ($p<0.001$) and 46% ($p<0.001$), respectively. We also identified a positive correlation between the NO production and the resistance exercise intensity ($r = 0.93$, $p = 0.03$). In a complementary approach, the rings of the superior mesenteric artery were stimulated with insulin (10 nmol/L) to

measure the capacity of NO additional synthesis. In this experimental condition, we observed a reduction in the supplementary production of NO in an intensity-dependent way (Fig. 2B).

Discussion

In the present study, we demonstrate that different resistance exercise intensities in a single session of resistance exercise promote acute alterations in the vascular physiology of healthy animals in an intensity-dependent way. According to the American College of Sports Medicine (2011), and used in this study, resistance exercise intensities were considered as light (30%), moderate (50%) and vigorous (70%) from the values obtained in the 1RM test. The results of the present study indicate that the resistance exercise performed in the intensities of 50% and 70% of 1RM promotes an improvement in the arterial relaxation resulting from vascular adjustments caused by a higher enzymatic activation of $eNOS^{ser1177}$ and a subsequent increase in the NO bioavailability.

The literature suggests that the model of the resistance exercise using the squat equipment as described by Tamaki et al. (1992) promotes beneficial cardiovascular effects both in healthy and hypertensive animals (Lizardo et al. 2008; Harris et al. 2010; Faria et al. 2010; Fontes et al. 2014). However, for the animal to accomplish the exercise movement, caudal electro-stimulation is necessary. The electro-stimulation patterns adopted in this study were similar to those used in a recent study of our laboratory, where it was demonstrated that those electrical stimuli do not promote alterations in the insulin-induced relaxation (Fontes et al. 2014), thus suggesting that the vascular effects observed in the present study solely stem from the resistance exercise.

Insulin is a hormone that is essential for the maintenance of the metabolic homeostasis and also an important cardiovascular system regulator (Gündüz et al. 2010). In endothelial cells, insulin causes a rapid and concentration-dependent increase in the production of NO through the activation of eNOS (Salt 2013). Besides that, insulin stimulates the sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase), causing hyperpolarization of smooth muscle cells, intracellular calcium reduction and consequently vasodilatation (Ghafouri et al. 2011). In our study, the light acute resistance exercise was not able to promote an increase in the vascular relaxation in an assay of concentration-response for insulin. On the other hand, increments in the intensity of the resistance exercise produced, in an intensity-dependent way, an increase in the vasodilatator response in superior mesenteric artery.

In order to investigate the participation of NOS in the endothelium-dependent relaxation, concentration-response curves for insulin in the presence of L-NAME were obtained. In this experimental condition, L-NAME antagonized the insulin-induced relaxation in all groups, demonstrating the participation of NO in the arterial relaxation promoted by insulin. The blockade of those relaxations increased as the intensity of the resistance exercise was increased, producing a reversion of the curve in the animals undergoing a session of resistance exercise in the intensity of 70% of 1RM. Likewise, Fontes et al. (2014) showed that a session of resistance exercise in the intensity of 70% of 1RM was able to improve the insulin-induced relaxation in the mesenteric arteries of healthy rats through mechanisms that involve the activation of the via PI3K/eNOS.

The endothelial production of the NO derived from the eNOS has been shown to play a critical role in the relaxation and remodeling of the vessels under physiological and physiopathological conditions (Förstermann and Sessa 2012). However, the activation of eNOS involves, among others, the phosphorylation of residues of serine/threonine (Alderton et al. 2001). Among these numerous phosphorylation sites, several mechanical (ex.: shear stress) and chemical (ex.: hormones) factors can activate reaction cascades that result in the phosphorylation of the serine 1177 residue, elevating the eNOS activity from 2 to 3 times and, consequently, increasing the NO production (Dimmeler et al. 1999; Fleming and Rudi, 1999; Alderton et al. 2001). According to all that, the present study revealed an increase in the $eNOS^{ser1177}$ phosphorylation levels immediately after a session of resistance exercise in the intensities of 50% and 70% of 1RM. Nevertheless, the regulation of negative regulatory sites such as Threonine 495 (Thr^{495}) constitutively phosphorylated by protein kinase C (PKC) and the activity of phosphatase proteins are equally important; nevertheless, that mechanism was not investigated in the present study.

Similarly, Zhang et al. (2009) showed that after a vigorous running session on treadmill during 50 minutes, there was an increase in the $eNOS^{ser1177}$ phosphorylation levels in arteries directly involved in the exercise (ex.: aorta, femoral and iliac arteries). Our findings also pointed to an increase in the eNOS enzymatic activity in arteries that are not directly involved in the resistance exercise (superior mesenteric artery), suggesting that this modality of exercise shares systemic adjustments through similar molecular mechanisms.

The mesenteric artery, used in our study, regulated around 20% of the blood flow and effectively participates in the total peripheral resistance (Blanco-Rivero et al. 2013). That artery is located in the splenic region that is in constriction during the performance of the exercise due to the abrupt change in blood flow (reactive hyperaemia) towards the tissues (ex.: skeletal striated muscle) directly involved in the execution of the

movement (Higashi et al. 1999a; Kingwell 2000). Many an evidence suggest that the vascular wall striation associated to the pulsatile flow, hypoxia and the release of catecholamines are all factors that determine the high production of NO during the performance of the acute exercise (Balon and Nadler 1994; Roberts et al. 1999; Momken et al. 2004). That response occurs due to the activation of multiple transduction signals, such as Ras/Raf/MEK/ERK, proto-oncogene c-Src (c-Src), PI3K/Akt, 5' adenosine monophosphate-activated protein kinase (AMPK), Heat shock protein (HSP) and hypoxia-inducible factor-1 (HIF-1), which rapidly lead to the increase in eNOS activity and improvement of endothelial function (Davis et al. 2003; Higashi and Yoshizumi 2004; Cacicedo et al. 2011).

The literature presents several studies that demonstrate the protective cardiovascular role of the aerobic exercise (Niebauer and Cooke 1996; Higashi et al. 1999a; Higashi et al. 1999b). Nonetheless, the acute adjustments and the insertion of the resistance exercise are still not understood. Specifically, in the mesenteric arteries of post-infarcted animals that presented vascular dysfunction due to the decrease in the eNOS expression and NO production, it was demonstrated that 8 weeks of aerobic training is able to restore the vascular function with a concomitant increase in the expression and activity of eNOS, suggesting a cause-effect relation in the decrease of the eNOS/NO signaling in the vascular dysfunction (Wang et al. 2010). Similarly, Faria et al. (2010) observed that a single session of resistance exercise in the intensity of 50% of 1RM increased the NO-dependent relaxation in the caudal artery, promoting a reduction in the arterial pressure in spontaneously hypertensive rats. Along with all that, it is suggestive to propose that the improvement in the vascular relaxation dependent on the eNOS/NO axis restores the systemic endothelial function both in healthy animals and in animals under pathological conditions.

In the present study, our results led to the assessment of the *in situ* production of NO in the endothelium of the superior mesenteric artery of animals exercised in different intensities. We demonstrated in a pioneering way that the production of NO was gradually increased in the animals that underwent the resistance exercise in the intensities of 50% and 70% of 1RM. Interestingly, in rings marked with DAF and stimulated with insulin, the synthesis of additional NO production was gradually reduced in the animals exercised in those intensities. That response is easily supported by the fact that the rings obtained from the animals exercised at 50% and 70% of 1RM already presented increased basal activity of after-exercise eNOS and, hence, a higher basal production of NO. On the other hand, the stimulation with insulin in mesenteric rings of the control group and the group exercised at 30% of 1RM promoted a substantial increment in the DAF fluorescence. This way, our evidences

allow us to suggest that the increase in the basal activity of eNOS led to a situation of sub-maximal activation of that enzyme and a consequent reduction in the responsiveness towards insulin.

Similarly, Sun et al. (2008) showed that the aerobic exercise on treadmill in the intensity of 50% of the maximal speed also promoted an increase in the $eNOS^{ser1177}$ phosphorylation and an increase in endothelial production of NO. Such findings highlight the fact that the model of resistance exercise employed in the present study promotes, acutely and in healthy animals, vascular adjustments that are similar to those found in animals undergoing treadmill running exercises.

To conclude, we demonstrated that a session of moderate and/or vigorous resistance exercise improves the insulin-induced, endothelium-dependent relaxation probably associated to an increase in the eNOS phosphorylation levels in the serine1177 residues and, consequently, an increment in the endothelial production of NO in healthy animals. Therefore, our results suggest that the magnitude of these vascular adjustments is strongly related to an increase in the resistance exercise intensity from the intensity of 50% of 1 RM. Finally, it is possible that these exercise intensities promote positive repercussions in the auxiliary treatment of pathological conditions that involve the reduction of the NO bioavailability, as for example, diabetes mellitus and arterial hypertension.

Acknowledgements

We thank CNPq, CAPES and FAPITEC-SE for the financial support.

Potential conflict of interests

We declare that there are no conflicts of interests concerning the present study.

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Table 1 – Values of Rmax obtained from concentration-response curves for insulin before and after the pre-treatment with L-NAME.

Groups	Insulin (%)	L-NAME (%)
Control	6.5 ± 0.6	1.2 ± 0.3 ***
Ex30%	7.2 ± 0.7	5.0 ± 0.6 ***
Ex50%	11.1 ± 0.6$^{\#,\dagger}$	2.9 ± 0.8 ***
Ex70%	16.7 ± 0.8$^{\#,+}$	-5.0 ± 0.5 ***

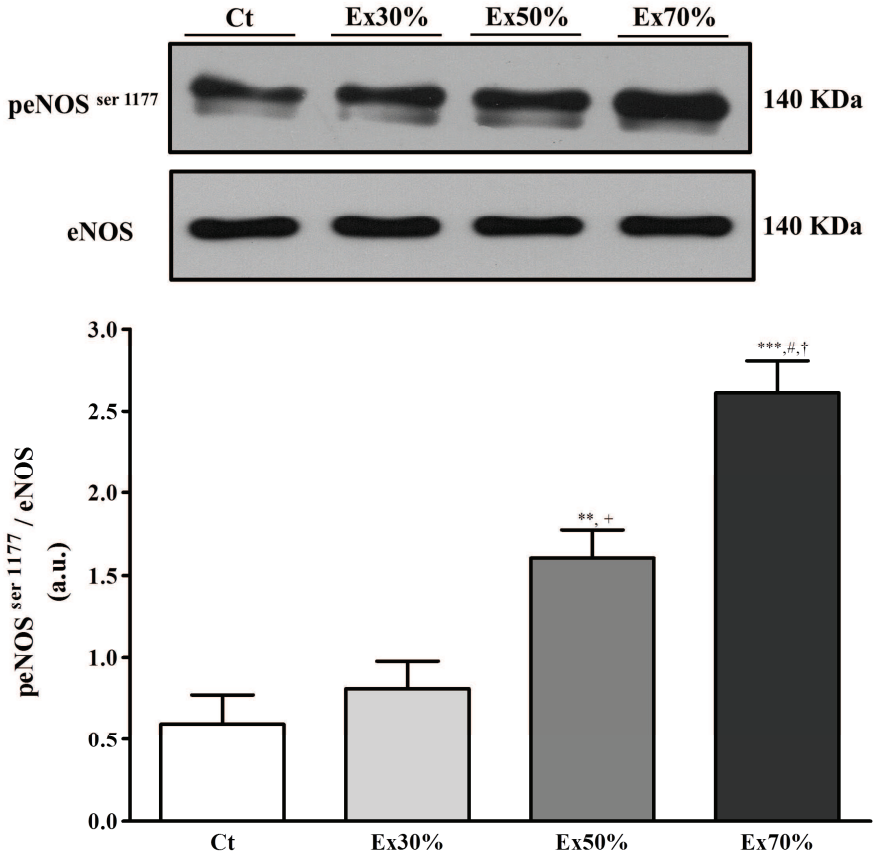
The rings were obtained from rats of the control groups - Ct; exercised at 30% of 1RM – Ex30%, exercised at 50% of 1RM – Ex50% and exercised at 70% of 1 RM – Ex70%. The experiments were performed in the absence of L-NAME (Insulin) and in the presence of 100 μM of L-NAME (L-NAME). The data represent the means ± SEM. The statistical differences were determined through the one-way ANOVA followed by the Bonferroni post hoc test. $^{\#}p<0.001$ *vs* Ct; $^{\dagger}p<0.01$ *vs* Ex30%; $^{+}p<0.001$ *vs* Ex30% and Ex50%; $^{***}p<0.001$ *vs* Insulin.

Figure captions

Figure 1. Acute effects of the resistance exercise over the enzymatic expression of the phosphorylated endothelial nitric oxide synthase ($eNOS^{ser1177}$) in rings isolated from the superior mesenteric artery. Representative image of the Western blot (top) and quantitative analysis (bottom) of the enzymatic expression of the phosphorylated endothelial nitric oxide synthase $eNOS^{ser1177}$. The data are expressed as mean ± SEM. The data are expressed as mean ± SEM. One-way variance analysis (ANOVA) followed by the Bonferroni post hoc test were performed to assess the significance of the differences among the means. Ct: Control, Ex30%: exercised at 30% of 1RM, Ex50%: exercised at 50% of 1RM and Ex70%: exercised at 70% of 1RM. $^{**}p<0.01$ or $^{***}p<0.001$ *versus* Ct, $^{\#}p<0.05$ or $^{\#\#\#}p<0.001$ *versus* Ex30% and $^{\dagger}p<0.05$ *versus* Ex50%.

Figure 2. Acute effects of the resistance exercise over the endothelial production of nitric oxide in rings isolated from the superior mesenteric artery. Representative image of the NO production (top), quantitative analysis (A) and variation between the rings in the basaline condition and the rings stimulated with 10 nmol/L of insulin (B). The arrows indicate the fluorescent staining of NO in the vascular endothelium. Scale bar: 20μm. The data are expressed as mean ± SEM. One-way variance analysis (ANOVA) followed by the Bonferroni post hoc test were performed to assess the significance of the differences among the means. Ct: Control, Ex30%: exercised at 30% of 1RM, Ex50%: exercised at 50% of 1 RM and Ex70%: exercised at 70% of 1 RM. $^{***}p<0.001$ *versus* Control, $^{\#}$ $p<0.001$ *versus* 30%. and $^{\dagger}p<0.001$ *versus* 50%.

Figure 1


Ct
Ex30%
Ex50%
Ex70%
peNOS ser 1177
140 KDa
eNOS
140 KDa
peNOS ser 1177 / eNOS (a.u.)
3.0
2.5
2.0
1.5
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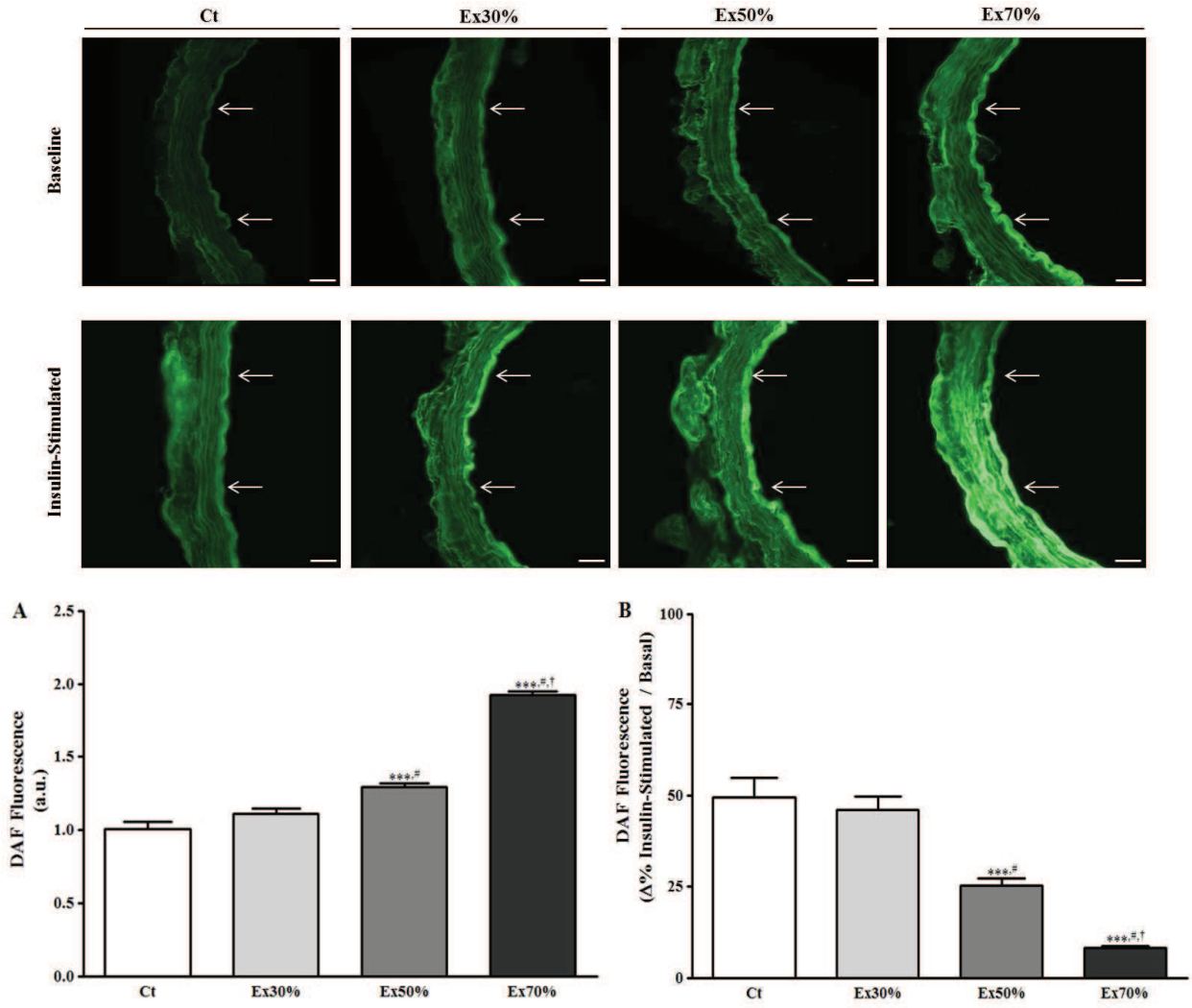
***,#,†
**,+
Ct
Ex30%
Ex50%
Ex70%

Figure 2


Ct
Ex30%
Ex50%
Ex70%
Baseline
Insulin-Stimulated
A
DAF Fluorescence (a.u.)
2.5
2.0
1.5
1.0
0.5
0.0
***,#,†
***,#
Ct
Ex30%
Ex50%
Ex70%
B
DAF Fluorescence (Δ% Insulin-Stimulated / Basal)
100
75
50
25
0
***,#
***,#,†
Ct
Ex30%
Ex50%
Ex70%

4. CONCLUSÃO

O presente estudo foi o primeiro a avaliar os efeitos do exercício resistido sobre a reatividade vascular desencadeada pela insulina em artérias mesentéricas superiores de ratos. Uma sessão de exercício resistido aumentou os vasorelaxamentos via IR/PI3K/eNOS. Este aumento, se deve em parte a uma maior produção de NO, associado a um aumento da participação dos canais para K^+ e da Na^+/K^+-ATPase. Além disso, foi observado um discreto aumento da via IR/MAPK/ET-1, entretanto sem promover prejuízos no vasorelaxamento destes animais induzidos pela insulina. Conjuntamente, estes resultados demostram que o exercício resistido agudo é capaz de promover ajustes vasculares importantes que atuam diretamente no favorecimento do melhor controle da manutenção do tônus vascular.

Demonstramos também que uma sessão de exercício resistido moderado e/ou vigoroso melhora o relaxamento dependente do endotélio induzido por insulina devido a um aumento nos níveis de fosforilação da eNOS nos resíduos serina1177 e, consequente, incremento da produção endotelial de NO em animais saudáveis. Portanto, nossos resultados sugerem que a magnitude destes benéficos ajustes vasculares está fortemente relacionados ao aumento da intensidade do exercício resistido a partir da intensidade de 50% de 1 RM. Finalmente, é possível que estas intensidades de exercício promovam repercussões positivas no tratamento coadjuvante de condições patológicas que envolvam a redução da biodisponibilidade de NO, como por exemplo, o diabetes mellitus e a hipertensão arterial.

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ANEXO A- Comprovante de Submissão.

European Journal of Applied Physiology

Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals

--Manuscript Draft--

Manuscript Number:	
Full Title:	Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals
Article Type:	Original Articles
Keywords:	Resistance exercise; eNOS phosphorylation; Nitric oxide; Vascular reactivity.
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	Rogério Brandão Wichi, Ph.D.
	Marcio Roberto Viana Santos, Philosophy Doctor
Order of Authors Secondary Information:	
Abstract:	The aim of the present study was evaluate the acute effects of different intensities of resistance exercise over the endothelium-dependent vasodilatation, the eNOSser1177 phosphorylation level and the endothelial production of NO in superior mesenteric artery of healthy rats. Groups: control (Ct), resistance exercise in the intensities of 30% (Ex30%), 50% (Ex50%) and 70% (Ex70%) of the maximal load established by the maximal repetition test (1RM). Exercise protocol: 15 sets of 10 repetitions. The maximal response of the relaxation induced by insulin was not altered in the animals of the Ex30% group when compared to the Ct group. However, the animals of the Ex50% and Ex70% groups presented an increase in this response ($p<0.001$) (11.1 ± 0.6% and 16.7 ± 0.8%, respectively) when compared to the group (Ct 6.5 ± 0.6%). The eNOSser1177 phosphorylation levels showed a increase in Ex50% and Ex70% groups when compared to the Ct, 166% ($p<0.01$) and 333% ($p<0.001$) groups, respectively. In the endothelial production of nitric oxide, it was observed that the Ex30% group did not show alteration in the NO production when compared to the Ct group. On the other hand, the animals exercised in the Ex50% and Ex70% groups showed increase in the

	NO synthesis when compared to the animals in the Ct ($p<0.001$) group. Our results suggest that the magnitude of the beneficial vascular endothelium adjustments is strongly related to the increase of the resistance exercise intensity from the intensity of 50% of 1 RM on.
Suggested Reviewers:	Luciana Venturini Rossoni, Philosophy Doctor University of Sao Paulo lrossoni@icb.usp.br Expert in the area.
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ANEXO B- Instruções aos Autores.

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Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

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EMG	Electromyography
MU	Motor unit
MVC	Maximal voluntary contraction
RMS	Root mean square
sEMG	Surface electromyography

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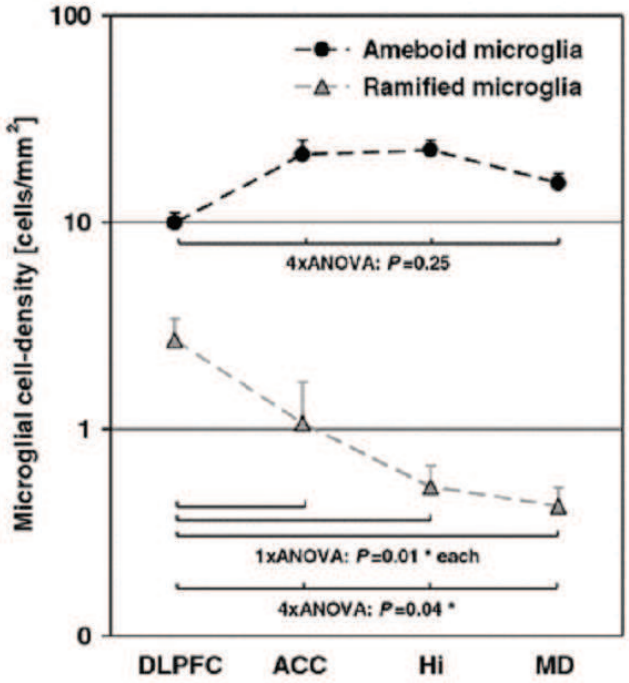

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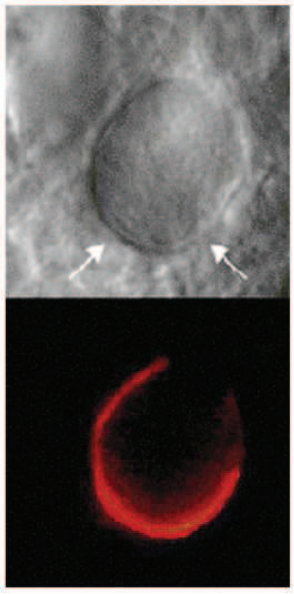
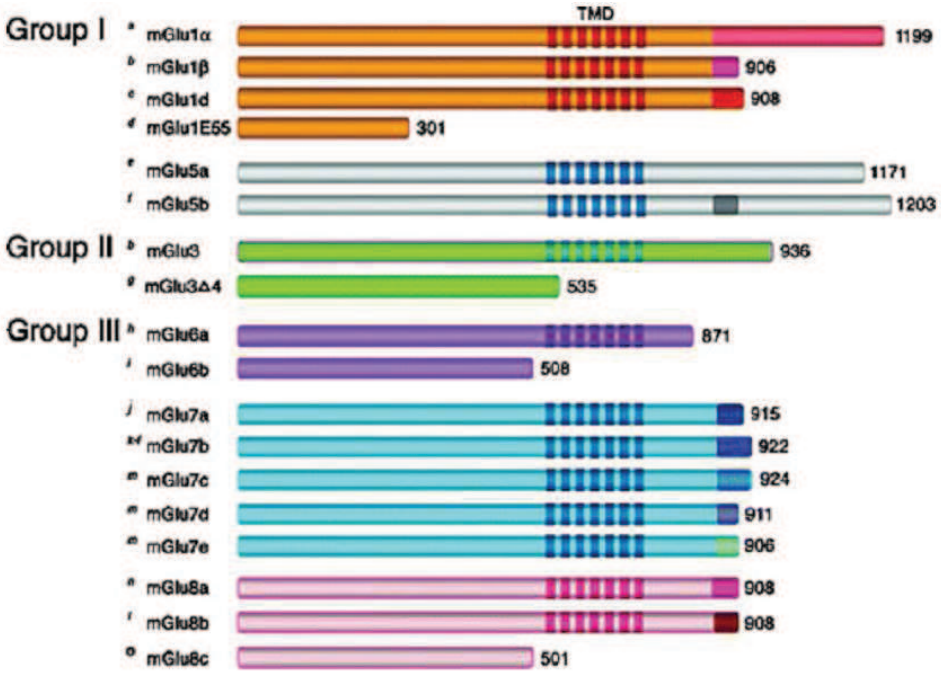


Combination Art



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ANEXO C- Aprovação do comitê de ética.

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COORDENAÇÃO DE PESQUISA
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado "Efeitos da freqüência do exercício resistido sobre a reatividade vascular de ratos", sob coordenação do Prof. Dr. Márcio Roberto Viana Santos (protocolo CEPA 80/2010), foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 10/12/2010.

São Cristóvão, 17 de dezembro de 2010

Profª. Drª. Flavia Teixeira Silva
Presidente do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
49100-000
Fones: 3212 6661/6606

ANEXO D- Artigo publicado nos Arquivos Brasileiros de Cardiologia "Resistance Training Controls Arterial Blood Pressure in Rats with L-NAME- Induced Hypertension".

Original Article

Resistance Training Controls Arterial Blood Pressure in Rats with L-NAME- Induced Hypertension

Ayslan Jorge Santos de Araujo[1], Anne Caroline Veríssimo dos Santos[1], Karine dos Santos Souza[2], Marlúcia Bastos Aires[2], Valter Joviniano Santana-Filho[3], Emerson Ticona Fioretto[2], Marcelo Mendonça Mota[1], Márcio Roberto Viana Santos[1]

Departamento de Fisiologia, Universidade Federal de Sergipe[1]; Departamento de Morfologia, Universidade Federal de Sergipe[2]; Departamento de Fisioterapia, Universidade Federal de Sergipe[3], São Cristovão, Sergipe – Brazil

Abstracts

Background: **Arterial hypertension is a multifactorial chronic condition caused by either congenital or acquired factors.**

Objective: **To evaluate the effects of Resistance Training (RT) on arterial pressure, and on vascular reactivity and morphology, of L-NAME-treated hypertensive rats.**

Methods: **Male Wistar rats (200 – 250 g) were allocated into Sedentary Normotensive (SN), Sedentary Hypertensive (SH) and Trained Hypertensive (TH) groups. Hypertension was induced by adding L-NAME (40 mg/Kg) to the drinking water for four weeks. Arterial pressure was evaluated before and after RT. RT was performed using 50% of 1RM, 3 sets of 10 repetitions, 3 times per week for four weeks. Vascular reactivity was measured in rat mesenteric artery rings by concentration-response curves to sodium nitroprusside (SNP); phenylephrine (PHE) was also used for histological and stereological analysis.**

Results: **Resistance training inhibited the increase in mean and diastolic arterial pressures. Significant reduction was observed in Rmax (maximal response) and pD_2 (potency) of PHE between SH and TH groups. Arteries demonstrated normal intima, media and adventitia layers in all groups. Stereological analysis demonstrated no significant difference in luminal, tunica media, and total areas of arteries in the SH and TH groups when compared to the SN group. Wall–to-lumen ratio of SH arteries was significantly different compared to SN arteries ($p<0.05$) but there was no difference when compared to TH arteries.**

Conclusions: **RT was able to prevent an increase in blood pressure under the conditions in this study. This appears to involve a vasoconstrictor regulation mechanism and maintenance of luminal diameter in L-NAME induced hypertensive rats (Arq Bras Cardiol. 2013;100(4):339-346).**

Keywords: **Hypertension / physiopathology; Exercise; Rats; Arterial Pressure / drug effects; Vasodilatation / physiology.**

Introduction

Arterial hypertension (AH) is a multifactorial chronic condition[1], caused by either congenital or acquired factors[2]. Its treatment involves pharmacological and non-pharmacological methods[3]. Among non-pharmacological methods, physical training (PT) has been one of the most important interventions indicated for preventing or controlling AH[4,5].

There is a positive relationship between hypertension and sedentary lifestyle[6]. Beunza et al[6] demonstrated that the interaction of sedentary behaviors, such as driving and computer use, was associated with a higher risk of hypertension. On the other hand, the noninteractive subtype, such as television viewing and sleeping, was not associated with AH. This data shows the great importance of long-term PT for the primary prevention and treatment of high blood pressure[4].

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Manuscript received May 16, 2012; revised manuscript October 19, 2012; accepted November 08, 2012.

DOI: 10.5935/abc.20130051

Many studies have focused on the effects of aerobic exercise[2-5], and it has been recommended as an adjuvant treatment for hypertension; however, knowledge regarding the benefits of resistance training (RT) has been increasing. Positive effects of long-term RT, such as increased skeletal muscle tone, inhibition of risk factors and prevention of obesity have been found[7]. Studies suggest the adoption of a moderate strength training program and assert that it can have a positive effect on chronically increased BP[8].

According to Pollock et al[9], there is some trepidation about the prescription and implementation of RT due to the possibility of vascular brain or cardiac events. On the other hand, in normotensive individuals, Ciolac and Guimarães[10] indicate that there is no justification for fearing RT implementation as many negative effects on blood pressure before the execution of RT have been observed. Furthermore, acute dynamic resistance exercise in spontaneously hypertensive rats decreased resting BP and reactivity to PHE, in addition to increasing endothelium-dependent relaxation[11,12]; however, the long-term effect of RT on BP and reactivity and morphology

ANEXO E- Prêmio ABC de Publicação Científica (Melhor Artigo Original 2013), Sociedade Brasileira de Cardiologia "Resistance Training Controls Arterial Blood Pressure in Rats with L-NAME- Induced Hypertension".

ANEXO F- Artigo publicado na Revista de Ciências Médicas e Biológicas "Suplementação com L-arginina associada ao exercício resistido melhora a força muscular e impede o aumento da glicemia de ratos diabéticos".

ARTIGO ORIGINAL
ISSN 1677-5090

Suplementação com L-arginina associada ao exercício resistido melhora a força muscular e impede o aumento da glicemia de ratos diabéticos

L-arginine Supplementation associated with resistance exercise improves muscle strength and prevents the increase in blood glucose in diabetic rats

Tharciano Luiz Teixeira Braga da Silva[1], Marcelo Mendonça Mota[1], Milene Tavares Fontes[2], Ana Paula dos Santos Soares[3], André Sales Barreto[4], Anderson Carlos Marçal [5], Márcio Roberto Viana Santos[6]

[1]Professor de Educação Física, Doutorando do Núcleo de Pós-Graduação em Medicina da UFS, [2] Professora de Educação Física, Mestranda do Núcleo de Pós-Graduação em Medicina da UFS, [3]Professora de Educação Física pela UFS, [4]Fisioterapeuta, Professor Assistente do Núcleo de Educação em Saúde da UFS, [5]Licenciado em Ciências, Professor Adjunto de Anatomia Humana da UFS, [6] Biólogo, Professor Adjunto do Departamento de Fisiologia da UFS.

Resumo

Introdução: Diversas terapêuticas têm sido empregadas no controle do diabetes. **Objetivo:** O objetivo deste estudo foi avaliar os efeitos da suplementação com L-arginina e do exercício resistido, isolado ou combinado sobre a massa corporal, glicemia e a força muscular de ratos diabéticos. **Metodologia:** Ratos Wistar foram divididos em 6 grupos: Controle (CON, n = 5), estimulado eletricamente (EE, n = 5), diabético sedentário (DS, n = 5), diabético L-arginina (DL-Arg, n = 5), diabético treinado (DT, n = 5) e diabético treinado + L-arginina (DT + L-Arg, n = 5). O diabetes foi induzido através da administração de aloxano na dose única de 40 mg/kg, i.v., duas semanas antes do início dos protocolos. Foi avaliada a massa corporal, glicemia e a força muscular no início, a cada duas semanas e no final das 6 semanas dos procedimentos experimentais. **Resultados:** No início do estudo, o DS apresentou um aumento significativo ($p < 0,001$) da glicemia quando comparado com o CON. Após as 6 semanas de estudo os animais do grupo DT e DT + L-Arg obtiveram um aumento significativo ($p < 0,01$ e $p < 0,001$; respectivamente) nos níveis de força quando comparado com o DS. Os animais DT + L-Arg apresentaram uma redução significativa ($p < 0,001$) da glicemia plasmática ao longo do tratamento quando comparado com o DS. **Conclusão:** A suplementação com L-arginina associada ao exercício resistido aumenta a força muscular e promove um equilíbrio metabólico em animais diabéticos.
Palavras-chave: Diabetes Mellitus. Arginina. Treinamento de resistência.

Abstract

Introduction: *Several therapies have been used to control diabetes.* ***Objective:*** *The aim of our study was evaluate the effects of L-arginine supplementation and resistance exercise, alone or in combination on body weight, blood glucose and muscle strength in diabetic rats.* ***Methodology:*** *Wistar rats were divided into 6 groups: control (CON, n = 5), electrically stimulated (ES, n = 5), sedentary diabetic (SD, n = 5), diabetic L-arginine (DL-Arg, n = 5), trained diabetic (TD, n = 5) and trained diabetic + L-arginine (TD + L-Arg, n = 5). Diabetes was induced by administration of alloxan in a single dose of 40 mg / kg, iv, two weeks before the start of the protocols. Was evaluated the body mass, blood glucose and muscle strength at the beginning of the experiment, every two weeks and at the end of the experimental procedures.* ***Results:*** *At baseline, the DS showed a significant increase ($p < 0.001$) glucose when compared with the CON. After 6 weeks of study animals from group TD and TD + L-Arg had a significant increase ($p < 0.01$ and $p < 0.001$, respectively) at muscle strength. The animals TD + L-Arg presented a significant reduction ($p < 0.001$), plasma glucose during the treatment group compared to SD.* ***Conclusions:*** *L-arginine Supplementation associated with resistance exercise increases muscle strength and promotes a metabolic balance in diabetic animals.*
Keywords: *Diabetes mellitus. Arginine. Resistance training.*

INTRODUÇÃO

O diabetes mellitus (DM) pode ser definido como um grupo heterogêneo de distúrbios metabólicos caracterizados pela hiperglicemia, causado por uma disfunção na secreção da insulina ou na ação desta, ou por ambas as coisas (SHI et al., 2006; ADA, 2008). Existem dois tipos principais de DM, o DM tipo 1 (DM1) onde os portadores são dependentes do uso da insulina, e o DM tipo 2 (DM2), em que os pacientes apresentam uma concentração plasmática do hormônio mas este é ineficaz (SBD, 2007). Em condições crônicas o DM1 descompensado resulta em disfunção, lesão e, em última instância, insuficiência de vários órgãos (ADA, 2008).

Dentre as diversas terapêuticas empregadas na melhora do DM, tem sido indicada a prevenção primária que inclui mudanças na dieta alimentar e a prática de

Correspondência / Correspondence: Márcio Roberto Viana dos Santos. Universidade Federal de Sergipe, Centro de Ciências Biológicas e da Saúde. Campus Universitário, S/N. Cidade Universitária. CEP: 49100-000 - Aracaju, SE – Brasil. Telefone: (79) 21056842. http://www.ufs.br

ANEXO G- Manuscrito aceito para publicação nos Arquivos Brasileiros de Cardiologia "Exercício resistido restaura a função endotelial e reduz a pressão arterial de ratos diabéticos tipo 1".

SOCIEDADE BRASILEIRA DE CARDIOLOGIA

Luiz Felipe P. Moreira
Editor-Chefe

Rio de Janeiro, 16 de dezembro de 2013.

DECLARAÇÃO

Declaro para os devidos fins, que o artigo **"Exercício Resistido Restaura a Função Endotelial e Reduz a Pressão Arterial de Ratos Diabéticos Tipo 1",** dos autores Marcelo Mendonça Mota, Tharciano Luiz Teixeira Braga da Silva, Milene Tavares Fontes, André Sales Barreto, João Eliakim dos Santos Araújo, Antônio César Cabral de Oliveira, Rogério Brandão Wichi e Márcio Roberto Viana Santos, foi aceito para publicação no periódico Arquivos Brasileiros de Cardiologia.

Luiz Felipe P. Moreira
Editor-Chefe

Artigo Original

Exercício resistido restaura a função endotelial e reduz a pressão arterial de ratos diabéticos tipo 1

Resistance exercise restores endothelial function and reduce blood pressure in type 1 diabetic rats

Título abreviado: Exercício resistido e ratos diabéticos tipo 1.

Quantidade Total de Palavras: 4721.

Marcelo M. Mota[1]; Tharciano Luiz T. B. da Silva[1]; Milene T. Fontes[1], André S. Barreto[1]; João Eliakim dos Santos Araújo[1]; Antônio Cesar Cabral de Oliveira[2]; Rogério Brandão Wichi[2]; Márcio R. V. Santos[1*]

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RESUMO

Fundamento: Os efeitos do exercício resistido sobre os parâmetros cardiovasculares não são consistentes.

Objetivo: Foram avaliados os efeitos do exercício resistido sobre as alterações na glicemia, reatividade vascular e pressão arterial de ratos diabéticos.

Métodos: Ratos Wistar foram divididos em 3 grupos: grupo controle (C, n = 8), diabético sedentário (DS, n = 8) e diabético treinado (DT, n = 8). O exercício resistido foi realizado no aparelho de agachamento para ratos e consistiu em 3 séries de 10 repetições com uma intensidade de 50%, 3 vezes por semana, durante 8 semanas. As alterações na reatividade vascular foram avaliadas em anéis de artéria mesentérica superior.

Resultados: Foi observado uma redução significativa da resposta máxima (Rmax) dos relaxamentos induzidos por acetilcolina (ACh) no DS (78,1% ± 2) e um aumento do DT (95 ± 3%), sem alterar a potência (pD_2). Na presença de N^G-nitro L-arginina metil éster (L-NAME), os relaxamentos induzidos por ACh foram significativamente reduzidos nos grupos C e DT, mas não no grupo DS. Além disso, foi observado um aumento significativo ($p < 0,05$) da pressão arterial média (PAM) no grupo DS de 104,9 ± 5 para 126,7 ± 5 mmHg, quando comparado ao grupo C. Por outro lado, o grupo DT apresentou uma redução significativa ($p < 0,05$) nos níveis da PAM de 126,7 ± 5 mmHg para 105,1 ± 4 mmHg, quando comparado ao DS.

Conclusão: O exercício resistido foi capaz de restaurar a funcionalidade endotelial e impedir o aumento da pressão arterial em ratos diabéticos tipo 1.

Palavras-Chave: Hiperglicemia; exercício resistido; reatividade vascular.

ABSTRACT

Background: The effects of resistance exercise on cardiovascular parameters are not consistent.

Objective: We evaluated the effects of resistance exercise on changes in blood glucose, blood pressure and vascular reactivity in diabetic rats.

Methods: Wistar rats were divided into 3 groups: control group (C, n = 8), sedentary diabetic (SD, n = 8) and trained diabetic (TD, n = 8). Resistance exercise was carried out in rats squat apparatus and consisted of 3 sets of 10 repetitions with an intensity of 50%, 3 times per week for 8 weeks. Changes in vascular reactivity were evaluated in rings of superior mesenteric artery.

Results: Was observed a significant reduction of the maximum response (Rmax) of relaxation induced by acetylcholine (ACh) on the SD (78.1 ± 2%) and an increase in TD (95 ± 3%) without changing the power (pD_2). In the presence of N^G-nitro-L-arginine methyl ester (L-NAME), the ACh-induced relaxation was significantly reduced in group C and TD, but not in SD group. Furthermore, we observed a significant increase ($p < 0.05$) in mean arterial pressure (MAP) in the SD 104.9 ± 5 to 126.7 ± 5 mmHg, compared to group C. On the other hand, the TD group showed a significant decrease ($p < 0.05$) in the levels of MAP 126.7 ± 5 mmHg to 105.1 ± 4 mmHg when compared to SD.

Conclusion: The resistance exercise was able to restore endothelial function and prevent an increase in arterial pressure in type 1 diabetic rats.

Keywords: Hyperglycemia; resistance exercise; vascular reactivity.

Introdução

O diabetes mellitus é caracterizado como um grupo heterogêneo de desordens metabólicas que apresentam em comum à hiperglicemia associada a complicações secundárias no sistema cardiovascular [1,2]. O aumento nos níveis glicêmicos está associado à disfunção endotelial *in vivo* e *in vitro* [3,4]. A disfunção endotelial é um fenômeno sistêmico e refere-se ao desequilíbrio da produção endotelial de mediadores que regulam o tônus vascular e contribui em parte para o aumento nos níveis de pressão arterial [5]. A disfunção endotelial no diabetes mellitus do tipo 1 pode ser considerada um marcador precoce de doenças cardiovasculares [6].

Muitos fatores podem explicar a disfunção endotelial no diabetes mellitus do tipo 1, tais como hiperlipidemia, a resistência à insulina, hiperglicemia e hipertensão [7]. Em adição, a literatura indica que o exercício resistido contribui na prevenção/tratamento de patologias que acometem o metabolismo e a função cardiovascular [8,9,10]. O exercício resistido tem demonstrado ter um importante potencial terapêutico por promover um ganho de massa muscular esquelética, um aumento na sensibilidade à insulina e uma redução da glicemia plasmática em ratos diabéticos [8,11]. Estes efeitos também são apresentados pelo exercício aeróbio [11,12].

Estudos sugerem que o exercício aeróbio é eficaz no tratamento da disfunção endotelial no diabetes [13,14,15]. Por outro lado, pouco se sabe sobre os efeitos crônicos do exercício resistido na pressão arterial e na função endotelial de ratos diabéticos do tipo 1. Nós hipotetizamos que a utilização do exercício resistido em longo prazo pode minimizar os efeitos deletérios que acometem o sistema cardiovascular e o controle metabólico apresentados por animais induzidos ao diabetes mellitus do tipo 1. Dentro desta perspectiva, o

objetivo do presente estudo foi avaliar os efeitos crônicos do exercício resistido sobre as alterações na glicemia, reatividade vascular e pressão arterial de ratos diabéticos.

Material e métodos

Animais e delineamento experimental

Ratos Wistar machos (*Rattus norvegicus*), com idade de 3 meses, pesando entre 250 e 300 g foram utilizados em todos os experimentos. Os animais foram mantidos sob condições controladas de temperatura (22±1ºC) e ciclo claro-escuro de 12 horas, tendo livre acesso à água e ração específica para roedores, Labina da marca Purina. Todos os procedimentos descritos no presente trabalho foram aprovados pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, Brasil (Protocolo número 01/2008). Os animais foram divididos em 3 grupos com 8 animais cada: grupo controle (C), diabético sedentário (DS) e diabético treinado (DT). Os animais dos grupos C e DS foram mantidos em suas caixas sem exposição ao exercício, enquanto que os animais do grupo DT foram submetidos a 8 semanas de exercício resistido.

Drogas

As drogas utilizadas foram: cloreto de acetilcolina (ACh), L-fenilefrina (FEN), N^G-nitro L-arginina metil éster (L-NAME), aloxano (Todos da SIGMA, USA), tiopental sódico (Thiopentax, Cristália, Itapira, SP, Brasil).

Indução do diabetes e medida da glicemia

A indução do diabetes experimental foi realizada conforme descrito por Da Silva Costa e cols [16]. Os animais, após jejum prévio de 24 horas, foram induzidos ao diabetes através da administração de aloxano na dose única de 40 mg/kg, i.v. (veia peniana), duas semanas antes do início do protocolo de exercício. Os animais com glicemia ≥ 200 mg/dL foram selecionados como diabéticos. A glicemia foi medida uma semana após o tratamento com aloxano utilizando fitas reagentes (ACCU-CHEK Advantage II, Roche, São Paulo/SP, Brasil) acoplada a um glicosímetro portátil digital (ACCU-CHEK Advantage II, Roche, São Paulo, SP, Brasil).

Protocolo de exercício

O exercício resistido foi realizado em aparelho de agachamento segundo modelo de Tamaki e cols [17]. Os animais do DT, após 1 semana de familiarização, foram treinados através de 3 séries de 10 repetições, com intervalos de repouso de 60s, e intensidade de 50% da carga

estabelecida através do teste de uma repetição máxima (1RM), 3 vezes por semana. Para a determinação da força máxima, cargas sucessivas foram acrescentadas ao equipamento e os animais foram estimulados eletricamente a executar uma repetição. Entre os incrementos de carga, repousos adequados de cinco minutos foram aplicados na tentativa de permitir a recuperação da musculatura trabalhada. Foi considerada como carga máxima para cada animal, aquela que foi realizada com o maior peso e permitiu um movimento completo. As cargas de treinamento foram reajustadas a cada 2 semanas através de um novo teste de 1RM [18]. Os parâmetros de estimulação elétrica foram realizados conforme descrito por Pinter e cols [19]. Os animais foram estimulados a executar as séries através da aplicação de estímulos elétricos (20 V, 0.3 s de duração, 3 s de intervalo) [20] por eletrodos (Valu Trode, Modelo CF3200, Axelgaard, Fallbrook, CA, EUA) fixados na cauda e conectados a um eletroestimulador (BIOSET, Physiotonus Four, Modelo 3050, Rio Claro, SP, Brasil).

Procedimento cirúrgico e registro direto da pressão arterial média

Neste procedimento, os animais foram anestesiados com tiopental sódico (45 mg/kg, i.p.), e cateteres de polietileno (PE-10/50*, Intramedic, Becton Dickinson and Company, Sparks, MD, USA*), preenchidos com solução salina heparinizada (1:20 v/v), foram implantados, através de incisão inguinal, na artéria femoral esquerda para o registro da pressão arterial. Após a inserção e fixação, o cateter foi exteriorizado na região cervical posterior do animal (*scapulae*) e incisão suturada. Após as suturas das incisões e término dos procedimentos cirúrgicos, todos os animais receberam cloridrato de oxitetraciclina (antibiótico de ação prolongada) em dose única (0,2 g/Kg) pela via intramuscular e diclofenaco sódico na dose de 10 mg/kg/dia por via oral em seguida colocados em caixas individuais, onde permaneceram por um período mínimo de 24h (recuperação pós-operatória).

Após recuperação pós-operatória com os animais apresentando mobilidade espontânea, o cateter foi acoplado a um transdutor de pressão (Edwards Lifescience, Irvine, CA, USA) e após 30 minutos de estabilização do sinal foram registrados 5 minutos para análise dos dados. Após 24 horas da coleta dos registros de pressão arterial média os animais foram anestesiados e preparados para os experimentos de reatividade vascular.

Reatividade vascular da artéria mesentérica superior

A preparação do tecido foi realizada conforme descrito por Araujo e cols [10]. A funcionalidade do endotélio foi verificada pela habilidade medida em percentagem, de 1 μM de ACh em relaxar mais do que 75% os anéis pré-contraídos com 1μM de FEN [21].

As alterações na reatividade vascular foram avaliadas através da obtenção de curvas concentração-resposta para ACh (10^{-9}-10^{-4} M), um agonista muscarínico não-seletivo. Para avaliar a participação do óxido nítrico nos relaxamentos induzidos por ACh, as curvas para este agente foram também obtidas na presença de L-NAME (100 μM), um inibidor da NOS.

Análises estatísticas

Inicialmente, todos os dados foram submetidos ao teste de Kolmogorov-Smirnov, com o intuito de determinar se suas distribuições de probabilidade apresentavam-se como

paramétricas ou não paramétricas. Todos os dados apresentaram uma distribuição normal. Os valores foram expressos como a média ± erro padrão da média (E.P.M.). Quando necessário, os testes *t de Student* para amostras independentes e a análise de variância (ANOVA) para medidas repetidas ou de duas-vias seguidas do pós-teste de Bonferroni foram realizados para avaliar a significância das diferenças entre as médias. A correlação de Pearson foi utilizada para determinar a associação entre o relaxamento induzido por ACh e a glicemia. Os valores foram considerados estatisticamente significativos quando $p<0,05$. Para todos estes procedimentos foi utilizado o programa estatístico GraphPad Prism versão 3.02 (GraphPad Software, San Diego, CA, E.U.A.)

Fontes de Financiamento

O presente estudo foi financiado pela CNPq, CAPES e FAPITEC-SE.

Resultados

Força máxima

Pode ser observado que no início dos experimentos os níveis de força foram similares em todos os grupos (C: 956,3 ± 63,3, n=8; DS: 1022,2 ± 32,3, n=8 e DT: 945,4 ± 108,7 g, n=8). Após 8 semanas de experimento, os animais dos grupos C e DS não apresentaram diferenças estatisticamente significativas em seus níveis de força (1032,2 ± 44,0 e 1030,5 ± 61,2 g respectivamente). Além disso, é possível observar que o exercício resistido promoveu um aumento ($p<0,01$) nos níveis de força de 945,4 ± 108,7 para 1327,3 ± 98,7 g.

Glicemia

O efeito do exercício resistido está demonstrado na figura 1. Pode ser observado que o aloxano induziu o aumento ($p<0,001$) da glicemia em ambos os grupos experimentais. Além disso, é possível observar que o exercício resistido promoveu uma redução ($p<0,05$) da glicemia após oito semanas de tratamento (Figura 1).

Relaxamento dependente do endotélio

Como demonstrado na Figura 2, a ACh induziu relaxamento dependente da concentração em anéis isolados de artéria mesentérica superior com endotélio intacto em todos os grupos. Nem o diabetes, nem o exercício resistido interferiram na sensibilidade arterial, tendo em vista que a pD_2 (concentração que o agonista produz uma resposta igual a 50% da resposta máxima) permaneceu inalterada. No entanto, no grupo DS o diabetes promoveu redução ($p<0,001$) na resposta máxima (Rmax) quando comparado ao grupo C. Este feito foi revertido ($p<0,01$) nos animais treinados que receberam aloxano (DT). Além disso, a Figura 3 mostra uma forte correlação negativa entre o relaxamento induzido por ACh e a glicemia nos grupos DS ($r = -0,9710$, $p = 0,001$, $n = 8$) e DT ($r = -0,9874$, $p = 0,001$, $n = 8$).

Relaxamento dependente do endotélio na presença de L-NAME

Como observado na Tabela 1, o L-NAME foi capaz de reduzir ($p<0,001$) a sensibilidade arterial (pD_2) e a Rmax ($p<0,01$ e $p<0,001$) dos relaxamentos induzidos por ACh nos grupos C e DT respectivamente. Esta redução não foi observada no DS. Entre os grupos, a presença de L-NAME aumentou significativamente ($p<0,001$) a sensibilidade arterial dos relaxamentos induzidos por ACh do grupo DS em relação ao grupo C sem modificar a resposta máxima. Por outro lado, o L-NAME reduziu significativamente ($p<0,001$) a sensibilidade arterial e a resposta máxima ($p<0,01$) dos relaxamentos induzidos por ACh no grupo DT quando comparado ao grupo DS.

Pressão arterial média

A indução do diabetes com aloxano promoveu aumento ($p<0,05$) da pressão arterial média no grupo DS. Inversamente, o exercício resistido reduziu ($p<0,05$) a pressão arterial dos animais treinados que receberam aloxano (Figura 4).

Discussão

Os resultados indicam que o exercício resistido em animais diabéticos do tipo I promove redução da glicemia, restaura a função endotelial e diminui a pressão arterial após 8 semanas de treinamento.

No presente estudo, foi utilizado o exercício resistido que é descrito como um tipo de exercício físico caracterizado por movimentos intermitentes e por uma via metabólica predominantemente anaeróbia [22,23]. O exercício resistido foi realizado em um aparelho de agachamento para ratos que já demonstrou ser eficaz em mimetizar os benéficos efeitos cardiovasculares encontrados em humanos praticantes desta modalidade de exercício [19,20]. Para que o animal execute o movimento de agachamento e necessário a eletroestimulação caudal. É descrito na literatura que os parâmetros de eletroestimulação usado neste estudo não promovem alterações no sistema cardiovascular [20]. Com base na literatura, sugerimos que os efeitos observados nos animais diabéticos treinados são diretamente relacionados ao exercício resistido.

O teste de força máxima foi usado como um indicador de eficácia do treinamento. Dentro desta perspectiva, observamos que os animais diabéticos treinados ganharam força muscular após 8 semanas. Isto indica que o protocolo de treinamento foi capaz de promover ajustes crônicos provenientes do exercício resistido. Recentemente, está em destaque o importante potencial terapêutico desta modalidade de exercício [9]. O exercício resistido demonstra ter um efeito benéfico na melhora da ação insulínica, no ganho de massa muscular, na redução da massa gorda, no controle glicêmico e na redução da pressão arterial em indivíduos com diabetes [24-26].

Evidências experimentais e clínicas demonstram que os distúrbios metabólicos, principalmente a hiperglicemia crônica, estejam estritamente relacionados com as complicações cardiovasculares provenientes do diabetes mellitus [7,27]. Para uma melhor compreensão das complicações metabólicas e cardiovasculares provenientes do diabetes, diversos modelos experimentais de diabetes induzidos em ratos têm sido amplamente utilizados por diversos grupos de pesquisa [28]. É relatado que o aloxano causa a destruição de grande parte das células β-pancreáticas o que impossibilita a produção de insulina necessária

para demanda do organismo [28,29]. O modelo experimental de diabetes induzido pelo aloxano é do tipo 1 e apresenta sintomas semelhantes aos encontrados em humanos, tais como perda de peso, poliúria, polidipsia, polifagia, glicosúria, cetonúria, aumento da produção das espécies reativas de oxigênio, hipoinsulinemia e hiperglicemia [28-30].

Os níveis elevados de glicemia apresentados pelos animais diabéticos no início do estudo, foram reduzidos após 8 semanas de treinamento. Da mesma forma, Farrell e cols [8] demonstram que o tratamento com o exercício resistido ao final de 8 semanas reduziu a glicemia de animais com diabetes do tipo I. A literatura indica que a contração muscular realizada durante o exercício físico estimula a translocação de proteínas transportadoras de glicose (GLUT4), independente da ação da insulina, que resulta no aumento da captação de glicose periférica [31,32]. Portanto, uma possível explicação para a redução da glicemia dos animais treinados no presente estudo, pode estar relacionada a uma maior ativação das vias de sinalização envolvidas no transporte de glicose independentes da ação de insulina, uma vez que nossos animais apresentam deficiência ou ausência na produção de insulina por se tratarem de um modelo de diabetes mellitus tipo 1 [8,29,32].

Segundo Gross e cols [33], a hiperglicemia causa danos, disfunções e até falência de vários órgãos, envolvendo severas alterações micro e macrovasculares. Em alguns casos, a restauração da normoglicemia reverte os danos celulares. Em outros, no entanto, estes danos são irreversíveis, o que torna o controle glicêmico um parâmetro fisiológico de essencial importância, para evitar as sérias complicações crônicas do diabetes [31-34]. Estudos têm demonstrado que o diabetes mellitus promove alterações no relaxamento dependente do endotélio em diferentes leitos vasculares promovendo disfunção endotelial [35,36]. A disfunção endotelial é considerada um biomarcador de risco cardiovascular e a importância do endotélio na manutenção da saúde vascular é consenso na literatura [37].

Os animais diabéticos não exercitados do presente estudo apresentaram uma perda da funcionalidade vascular. Por outro lado, 8 semanas de exercício resistido foi capaz de restaurar a função vascular dos animais diabéticos. Tal situação pode ser justificada em virtude da redução nos níveis glicêmicos observada nos animais diabéticos exercitados. Uma vez que, nossos resultados evidenciam que o relaxamento induzido por ACh tem uma forte correlação inversa com os níveis glicêmicos. Os animais diabéticos não exercitados apresentaram um aumento dos níveis glicêmicos e uma importante perda da funcionalidade endotelial, em contrapartida, a redução da glicemia está associada à restauração da função vascular apresentada pelos animais diabéticos exercitados. Estes resultados reforçam os achados de vários outros estudos que apontam o exercício resistido como uma possível ferramenta para o tratamento e/ou prevenção de doenças que apresentam a perda da funcionalidade vascular como a hipertensão e o diabetes [8,10,19,20,38].

É relatado na literatura que o diabetes mellitus promove uma redução na produção endotelial de substâncias vasoativas responsáveis pelo controle do tônus vascular, como o óxido nítrico e as prostaglandinas [1]. Para investigar a participação do NO nos relaxamentos dependentes do endotélio, foram obtidas curvas concentração-resposta para ACh na presença de L-NAME. Nesta condição experimental, foi observado que L-NAME antagonizou os relaxamentos induzidos por ACh nos animais do C e DT, mas não modificou os relaxamentos dos animais do grupo DS, caracterizando uma redução na participação de um dos principais fatores relaxantes derivados do endotélio nos animais diabéticos sedentários. Estes achados estão de acordo com os resultados apresentados por Chen e cols [39], no qual demonstraram que as Rmax induzidas por ACh também foram reduzidas na presença de L-NAME nos animais submetidos ao exercício aeróbio por 8 semanas.

Interessantemente, os animais diabéticos exercitados apresentaram um percentual de inibição mais pronunciado nos relaxamentos realizados na presença de L-NAME quando

comparado aos animais saudáveis. Tal fenômeno pode ter ocorrido em decorrência de um possível aumento no relaxamento dependente de NO proporcionado pelo exercício resistido. O aumento dos relaxamentos apresentados neste estudo corrobora com outros achados onde houve um aumento na produção de NO mediada pelo exercício aeróbio no modelo experimental de diabetes tipo I [14].

Estudos com humanos portadores de diabetes tipo I também demonstraram que o exercício aeróbio melhorou a função endotelial em leitos vasculares que não estão diretamente envolvidos durante o exercício [13]. Em nosso estudo, os efeitos observados nas artérias dos animais exercitados sugerem também um possível efeito vascular generalizado, pois a artéria analisada encontra-se distante dos tecidos mais ativos durante execução do exercício. Este efeito vascular generalizado também foi observado por Faria e cols [38] onde uma única sessão de exercício resistido aumentou o relaxamento dependente de NO na artéria caudal, promovendo uma redução na pressão arterial em ratos espontaneamente hipertensos.

Em adição, observamos em nosso estudo que o exercício resistido reduziu a pressão arterial média nos animais diabéticos exercitados demonstrando ser eficaz no tratamento da disfunção endotelial relacionada à hiperglicemia. Dados recentes do nosso laboratório demonstraram que animais hipertensos induzidos por L-NAME também apresentaram uma redução nos valores de pressão arterial após 4 semanas de exercício resistido [10]. A redução da resistência vascular periférica e um aumento na condutância vascular sistêmica pode ser o mecanismo responsável pela queda da pressão arterial apresentadas pelos animais submetidos ao exercício resistido [40].

Desta forma, nossos resultados sugerem que o exercício resistido de baixa intensidade induz respostas metabólicas e cardiovasculares semelhantes às observadas em estudos que utilizaram animais diabéticos submetidos ao exercício aeróbio [14,15]. Mesmo se tratando de

modalidades de exercício com características diferentes, tais como, a via energética e a execução do movimento, ambas promovem efeitos cardiometabólicos benéficos que auxiliam no tratamento do diabetes mellitus tipo 1 e 2 [11,12].

Assim, este estudo indica que o modelo de exercício resistido utilizado foi capaz de reduzir a glicemia, restaurar a funcionalidade endotelial e reduzir a pressão arterial em animais diabéticos. Finalmente, é possível que o exercício resistido promova ajustes vasculares e metabólicos benéficos para o tratamento das disfunções presentes no diabetes mellitus tipo 1 em um modelo experimental.

Agradecimentos

Agradecemos a CNPq, CAPES e FAPITEC-SE pelo apoio financeiro.

Legendas das figuras

Figura 1 – Variação da glicemia de ratos no início (0) e ao final (8) de oito semanas de treinamento: grupos controle - C; diabético sedentário - DS e diabético treinado - DT. Os dados são expressos como média ± E.P.M. As diferenças estatísticas foram determinadas pela ANOVA para medidas repetidas seguidas pelo pós-teste de Bonferroni. $^{***}p<0,001$ *vs* C 0; $^{\dagger\dagger}p<0,01$ *vs* DS 0 e $^{\#}p<0,05$ *vs* DT 0.

Figura 2 – Curvas concentração-resposta para acetilcolina (ACh: 10^{-9} – 10^{-4} M) em anéis isolados artéria mesentérica superior com endotélio intacto e pré-contraídas com FEN (1μM). Os anéis foram obtidos de ratos dos grupos controle - C; diabético sedentário - DS e diabético treinado - DT. Os dados representam as médias ± E.P.M. As diferenças estatísticas foram determinadas pela ANOVA de duas-vias seguida do pós-teste de Bonferroni. $^{**}p<0,01$ e $^{***}p<0,001$ *vs* C; $^{\#\#}p<0,01$ *vs* DS.

Figura 3 – Correlação entre o percentual de resposta máxima dos relaxamentos induzidos por ACh e glicemia em anéis de artéria mesentérica dos grupos diabético sedentário (A) e diabético treinado (B).

Figura 4 – Pressão arterial média de ratos dos grupos controle – C; diabético sedentário - DS e diabético treinado - DT após 8 semanas de exercício resistido. Os dados representam as médias ± E.P.M. As diferenças estatísticas foram determinadas pela ANOVA para medidas repetidas seguidas pelo pós-teste de Bonferroni. $^{*}p<0,05$ DS *vs* C e $^{\#}p<0,05$ DT *vs* DS.

Tabela 1 – Valores de pD_2 e Rmáx obtidos de curvas concentração-resposta para ACh antes e após o pré-tratamento com L-NAME.

Grupos	Condição	pD_2	Rmax
C	- L-NAME	7,0 ± 0,0	99,0 ± 2,7
	+ L-NAME	5,5 ± 0,1***	70,0 ± 6,2**
DS	- L-NAME	7,2 ± 0,0	78,0 ± 1,8
	+ L-NAME	6,8 ± 0,1†	72,4 ± 3,1
DT	- L-NAME	7,0 ± 0,1	95,0 ± 3,5
	+ L-NAME	4,9 ± 0,2$^{***,\#\#}$	50,0 ± 3,6$^{***,\#}$

Os anéis foram obtidos de ratos dos grupos controle - C; diabético sedentário - DS e diabético treinado - DT. Os experimentos foram realizados na ausência L-NAME (- L-NAME) e na presença de 100 µM de L-NAME (+ L-NAME). Os dados representam as médias ± E.P.M. As diferenças estatísticas foram determinadas pelo teste *t de student* para amostras independentes (intra-grupo) ou pela ANOVA seguida do pós-teste de Bonferroni (inter-grupo). pD_2 - potência e Rmax - Resposta máxima. $^{**}p < 0,01$ ou $^{***}p < 0,001$ para valores - L-NAME *vs* + L-NAME; $^{\dagger}p<0,001$ *vs* C e $^{\#}p<0,01$ ou $^{\#\#}p<0,001$ *vs* DS.

Figura 1

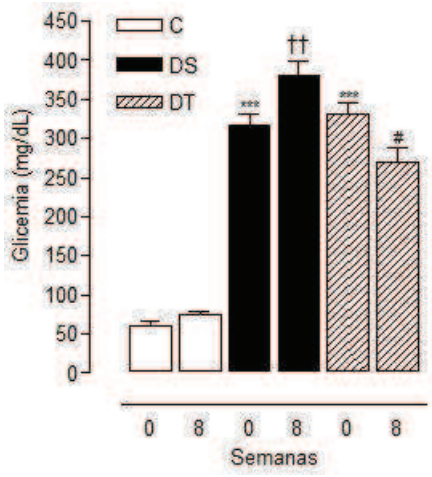

C
DS
DT
Glicemia (mg/dL)
450
400
350
300
250
200
150
100
50
0
***
††
***
#
0
8
0
8
0
8
Semanas

Figura 2

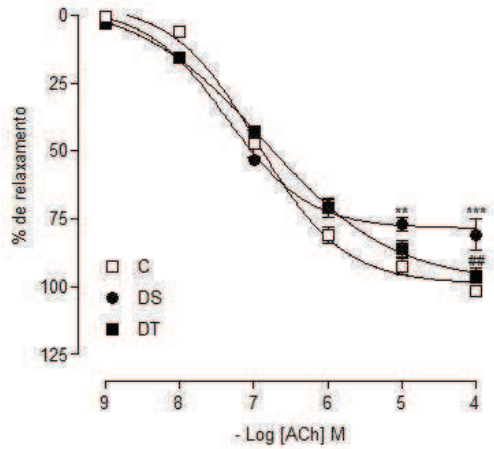


Figura 3

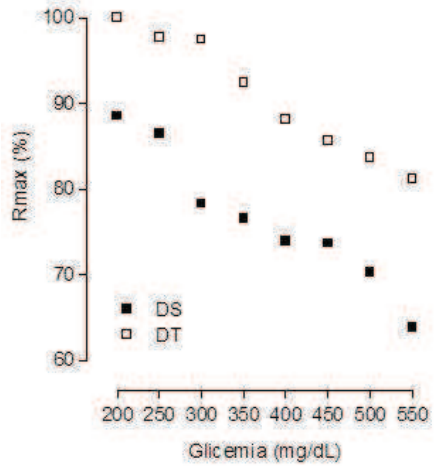
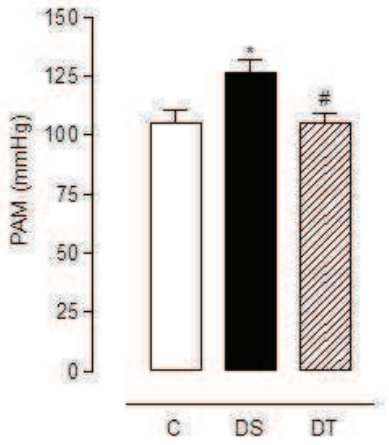

100
90
80
70
60
Rmax (%)
DS
DT
200 250 300 350 400 450 500 550
Glicemia (mg/dL)

Figura 4



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