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

ANDRÉ SALES BARRETO

TREINAMENTO FÍSICO RESISTIDO PREVINE HIPERTENSÃO ARTERIAL E MELHORA MODULAÇÃO AUTONÔMICA CARDÍACA EM RATOS DIABÉTICOS INDUZIDOS PELA ALOXANA

ARACAJU – SE
2010

ANDRÉ SALES BARRETO

TREINAMENTO FÍSICO RESISTIDO PREVINE HIPERTENSÃO ARTERIAL E MELHORA MODULAÇÃO AUTONÔMICA CARDÍACA EM RATOS DIABÉTICOS INDUZIDOS PELA ALOXANA

Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Núcleo de Pós-Graduação em Medicina da Universidade Federal de Sergipe como requisito parcial à obtenção do grau de Mestre em Ciências da Saúde. Área de concentração: Estudos Clínicos e Laboratoriais em Saúde.

Orientador: Prof. Dr. Márcio Roberto Viana Santos

ARACAJU – SE

2010

ANDRÉ SALES BARRETO

TREINAMENTO FÍSICO RESISTIDO PREVINE HIPERTENSÃO ARTERIAL E MELHORA MODULAÇÃO AUTONÔMICA CARDÍACA EM RATOS DIABÉTICOS INDUZIDOS PELA ALOXANA

Dissertação apresentada ao Núcleo de Pós-Graduação em Medicina da Universidade Federal de Sergipe como requisito parcial à obtenção do grau de Mestre em Ciências da Saúde.

Aprovada em ____/___/____

_____

Orientador: Prof. Dr. Márcio Roberto Viana Santos
Universidade Federal de Sergipe – DFS/UFS

_____

1º Examinador: Profª. Drª. Luciana Venturini Rossoni
Universidade de São Paulo – ICB/USP

_____

2º Examinador: Prof. Dr. José Augusto Soares Barreto Filho
Universidade Federal de Sergipe – DM/UFS

PARECER

_____

_____

_____

_____

_____

_____

_____

Dedico este trabalho aos meus pais (Valdecí e Eliel), aminha esposa (Rosana) e filha (Giovana) por terem acreditado junto comigo na realização desse sonho.

Amo vocês!!!

AGRADECIMENTOS

A Deus, todo poderoso, ao qual sempre me permitiu compartilhar de Sua maravilhosa presença e desfrutar da certeza de sua fidelidade em todos os momentos. A Ti dedico minha vida e minhas conquistas;

A minha mãe Valdecí, que me faz acreditar que é possível viver de forma ética e digna através de muita dedicação, trabalho, compromisso e sobretudo amor em tudo que faz. A valorização da família por ela demonstrada em toda sua vida foi fundamental para formação do meu caráter. A meu pai Eliel, pelo amor, carinho e incentivo aos estudos sempre;

A minha esposa Rosana, por seu amor, compreensão e dedicação à família. Pelo amor que tenho a você vivo a certeza de que Deus reservou o melhor dessa terra para mim. À minha filha Giovana por demonstrar-me a graça de renovar a esperança todos os dias;

A minha irmã Luciana por seu exemplo de garra e determinação em seus objetivos com ética e dignidade e ao meu cunhado e pastor Adlai pelo aconselhamento espiritual e inabalável fé em Deus que sempre evidenciou;

Aos meus sobrinhos amados Sarah, Davi, Israel, Sophia, Luana, Wendell, Arthur e Mariana pelo amor e carinho que demonstram por mim;

Ao meu avô Fileto, *in memorian*, pela firmeza de caráter e exemplo de vida pautada no evangelho. A minha avó Ianete, matriarca da família, por seu amor verdadeiro e carinho que sempre demonstrou por mim;

Ao meu amigo e prof. Lucindo que me incentivou a esse desafio, por seu exemplo de amor e dedicação a pesquisa científica e a sua esposa Jullyana, minha cunhada, que me recebeu como um irmão;

Ao amigo e parceiro de mestrado Marcelo, por seu companheirismo e dedicação ao trabalho realizado;

Aos professores Drs. José Ausgusto, Valter Santana e Msc José Melquíades pela orientação e colaboração na elaboração do trabalho;

Aos colegas do laboratório que contribuíram com o desenvolvimento dessa pesquisa Milene, Ana Paula Soares, Tharciano, Ana Paula Barbosa, Ayslan, Vagner e Fernando;

Ao Prof Dr. Márcio Roberto Viana Santos por ter aceitado o desafio de ser meu orientador e acreditado no meu potencial. Seu compromisso com o rigor metodológico e dedicação a pesquisa científica com qualidade mesmo na adversidade, foram fundamentais para minha formação como pesquisador e professor. Agradeço também por sua paciência e dedicação com as correções e sugestões que por muitas vezes sacrificou seu tempo pessoal. Muito obrigado por tudo;

Aos órgãos de fomento CNPq, CAPES, FAPITEC-SE, SES/SE e ao Ministério da Saúde que permitiram a realização desse trabalho.

BARRETO, A.S. **Treinamento físico resistido previne hipertensão e melhora balanço autonômico cardíaco em ratos diabéticos induzidos pela aloxana**. 2010. 89f. Dissertação (Mestrado em Ciências da Saúde) – Universidade Federal de Sergipe, Aracaju.

RESUMO

O objetivo desse estudo foi avaliar os efeitos do treinamento físico resistido sobre a pressão arterial, repolarização ventricular, sensibilidade barorreflexa e balanço autonômico cardíaco em ratos diabéticos. A avaliação cardiovascular foi realizada em animais conscientes treinados e sedentários, após 8 semanas do início do diabetes com aloxana ou nos animais controle. O treinamento físico resistido consistiu de 3 séries de 10 repetições realizada a 40% do teste de uma repetição máxima, 3 dias/semana durante 8 semanas em um aparato que simula o exercício de agachamento em humanos. Pressão arterial foi monitorada por 30 minutos 48 horas após a última sessão de treinamento físico ou tempo controle. A sensibilidade barorreflexa foi analisada pelo método da sequência e o balanço autonômico cardíaco foi avaliado pela variabilidade da freqüência cardíaca no domínio da freqüência. Após 8 semanas, o diabetes aumentou significativamente a glicemia (de 83 ± 8 para 381 ± 41 mg/dl), pressão arterial média (de 104.7 ± 5,4 para 125 ± 5,4 mmHg), intervalo QTc (de 4,4 ± 0,1 para 5,1 ± 0,1 ms), reduziu sensibilidade barorreflexa (de 2,01 ± 0,3 para 0,38 ± 0,1 ms/mmHg) e produziu um distúrbio sobre o balanço autonômico cardíaco. O treinamento físico resistido foi capaz de produzir significante redução sobre a glicemia (270 ± 17 mg/dl), preveniu o aumento da pressão arterial (100,8 ± 4,2 mmHg) e intervalo QTc (4,6 ± 0,1 ms), a redução da sensibilidade barorreflexa (2,63 ± 0,5 ms/mmHg) e distúrbio sobre a balanço autonômico cardíaco. Esses resultados sugerem que o treinamento físico resistido promove um melhor controle glicêmico, previne hipertensão e melhora a sensibilidade barorreflexa e balanço autonômico cardíaco em ratos diabéticos induzidos pela aloxana.

Palavras-chave: barorreflexo; diabetes mellitus; pressão arterial; sistema nervoso autônomo; treinamento resistido.

BARRETO, A.S. **Resistance training prevents hypertension and improves cardiac autonomic modulation in alloxan diabetic rats**. 2010. 89f. Dissertação (Mestrado em Ciências da Saúde) – Universidade Federal de Sergipe, Aracaju.

ABSTRACT

The aim of this study was to evaluate the effects of resistance training on blood pressure, ventricular repolarization, baroreflex sensitivity and cardiac autonomic balance in diabetic rats. Cardiovascular evaluation was performed in conscious trained and sedentary animals, 8 weeks after the onset of diabetes with alloxan or control animals. The resistance training consisted of 3 sets of 10 repetitions performed at 40% of one repetition maximum test, 3 days/wk over 8 wks in squat-training apparatus. Blood pressure was monitored for 30 min 48 h after the last training session or time control. Baroreflex sensitivity was analyzed by sequence method and cardiac autonomic balance was assessed by heart rate variability in the frequency domain. After 8 wks, the diabetes significantly increased glycemia (from 83 ± 8 to 381 ± 41 mg/dl, $p<0.05$), mean blood pressure (from 104.7 ± 5.4 to 125.1 ± 5.4 mmHg, $p<0.05$), QTc interval (from 4.4 ± 0.1 to 5.1 ± 0.1 ms, $p<0.05$), reduced baroreflex sensitivity (from 2.01 ± 0.3 to 0.38 ± 0.1 ms/mmHg, $p<0.05$) and impaired the cardiac autonomic balance. Resistance training was able to produce significant reduction on the glycemia (270 ± 17 mg/dl, $p<0.05$), prevented the increase of mean blood pressure (108 ± 3 mmHg, $p<0.001$) and QTc interval (4.6 ± 0.1 ms, $p<0.01$), the reduction of baroreflex sensitivity (2.63 ± 0.5 ms/mmHg, $p<0.01$) and disturbance on the cardiac autonomic balance. These results suggest that resistance training promotes a better glycemic control, prevents hypertension and improves baroreflex sensitivity and cardiac autonomic balance in alloxan diabetic rats.

Keywords: blood pressure, autonomic nervous system, diabetes mellitus, exercise, baroreflex sensitivity.

LISTA DE FIGURAS

Figure 1: Arterial baroreflex gain after experimental protocol in sedentary control (SC) (n = 8), Sedentary Diabetic (SD) (n = 8) and Trained Diabetic (TD) (n = 8).....51

Figure 2: Schematic picture of pulse interval (upper panel) and spectra of pulse interval (lower panel) of an animal representative from each group: Sedentary Control (SC), Sedentary Diabetic (SD) and Trained Diabetic (TD)…….…....…..... 52

Figure 3: Analysis of spectral power in normalized units (n.u.)(A) and in ms^2 (B) of cardiac autonomic balance in Sedentary Control (SC)(n = 8), Sedentary (SD)(n = 8) and Trained (TD)(n = 8) diabetic rats……53

LISTA DE TABELAS

Table 1: Cardiovascular evaluation in Sedentary Control, Sedentary and Trained Diabetic rats…….……..…… 54

Table 2: PR, QRS, QT and QTc intervals from ECG in sedentary control, diabetic sedentary and trained rats..... 55

LISTA DE ABREVIATURAS

ADP: difosfato de adenosina

ALX: aloxana

ATP: trifosfato de adenosina

BP: pressão sanguínea

BRS: sensibilidade barorreflexa

DAC: doença arterial coronariana

DBP: Pressão sanguínea diastólica

DM: Diabetes *Mellitus*

DP: duplo-produto

ECG: eletrocardiografia

EROs: espécies reativas de oxigênio

ET: treinamento físico aeróbico

FFT: transformação rápida de Fourier

HbA1c: hemoglobina glicada

HF: banda de alta frequência

HR: freqüência cardíaca

HRV: variabilidade da freqüência cardíaca

LF/HF: balanço simpato-vagal

LH: banda de baixa frequência

MAP: pressão arterial média

NAC: neuropatia autonômica cardíaca

NAD^+: nicotinamida adenina dinucleotídeo

PAM: pressão arterial média

RM: repetição máxima

RT: treinamento físico resistido

SBP: pressão sanguínea sistólica

STZ: Estreptozotocina

TFA: treinamento físico aeróbico

TFR: treinamento físico resistido

SUMÁRIO

1 INTRODUÇÃO..... 12
2 FUNDAMENTAÇÃO TEÓRICA..... 16
2.1 Diabetes Mellitus (DM)..... **17**
2.2 DM e alterações cardiovasculares..... **19**
2.3 DM experimental..... **22**
2.4 DM e exercício físico..... **24**
3 OBJETIVOS..... 28
3.1 Objetivo geral..... **29**
3.2 Objetivos específicos..... **29**
4 RESULTADOS..... 30
4.1 Resistance training prevents hypertension and improves cardiac autonomic modulation in alloxan diabetic rats.....…… **31**
5 CONCLUSÃO……..……..….. 55
6 PERSPECTIVAS…….……..….. 57
REFERÊNCIAS…….……..... 59
ANEXO A – Aprovação do Comitê de Ética**……...……..….. 68**
ANEXO B – Cronologia dos experimentos**..……..……..….. 70**
ANEXO C – Normas de Submissão da Revista...**…….….. 72**
ANEXO D – Comprovante de submissão de artigo**..... 74**

INTRODUÇÃO

1 INTRODUÇÃO

O diabetes *mellitus* (DM) é uma síndrome heterogênea de etiologia múltipla decorrente da falta e/ou resistência a ação da insulina, caracterizada por hiperglicemia crônica com distúrbios do metabolismo intermediário dos carboidratos, lipídios e proteínas. Esses distúrbios estão associados a complicações crônicas no sistema cardiovascular e no controle autonômico de vários órgãos em humanos e animais (De ANGELIS et al, 2000; NEGRÃO; BARRETTO, 2006; SBD, 2007).

A hiperglicemia é acompanhada por sintomas como poliúria, polidipsia, perda de peso, polifagia e visão turva. Pode evoluir com complicações agudas como a cetoacidose diabética, estado hiperosmolar não-cetótico e hipoglicemia, ou complicações crônicas como doença macro e microvascular e neuropatia, as quais proporcionam dano, disfunção e falência de vários órgãos, especialmente olhos, rins, nervos, coração e vasos sanguíneos (POWERS, 2001; GROSS et al, 2002; SBD, 2007).

Atualmente no Brasil, o DM se apresenta como uma doença fortemente associada à alta mortalidade e morbidade, gerando um elevado custo médico-sócio-econômico. Os custos diretos com DM variam entre 2,5% e 15% do orçamento anual da saúde, dependendo de sua prevalência e do grau de sofisticação do tratamento disponível. Constitui-se a principal causa dos internamentos por problemas cardiovasculares, entre elas a hipertensão, sendo também a principal causa de amputação de membros e cegueira adquirida. Além disso, os custos intangíveis (dor, ansiedade, inconveniência, incapacidade para o trabalho ou perda da produtividade e de qualidade de vida) também apresentam grande impacto na vida das pessoas com diabetes (SBD, 2007).

A evolução clínica dos portadores de DM é bastante limitante. Alterações como vasculopatia, nefropatia, neuropatia autonômica e hipertensão arterial além dos níveis plasmáticos de glicemia são critérios importantes no prognóstico dessa patologia. A avaliação portanto de parâmetros hemodinâmicos e bioquímicos são de grande utilidade no acompanhamento da evolução dessa morbidade (LERARIO, 2002; SCHEFFEL et al, 2004; ALVARENGA, 2005).

Movidos pela busca de soluções para essa problemática social, diversos estudos têm sido continuamente desenvolvidos no sentido de esclarecer as causas, os efeitos e o tratamento desta doença. O desenvolvimento de modelos experimentais em animais de laboratório tem dado contribuições significativas para o desenvolvimento de estudos que definem com acurácia e precisão a patologia desta doença, o que tem possibilitado o estudo de métodos preventivos, paliativos e curativos (MACHADO et al, 2000; DUNCAN & SCHIMIDT, 2001; SCHAAN et al, 2004; TOSCANO, 2004; BARRETO et al, 2005; PASSOS et al, 2005; RUSSELL et al, 2005).

O exercício físico tem sido indicado como uma conduta não-farmacológica no tratamento de diversas patologias como o diabetes, hipertensão arterial e insuficiência cardíaca devido aos benefícios cardiovasculares, metabólicos e no sistema nervoso autonômico (TIPTON et al, 1991; WALLBERG et al, 1998). Estudos recentes demonstraram que é possível diminuir significativamente a incidência de novos casos de DM através de mudanças no estilo de vida, como por exemplo, realizar exercício físico regular e educação alimentar (EDELSTEIN et al, 1997; BARZILAV et al, 1999; TUOMILEHTO et al, 2001).

O treinamento físico (TF) adequado proporciona importantes ajustes metabólicos, neuroendócrinos e cardiovasculares, que contribuem para prevenção, redução e reversão das alterações metabólicas nos diabéticos, as quais melhoram a qualidade de vida desses indivíduos. As alterações persistentes na estrutura ou na função do organismo são particularmente ocasionadas em resposta ao estímulo gerado pelo exercício físico repetido sistematicamente e ao longo do tempo (TANCREDE et al, 1982; DORN et al, 2001; ADA, 2004; LaMONTE et al, 2005).

A maioria dos estudos que investigaram as repercussões metabólicas e cardiovasculares do treinamento físico no diabetes utilizaram protocolos aeróbicos. Estes estudos tem observado um aumento na sensibilidade à insulina, redução na sua dose diária, melhora na cinética e no consumo de oxigênio, melhora no controle de fatores de risco para doenças cardiovasculares como redução na pressão arterial, peso corporal e estresse oxidativo (ACSM, 2000a; CIOLAC & GUIMARÃES, 2002 e 2004; GOTO et al, 2003; ADA, 2004; SASAKI & SANTOS, 2006).

Recentemente, alguns estudos têm demonstrado o valor potencial e relativa segurança do treinamento resistido no portador de DM. O principal mecanismo

biológico aparente envolvido é a hipertrofia da musculatura esquelética, com um aumento associado na absorção de glicose e sensibilidade à insulina, além de adaptações cardiovasculares como o efeito hipotensor arterial. (POLLOCK et al, 2000; FLETCHER et al, 2001; CIOLAC & GUIMARÃES, 2004; SANCHEZ & LEON, 2006).

Portanto, existem evidências na literatura de que o treinamento físico contribui de maneira significativa para a melhora da disfunção metabólica e cardiovascular do diabetes *mellitus*, entretanto os estudos concentram-se nos benefícios advindos do treinamento aeróbico. Tanto atividades aeróbias quanto resistidas devem ser consideradas na elaboração do TF do portador de DM. Além disso, existe carência na literatura sobre possíveis contribuições do TFR sobre parâmetros hemodinâmicos. Por conseguinte, a realização desse trabalho com a finalidade de ofertar mais uma modalidade de tratamento, que represente uma significativa melhora na função metabólica e cardiovascular nesse grupo populacional, é mais que justificado.

FUNDAMENTAÇÃO TEÓRICA

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Diabetes Mellitus

O diabetes *mellitus* (DM) não é uma única doença, mas um grupo heterogêneo de distúrbios metabólicos que apresentam em comum a hiperglicemia crônica. Esta hiperglicemia é o resultado de defeitos na ação e/ou secreção da insulina, que gera distúrbios do metabolismo intermediário dos carboidratos, lipídios e proteínas (SBD, 2007).

O número de pessoas com diabetes está aumentando devido ao crescimento, envelhecimento e urbanização populacional e aumento da prevalência de obesidade e sedentarismo, bem como a maior sobrevida do paciente com DM (ADA, 2006). Segundo a Sociedade Brasileira de Diabetes (SBD) uma epidemia de DM está em curso. Em 1985 estimava-se que existissem 30 milhões de adultos com DM no mundo, esse número cresceu para 135 milhões em 1995, atingindo 173 milhões em 2002, com projeção de chegar a 300 milhões no ano de 2030. Cerca de dois terços desses indivíduos com DM vivem em países em desenvolvimento, onde a epidemia tem maior intensidade, com crescente proporção de pessoas afetadas em grupos etários mais jovens (WILD et al, 2004).

No Brasil, no final dos anos 1980, a prevalência de DM na população adulta foi estimada em 7,6%. Dados mais recentes, levantados por Torquato et al (2003), apontam para taxas mais elevadas, como 12,1% no município de Ribeirão Preto, SP. De acordo com o Sistema de Informação de Atenção Básica - SIAB, o Estado de Sergipe possui 30.520 diabéticos cadastrados numa perspectiva total de 60.000 doentes. Nesse mesmo estado, em 2006 foram contabilizados 1.266 casos de hospitalizações devido complicações do DM, em 2008 foram 1.235 casos e até julho de 2009 foram 735 hospitalizações (MINISTÉRIO DA SAÚDE, 2009).

A natureza crônica do DM, a gravidade de suas complicações e os meios necessários para controlá-las, tornam-a uma doença muito onerosa, não apenas para os indivíduos afetados e suas família, mas também para o sistema de saúde. Os custos diretos com DM variam entre 2,5% e 15% do orçamento anual de saúde, dependendo de sua prevalência e do grau de sofisticação do tratamento disponível. Estimativas do custo no Brasil estão em torno de 3,9 bilhões de dólares americanos,

em comparação com 0,8 bilhão para Argentina e 2 bilhões para o México (BARCELÓ et al, 2003; SBD, 2007).

Os custos do DM não são apenas um problema econômico. Os custos intangíveis como dor, ansiedade, inconveniência e perda da qualidade de vida também representam grande impacto na vida desses indivíduos e de suas famílias (SBD, 2007).

A classificação atual do DM proposta pela Organização Mundial da Saúde (OMS) e pela Associação Americana de Diabetes (ADA) (2006) é baseada na sua etiologia, estabelecida em quatro classes clínicas: DM tipo 1 (DM 1), DM tipo 2 (DM2), outros tipos específicos de DM e DM gestacional. O DM 1 caracteriza-se pela ausência ou diminuição da secreção de insulina pelas ilhotas de Langerhans, causada ou por processo auto-imune, desencadeado após interação complexa entre fatores genéticos e ambientais que destroem células β- pancreáticas responsáveis pela produção da insulina, ou por forma idiopática. Já o DM 2 ocorre devido a uma resistência à ação da insulina e sua etiologia está relacionada, principalmente, com a obesidade andróide (NOTKINS, 1979; WHO, 1999; De FRONZO, 2004; SKYLER, 2004; ADA, 2006).

As complicações do diabetes podem ser categorizadas como agudas e crônicas. As complicações agudas envolvem a cetoacidose diabética, o estado hiperosmolar não cetótico e a hipoglicemia que está associada ao tratamento inadequado. A cetoacidose predomina no DM tipo 1, enquanto que o quadro hiperosmolar, no tipo 2. Já a hipoglicemia é um evento ocasional para os usuários de hipoglicemiantes orais (DM2), mas relativamente freqüente para os usuários de insulina (DM1) (POWERS, 2001).

A cetoacidose e o estado hiperosmolar são condições graves causadas por extrema deficiência da ação insulínica e caracterizados por níveis circulantes elevados de glicose (> 400 mg/dL). A deficiência total ou parcial da insulina permite excessiva produção de glicose pelo fígado, além de catabolismo de proteínas e gorduras dos tecidos muscular e adiposo, os quais geram aminoácidos e ácidos graxos livres que são utilizados pelo fígado para produzir mais glicose e corpos cetônicos. A hiperglicemia leva a glicosúria, que induz diurese osmótica, depleção de volume, taquicardia, hipotensão e choque. A hipoglicemia (glicemia <45mg/dL) induz privação de glicose ao cérebro podendo produzir tremores, sudoreses,

taquicardia, palidez, tonturas, distúrbios visuais, confusão mental, coma e convulsões (FREITAS-FOSS & FOSS, 2003; NEGRÂO & BARRETO, 2006).

As complicações crônicas da DM advém da hiperglicemia crônica que pode causar lesões por meio de alterações metabólicas. Tais alterações produzem acúmulo de sorbitol que causa edema celular. A pseudo-hipóxia intracelular e diminuição do mioinositol reduzem a velocidade de condução dos nervos. Além disso, a glicação de proteínas circulantes ou intracelulares afetam suas propriedades e propicia o dano tissular (CEFALU, 2001; POWERS, 2001; ROITH, 2001).

A ligação dos produtos finais de glicação avançada nas paredes dos vasos sanguíneos predispõe a liberação de citoquinas e fatores de crescimento, à aterosclerose, à destruição da estrutura e à alteração da composição vascular, contribuindo para o aumento da permeabilidade do espessamento arterial. Há também diminuição da síntese de oxido nítrico, principal produto vasodilatador derivado do endotélio, aumento da viscosidade sanguínea e agregação plaquetária, comprometendo assim o fluxo na microcirculação. O aumento do fluxo sanguíneo e da pressão estimulam o espessamento e a permeabilidade capilares. As mudanças estruturais e funcionais macro e microvasculares alteram a irrigação, a permeabilidade e tônus vasomotor, limitando a perfusão dos tecidos e causando dano tecidual (CEFALU, 2001; POWERS, 2001; ROITH, 2001).

Estas complicações crônicas são classificadas, portanto como macrovasculares ou microvasculares. As macrovasculares se manifestam clinicamente principalmente através de cardiopatia isquêmica, acidente vascular cerebral e doença vascular periférica. Já a microvascular pode se manifestar sob a forma de retinopatias, nefropatias e neuropatias sensitiva-motora ou autonômica gastrointestinal, urogenital e cardiovascular (NEGRÂO & BARRETTO, 2006).

2.2 Diabetes e alterações cardiovasculares

Os distúrbios do metabolismo da glicose podem causar complicações que envolvem doenças cardiovasculares, incluindo hipertensão arterial, doença arterial coronariana (DAC) e insuficiência cardíaca (IC), sendo que 75% dos pacientes diabéticos morrem por algum evento cardiovascular (KANNEL et al, 1974; GIULIANO et al, 1996; GU et al, 1999).

Observa-se que dentre as complicações cardíacas apresentadas por pacientes diabéticos, denominada cardiomiopatia diabética, estão a fibrose do miocárdio, lise de miócitos, alterações ultra-estruturais (FACTOR et al, 1981; FACTOR et al, 1984; FANG et al, 2004), depressão da contratilidade e do relaxamento cardíaco (RODGERS, 1986; RODRIGUES & McNEILL, 1986; FANG et al, 2004; BORGES et al, 2006; WICHI et al, 2007), e conseqüente disfunção sistólica e diastólica (VENCO et al., 1987; GROSSMAN et al., 1992; FANG et al, 2004; NEMOTO et al, 2006).

Todas estas alterações manifestam-se de forma mais severa em indivíduos hipertensos-diabéticos do que naqueles que apresentam hipertensão ou diabetes isoladamente, ressaltando a gravidade da associação entre as duas doenças (ROSSING et al, 1996). Nesses indivíduos, o aumento da resistência vascular periférica devido à disfunção arterial induz sobrecarga ao coração com consequente hipertrofia patológica. Esta hipertrofia é observada principalmente no ventrículo esquerdo em diabéticos de longo curso, com precário controle glicêmico e de pressão arterial (POORNIMA et al, 2006).

As alterações promovidas pelo catabolismo de carboidratos e de lipídeos e mudanças eletrolíticas de cálcio e potássio no sangue podem causar modificações na estrutura e fisiologia cardiovascular, que, por sua vez, alteram o registro do eletrocardiograma (ECG) (PYE et al, 1992; TOMASELLI, et al, 1994).

Entre as principais alterações observadas no ritmo cardíaco no paciente diabético, encontra-se o aumento do intervalo QT, também chamado síndrome do QT longo. O prolongamento do intervalo QT se deve ao retardo na despolarização e/ou na repolarização do potencial de ação cardíaco, provocando o aumento do risco de arritmias e de morte súbita. O intervalo QT varia inversamente com a freqüência cardíaca (FC), de modo que ele deve ser corrigido em relação à FC, gerando o QT corrigido (QTc), que é preferencialmente utilizado. (FEUVRAY, 1997; GIUNTI et al, 2005; KLEIN et al, 2005).

Outros estudos demonstraram no ECG de pacientes diabéticos um quadro compatível com doença arterial coronariana (DAC) e consequente processo isquêmico silencioso, como presença de depressão do segmento ST (NASS et al, 1998; LEE et al, 2001). Portanto, a avaliação do ECG em diabéticos é um valioso instrumento para avaliar comprometimento da condução do ritmo elétrico do coração

e identificar também anormalidades subclínicas (LEE et al, 2001; KLEIN et al, 2005; NYGREN et al, 2007).

A neuropatia autonômica cardiovascular (NAC) é outra séria e freqüente complicação do diabetes. A ocorrência de NAC é um fator preditivo para isquemia miocárdica silenciosa, desenvolvimento de acidente vascular cerebral e morte. Está associada com prognóstico ruim e pode resultar em manifestações clínicas como taquicardia de repouso, hipotensão postural, intolerância ao exercício e instabilidade cardiovascular peri e intra-operatória (VINIK et al, 2007). Por causa da associação com uma variedade de resultados adversos, incluindo morte, a NAC é a mais importante e bem estudada forma de neuropatia autonômica diabética (VINIK et al, 2003 e 2007).

Em estudos com diabetes experimental em ratos, Lin et al (2008), demonstrou que a NAC pode preceder alterações funcionais e estruturais do sistema cardiovascular como rigidez arterial e hipertrofia cardíaca. Além disso, vários estudos têm comprovado o envolvimento do sistema nervoso autonômico cardíaco como o comprometimento do barorreflexo, quimiorreflexo, reflexo cardiopulmonar, balanço autonômico cardiovascular e atividade neural simpática tanto em humanos quanto em ratos diabéticos (DALL'AGO, 1997; HICKS et al, 1998; OLIVEIRA, 1999; USTINOVA, 2000; De ANGELIS, 2002 e 2007; HARTHMANN, 2007).

Portanto o diagnóstico precoce da NAC pode sugerir grau de evolução do comprometimento do sistema cardiovascular na história clínica do portador de diabetes. A avaliação, por conseguinte do balanço do sistema nervoso autônomo cardiovascular pelo uso de diferentes técnicas de medida tem permitido estimar a contribuição desse sistema nas respostas alteradas do diabetes associado às doenças cardiovasculares (De ANGELIS et al, 2004a).

Os parâmetros mais utilizados para estimar a funcionalidade do sistema nervoso autônomo cardiovascular nos diferentes níveis constituem-se na sensibilidade dos reflexos barorreceptor e quimiorreceptor arterial, cardiopulmonar, análise da variabilidade da freqüência cardíaca e pressão arterial, além da atividade neuronal simpática (COSTA et al, 2004; Dall'AGO et al, 1997; De ANGELIS et al, 2002; MIKI et al, 2004). A avaliação de tais parâmetros pode servir, portanto, como preditor precoce de envolvimento do sistema cardiovascular nas complicações do diabetes.

Diante das limitações impostas pelo diabetes, torna-se imprescindível a melhor compreensão dos mecanismos patológicos do diabetes e de suas complicações, à procura de um tratamento capaz de contribuir para a melhora das alterações endócrino-metabólicas causadas pela doença e, principalmente, as lesões crônicas sobre os diferentes órgãos. Neste particular, os estudos experimentais sobre o diabetes têm sido extremamente necessários.

2.3 Diabetes *Mellitus* experimental

Estudos envolvendo pacientes diabéticos são frequentemente inconclusivos em relação aos mecanismos patológicos da doença e de suas complicações a longo prazo. Isso deve-se a complexidade da síndrome e alta variabilidade do controle glicêmico entre os pacientes. Além disso, estudos em humanos possuem várias limitações práticas e éticas que podem ser contornadas pelo uso de modelos animais, os quais têm fornecido importantes informações em relação aos mecanismos celulares e moleculares fundamentais para a compreensão da fisiopatologia do diabetes (SURWIT & WILLIAMS, 1996; RODRIGUES et al, 1999).

Existem vários modelos experimentais de diabetes. O diabetes em animais pode ser produzido por cirurgia, agentes químicos e alterações genéticas. Dentre as formas de indução mais utilizadas está a indução química. A indução química do DM experimental é amplamente utilizada para se investigar as complicações causadas pelo DM e avaliar possíveis efeitos de intervenções terapêuticas nesse grupo populacional. Os agentes químicos diabetogênicos mais utilizados pela comunidade científica são a streptozotocina (STZ) e a aloxana (ALX) (BATTELL et al, 1999; SZKUDELSKI, 2001).

O mecanismo de ação tóxico da STZ inicia-se pela entrada nas células β-pancreáticas. Isto ocorre devido à alta afinidade da STZ aos receptores GLUT2 presentes na membrana plasmática. A entrada da STZ provoca alquilação do DNA. O dano ao DNA induz ativação de poli ribozilação de ADP, um processo que é mais importante para o efeito diabetogênico da STZ do que o dano ao DNA diretamente. A poli ribozilação do ADP conduz a depleção de NAD^+ e ATP. O aumento da defosforilação do ATP fornece substrato para a enzima xantina oxidase resultando na formação de radicais superóxidos. Consequentemente, peróxido de hidrogênio e

radicais hidroxilas são também gerados. Ademais, STZ libera quantidades tóxicas de oxido nítrico, o qual inibe a atividade da enzima aconitase e participa do dano ao DNA. Como resultado as células células β-pancreáticas sofrem destruição por necorse (BATTELL et al, 1999; SZKUDELSKI et al, 1998 e 2001).

A ação citotóxica do agente diabetogênico ALX, que possui alta afinidade pelas células β-pancreáticas, é mediada por EROs. ALX e o produto de sua redução, o ácido dialurônico, estabelecem um ciclo redox com a formação de radicais superóxidos. Esses radicais são dismutados a peróxido de hidrogênio, posteriormente são formados radicais altamente reativos de hidroxila pela reação de Fenton. A ação das EROs associado ao aumento maciço na concentração de cálcio citosólico causam rápida destruição das células β-pancreáticas. O aumento maciço da concentração de cálcio deve-se tanto pelo aumento do influxo extracelular, quanto pela liberação dos estoques intracelulares de cálcio e limitada eliminação do citosol (BATTELL et al, 1999; SZKUDELSKI et al, 1998 e 2001).

A destruição de grande parte das células β-pancreáticas pela aloxana impossibilita a produção de insulina necessária para demanda do organismo. A ausência de insulina produz um estado hiperglicêmico, característico do diabetes mellitus, associado a sintomas como poliúria, polidpsia e polifagia (LEZEN, 1988; SKUDELSKI, 2001).

Apesar da seletividade pelas células β-pancreáticas, segundo Szkudelski (1998), a ALX também causa sobrecarga oxidativa em outros órgãos como no fígado. Entretanto a capacidade antioxidante hepática é maior que a dose necessária para citotoxidade das células β-pancreáticas. Doses excessivas de ALX podem levar a morte decorrente de necrose tóxica da célula tubular renal (LENZEN et al, 1996).

O modelo experimental de DM induzido pela aloxana é portanto do tipo 1, caracterizado pela ausência ou significativa redução de insulina proporcionando desequilíbrio na homeostase glicêmica. Segundo Lenzen e Panten (1988), os animais diabéticos induzidos pela aloxana apresentam sintomas semelhantes aos encontrados no DM humano, tais como perda de peso, poliúria, polidipsia, polifagia, glicosúria, cetonúria, hipoinsulinemia, hiperglicemia e cetonemia. Esse modelo também induz o desenvolvimento do diabetes associado a um quadro de hipertensão arterial sistêmica (VADLAMUDI, 1983; KULKARNI et al, 2002).

2.4 DM e exercício físico

Os objetivos principais no tratamento do DM são controlar os níveis de glicemia e os fatores de risco coexistente de doenças cardiovasculares, além de prevenir ou reduzir a progressão de complicações crônicas. Isto pode ser conseguido pela combinação de dieta, interrupção do tabagismo, utilização de insulina ou de medicamentos hipoglicemiantes e prática regular de exercício físico (PAN et al, 1997).

Os benefícios cardiovasculares, metabólicos e autonômicos observados com o exercício físico agudo e crônico têm levado muitos investigadores a sugerir o treinamento físico como uma conduta não farmacológica importante no tratamento de diferentes patologias como o diabetes, a hipertensão arterial e a insuficiência cardíaca (JENNINGS et al, 1986; TIPTON et al, 1991; WALBERGHERIKSSON et al, 1998).

Estudos epidemiológicos e de intervenção têm demonstrado que é possível diminuir significativamente a incidência de novos casos de DM ou diminuir suas complicações através de medidas de intervenção como a realização de exercício físico regular e educação alimentar (EDELSTEIN et al, 1997; BARZILAV et al, 1999; TUOMILEHTO et al, 2001; CASTANEDA et al, 2001 e 2002).

O treinamento físico (TF) adequado proporciona importantes ajustes metabólicos, neuroendócrinos e cardiovasculares, contribuindo para prevenção, redução e reversão das alterações metabólicas nos diabéticos e melhorando a qualidade de vida desses indivíduos. As alterações persistentes na estrutura ou na função do organismo são particularmente ocasionadas em resposta ao estímulo gerado pelo exercício físico repetido sistematicamente e ao longo do tempo (DORN et al, 2001; TANCREDE et al, 2002; ADA, 2004; LaMONTE et al, 2005).

Vários estudos têm demonstrado a importância do TF no manejo da glicemia plasmática de diabéticos (TUOMILEHTO et al, 2001; CASTANEDA et al, 2001 e 2002; CAMACHO et al, 2005). Entre os fatores que melhoram a homeostase glicêmica por aumentar a captação de glicose celular durante o exercício, pode-se destacar principalmente, o aumento da translocação de vesículas contendo GLUT4 para a membrana da célula muscular. Este mecanismo intracelular de translocação não é completamente compreendido, mas é independente de insulina. O aumento da

concentração de cálcio, o estímulo da enzima sintase do oxido nítrico, ou até mesmo a hipóxia proporcionadas pela contração muscular podem estar envolvidas nesse processo de sinalização (GOODYEAR & KAHN, 1998; HENRIKSEN, 2002; KROOK et al, 2004; JESSEN & GOODYEAR, 2005).

Esses resultados são reforçados por pesquisas que verificaram a ausência de envolvimento na ativação de sinalização insulínica para a entrada de glicose na célula, demonstrada pela carência de fosforilação do receptor de insulina, de seus substratos (IRS-1 e IRS-2) e nem da enzima fosfatidilinositol-3-quinase (PI3K) durante o exercício (GOODYEAR & KAHN, 1998). Além disso, foi demonstrado que a proteína quinase estimulada por AMP (AMPK), que não participa da cascata insulínica, também é estimulada pela contração muscular (GOODYEAR & KAHN, 1998; MUSI et al, 2001; RYDER, 2001; HENRIKSEN, 2002; SAKAMAOTO & GOODYEAR, 2002).

Portanto observa-se que a via insulino-independente para a captação da glicose durante o exercício é fundamental para o controle da glicemia plasmática em diabéticos até porque durante o exercício há diminuição da secreção de insulina e aumento dos hormônios contra-regulatórios (NEGRÃO & BARRETTO, 2006).

Entretanto os benefícios do TF na população diabética não se restringem apenas aos efeitos agudos. Dentre os efeitos crônicos do TF proporcionados por adaptações metabólicas pode-se observar a redução da resistência insulínica devido ao aumento da sensibilidade de seus receptores. O aumento da sensibilidade à insulina facilita a captação de glicose durante o repouso, contribuindo para a redução da quantidade de insulina exógena administrada. Além disso, alguns estudos demonstraram aumento dos estoques de glicogênio muscular e hepático (NEGRÃO & BARRETTO, 2006).

Estudos realizados em humanos têm demonstrado que diabéticos submetidos ao treinamento físico aeróbio (TFA) apresentaram aumento na sensibilidade à insulina, redução na sua dose diária, melhora na cinética e no consumo de oxigênio, diminuição das complicações crônicas do DM, melhora no controle de fatores de risco para doenças cardiovasculares associadas ao DM, como redução da pressão arterial (PA), frequência cardíaca (FC), peso corporal e stress oxidativo (ACSM, 2000; CIOLAC & GUIMARÃES, 2002; GOTO et al, 2003; CIOLAC & GUIMARÃES, 2004; ADA, 2004; SASAKI & SANTOS, 2006).

O TF também diminui os níveis circulantes de angiotensina, aldosterona, vasopresina e endotelina, como também reduz os níveis de citocinas, entre elas o fator de necrose tumoral alfa e a interleucina-6, que estão diretamente associadas à maior ativação do sistema renina-angiotensina e à disfunção endotelial (LEVINE et al, 1990; BRAITH et al, 1999; MAEDA et al, 2001).

Outros estudos demonstraram as contribuições do treinamento físico no sistema nervoso autônomo (SNA), tanto em animais normais, como na neuropatia autonômica cardiovascular (NAC) presente no diabetes. Dentre estas contribuições podem se citar melhora na sensibilidade barorreflexa, quimiorreflexa e receptores cardiopulmonares, além de melhora do balanço autonômico cardiovascular. Nesses estudos pôde-se observar uma redução na atividade simpática e aumento da atividade parassimpática contribuindo para redução dos fatores de risco cardiovasculares (De ANGELIS, et al, 2000; LOIMAALA et al, 2003; COSTA et al, 2004; ANGELIS, et al, 2004b; IRYGOYEN et al, 2005; HARTHMANN et al, 2007).

Por outro lado, o RT não está isento de efeitos desfavoráveis. Nesse sentido alguns estudos demonstraram que intensidades elevadas do treinamento físico resistido, que é caracterizado por um forte componente isométrico, podem induzir hipertrofia cardíaca concêntrica e rigidez arterial, ocasionada pela sobrecarga de pressão (KAWANO et al, 2007; MIHL, at al, 2008). Entretanto os estímulos moleculares e celulares para tais efeitos ainda não são claros. Esses resultados demonstram que intensidades elevadas de RT deveriam ser desencorajadas na reabilitação cardiovascular.

Segundo Graves e Franklin (2006) não há publicação de estudos epidemiológicos ou experimentos clínicos sobre os efeitos do exercício resistido na prevenção do DM tipo 2 ou na administração glicêmica do DM dos tipos 1 ou 2. Entretanto só recentemente um número limitado de estudos tem demonstrado o valor potencial e relativa segurança do treinamento resistido no portador de DM.

Este tipo de treinamento físico tem como principal mecanismo biológico, aparentemente envolvido no controle glicêmico, a hipertrofia da musculatura esquelética. Esta hipertrofia caracteriza-se pelo aumento das taxas de síntese protéica, com aumento associado na absorção de glicose e sensibilidade à insulina, além de adaptações cardiovasculares como o efeito hipotensor arterial (FARREL et al, 1998; POLLOCK et al, 2000; FLETCHER et al, 2001; CIOLAC &

GUIMARÃES, 2004; SANCHEZ & LEON, 2006). Segundo Honkola et al e Eriksson et al, (1997) o TFR em diabéticos também foi capaz de reduzir a HbA1c.

Esse tipo de treinamento é considerado relativamente seguro para aumentar a força muscular e melhorar a qualidade de vida tanto em adultos saudáveis quanto em idosos ou portadores de comprometimentos cardiovasculares. Um método seguro e não invasivo que tem sido utilizado para avaliar o trabalho do miocárdio, durante o repouso ou esforços físicos é o cálculo do duplo-produto (DP)(frequência cardíaca multiplicada pela pressão arterial sistólica), pois apresenta uma forte correlação com o consumo de oxigênio pelo miocárdio. Valores mais elevados representam maior estresse cardiovascular. Logo trata-se de uma variável estreitamente relacionada com segurança da atividade (GOBEL et al, 1994; FEIGENBAUM et al, 1999; POLLOCK et al, 1994; ACSM, 2000b; POLITO & FARINATTI, 2003).

Alguns estudos mostram que os valores do DP dos exercícios resistidos costumam ser menores do que os observados em atividades aeróbias de intensidade moderada em razão de uma menor resposta de pico para a FC (DEBUSK et al, 1978; POLLOCK et al, 2000).

Outro fator que justifica a utilização do TFR é que a progressão da doença torna o diabético mais debilitado e pode limitá-lo para prática de atividades aeróbicas. Baseado nestas observações postula-se que o treinamento resistido poderia ser útil no gerenciamento do DM.

Existem, portanto, evidências na literatura de que o treinamento físico contribui de maneira significativa para a melhora do manejo glicêmico e as complicações do DM. Por conseguinte devem ser consideradas tanto atividades aeróbias quanto resistidas na elaboração de um protocolo de treinamento para o portador de DM, maximizando assim, os benefícios advindos da prática regular do exercício físico. Estas atividades devem ter como objetivos tanto prevenção, quanto tratamento das complicações do DM, representando uma significativa melhora na função dos diversos sistemas orgânicos acometidos pelo diabetes.

OBJETIVOS

3 OBJETIVOS

3.1 OBJETIVO GERAL

- Avaliar os efeitos do treinamento físico resistido sobre o balanço autonômico cardíaco de ratos diabéticos induzidos pela aloxana.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar em ratos diabéticos os efeitos do TFR sobre:
    - Glicemia;
    - Pressão arterial (PA) e Freqüência Cardíaca (FC);
    - Sensibilidade barorreflexa arterial (SBA);
    - Variabilidade da frequência cardíaca (VFC);
    - Repolarização ventricular.

RESULTADOS

RESISTANCE TRAINING PREVENTS HYPERTENSION AND IMPROVES CARDIAC AUTONOMIC MODULATION IN ALLOXAN DIABETIC RATS

André Sales Barreto[1]; Marcelo Mendonça Mota[1]; Milene Tavares Fontes[1]; José Melquiades Resende-Neto[2]; MSc; Valter Joviniano Santana-Filho[3] P.T., PhD; José Augusto Soares Barreto-Filho[4] M.D., PhD and Márcio Roberto Viana Santos[1*], PhD

[1] Departament of Physiology, Federal University of Sergipe, São Cristovão, Sergipe, Brazil;

[2] Departament of Pharmacy, Tiradentes University, Aracaju, Sergipe, Brazil.

[3] Nucleo of Physical Therapy, Federal University of Sergipe, São Crsitovão, Sergipe, Brazil;

[4] Departament of Medicine, Federal University of Sergipe, Aracaju, Sergipe,Brazil;

Short title: Resistance training and autonomic function in DM

Word count total: 5436; A**bstract:** 248; **Figures**: 3; **Tables:** 2

Correspondence Author: Márcio Roberto Viana Santos, Ph.D., Department of Physiology, Federal University of Sergipe, Av. Marechal Rondon s/n. Roza Elze, São Cristovão, Sergipe, Brazil. Telephone: 55 79 21056842. FAX: 55 79 21056474. E-mail:marcio@infonet.com.br

ABSTRACT

This study aimed to evaluate the effects of resistance training on blood pressure, baroreflex sensitivity, cardiac autonomic balance and ventricular repolarization in diabetic rats. Cardiovascular evaluation was performed in conscious trained and sedentary animals, 8 weeks after the onset of diabetes with alloxan or control animals. The resistance training consisted of 3 sets of 10 repetitions performed at 40% of one repetition maximum test, 3 days/wk over 8 wks in squat-training apparatus. Blood pressure was monitored for 30 min 48 h after the last training session or time control. Baroreflex sensitivity was analyzed by sequence method and cardiac autonomic balance by heart rate variability in the frequency domain. After 8 wks, the diabetes significantly increased glycemia (from 83 ± 8 to 381 ± 41 mg/dl, $p<0.05$), mean blood pressure (from 104.7 ± 5.4 to 125.1 ± 5.4 mmHg, $p<0.05$), QTc interval (from 4.4 ± 0.1 to 5.1 ± 0.1 ms, $p<0.05$), reduced baroreflex sensitivity (from 2.01 ± 0.3 to 0.38 ± 0.1 ms/mmHg, $p<0.05$) and impaired the cardiac autonomic balance. Resistance training was able to produce significant reduction on the glycemia (270 ± 17 mg/dl, $p<0.05$), prevented the increase of mean blood pressure (108 ± 3 mmHg, $p<0.001$) and QTc interval (4.6 ± 0.1 ms, $p<0.01$), the reduction of baroreflex sensitivity (2.63 ± 0.5 ms/mmHg, $p<0.01$) and disturbance on the cardiac autonomic balance. These results suggest that resistance training promotes a better glycemic control, prevents hypertension and improves baroreflex sensitivity and cardiac autonomic balance in alloxan diabetic rats.

Keywords: diabetes mellitus, exercise, blood pressure, baroreflex sensitivity, autonomic nervous system.

INTRODUCTION

The incidence of diabetes mellitus (DM) in a modern society has been rising at epidemic rates, mostly related to an increase in the prevalence of obesity associated with sedentary lifestyle. DM is a chronic metabolic disorder associated to secondary complications in the cardiovascular system, such as microangiopathy, atherosclerosis, hypertension and autonomic neuropathy (1, 2).

Cardiac autonomic neuropathy is a major cause of cardiovascular complications in diabetes and it is closely with cardiovascular events as silent myocardial ischemia, stroke and sudden death (3). There are several reports showing that Cardiac autonomic neuropathy produce abnormalities in the heart control and cardiovascular dynamics (4), affecting the autonomic modulation of the sinus node, reducing heart rate variability (HRV) and impairing baroreflex sensitivity (BRS) (5, 6). Several methods of assessing cardiovascular function have been introduced for the diagnosis of autonomic neuropathy in diabetes increasing our knowledgement about autonomic control of the cardiovascular system and the associated pathophysiological mechanisms, both in patients (7) and in experimental models (8, 9).

Exercise training has been one of the non-pharmacological interventions indicated for preventing or controlling cardiovascular complications associated to diabetes (10,11), and there are several studiesshowing the effects of endurance training (ET) protocols on diabetes and its cardiovascular complications (12, 13). However, the cardiovascular adaptations induced by resistance training (RT) are not well documented. Thus the purpose of the present study was to determine the effects of chronic RT on blood pressure, heart rate, baroreflex sensitivity, cardiac autonomic balance and ECG in alloxan-diabetic rats.

METHODS

Animals

Experiments were performed in male wistar rats weighing between 250 and 300 g. The animals were housed in individual cages with free access to water and food, at a constant temperature of 22 ± 1^{o}C, on a 12 h light/dark cycle. All experimental protocols were in accordance with the Guidelines for Ethical Care of Experimental Animals and were approved by the Animal Research Ethics Committee of the Federal University of Sergipe.

Diabetes Induction

Diabetes was induced by a single injection of alloxan (ALX) diluted in saline (40 mg/kg of body weight, i.v., pH 4.5, Sigma Chemical Co, St Louis, MO, USA), administered 2 wks before the start of exercise training protocols. The sedentary control group (SC) (n = 8) received the same volume of vehicle. The rats were fasted for 12 h before ALX or vehicle injection. One week after, only the animals with plasma glucose above 200 mg/dl evaluated by glucose analyser (Accu-chek Advantage II, Roche, São Paulo, Brazil) were included in diabetic group. Before the beginning of experiments, the diabetic animals were randomized in sedentary (SD) (n = 8) or trained (TD) (n = 8) groups.

Exercise training

Diabetic animals allocated in the trained group followed a protocol where the hindlimbs of all exercised rats were trained with sessions of weight lifting as described in detail elsewhere (14). Before start the exercise training, rats were adapted for 1 week. Rats fitted with a canvas jacket were able to regulate the twisting

and flexion of their torsos and were suspended in a standard position on their hind limbs. An electrical stimulation (20 V, 0.3 s duration, at 3 s intervals) delivered by an electro stimulator (Bioset, Physiotonus four, mod 3050, Rio Claro, SP-Brazil) was applied to the tail of the animal through a self adhesive surface electrode (Valu Trode, model CF3200, size 3.2cm, Axekgeerd, Fallbrook, CA, EUA). As a result, the rats flexed their legs repeatedly, which lifted the weight-arm of the training apparatus. The exercise session involved 3 sets of 10 repetitions (lifts) with ~1 min rest between each set preformed 3 times a wk during 8 wks. The exercises were started after 1 week of adaptation. After measurement of the maximum weight lifted (1RM) with the squat-training apparatus, the training load was set at 40% of 1RM. The training load was adjusted each 15 days by 1RM test until finishing the exercise training to maintain the same load during all experimental protocol.

Cardiovascular assessments

At the end of the eighth week of exercise training or time control, rats were anaesthetized (45 mg/kg, i.p., Thiopentax, Cristália, Itapira, SP, Brazil) and a polyethylene catheter (PE), segment of PE-10 (internal and external diameter of 0.28 mm and 0.61, respectively) was placed into the right femoral artery for the direct measurement of blood pressure (BP). Thereafter, electrodes were subcutaneously implanted into the thorax for ECG records on lead II configuration. Catheters and wire from ECG electrodes were exteriorized through the back of the neck and the incision was sutured. Conscious rats were studied 24 h after surgical procedure and were allowed to move freely during the experiments. The arterial catheters was connected to a pressure transducer (Edwards Lifescience, Irvine, CA, USA) and after 30 min to stabilizing BP signals were recorded more 30 min by a microcomputer

equipped with an analogue-to-digital converter board (BioData, João Pessoa, PB, Brazil). The data were recorded with 2 kHz sampling frequency and were analyzed on a beat-to-beat basis to quantify changes in mean BP and HR.

The baroreflex control of HR was assessed through spontaneous changes in arterial pressure and pulse interval by the sequence method described by Bertinieri and coworkers (15). Ramps of progressive increases and decreases in systolic arterial pressure were automatically detected in 10^4 beats of pulsatile arterial pressure recordings using the freely available HemoLab computer software (http://www.intergate.com/_harald/ HemoLab/Hemolab.html). Sequences defined ramps of four or more systolic arterial pressure values associated with parallel changes in pulse interval, i.e., systolic arterial pressure increases and pulse interval lengthenings, as well as systolic arterial pressure decreases and pulse interval shortenings. The spontaneous BRS was calculated from the slope (ms/mmHg) of linear regression lines between the systolic arterial pressure and the subsequent pulse interval. Only regression lines with a correlation coefficient higher than 0.85 were considered (16).

The cardiac autonomic balance (CAB) was evaluated by heart rate variability through spectral analysis. The spectral density of the various frequency components of systolic arterial pressure and heart rate was calculated using Fast Fourier Transform (Kubios HRV, Department of Physics, University of Kuopio, Kuopio, Finland). This analysis requires data collected at equal time intervals. Therefore, the beat-to-beat systolic arterial pressure and heart rate data were converted into data points every 100 ms using a cubic spline interpolation. The interpolated series were divided into half-overlapping sequential sets of 512 data points. The spectra obtained for the different data sets was averaged in order to attenuate the contribution of

variable noise and to sharpen the reproducible spectral peaks. The averaged spectra were integrated in three frequency bands defined as VLF (0.01 to 0.04 Hz), LF (0.04 to 0.15 Hz) and HF (0.15 to 0.4 Hz). This procedure permitted the automatic quantification of the center frequency and the power of each relevant component in absolute as well as in normalized.

Electrocardiographic parameters

Subcutaneous electrodes were implanted to evaluate ventricular repolarization in DII derivation. At the end of blood pressure recording, the electrodes were connected to a bioelectric amplifier (bioData, Model BD-01, João Pessoa, PB, Brazil), the ECG was continuously sampled (2000 Hz) for a period of 30 min, and QRS, PR, QT and QTc intervals were evaluated. Interval QTc was calculated by using Bazetts formula.

Statistical Analysis

Values are expressed as mean ± standard error mean (SEM). When appropriate Student's test or one way ANOVA with Bonferroni post-test were conducted in order to evaluate the significance of differences between means. Significant values were considered those whose differences were lower than 5%. All statistical analyses were done by using Graph Pad Prism ^{TM} version 3.02 software.

RESULTS

Animals submitted to resistance training presented an increase in the workload (Initial: 900 ± 77 and final: 1340 ± 67 g, $p<0.001$ Student's test). ALX treated animals developed severe and significant hyperglycemia initial (SD: 320 ± 26 and TD: 340 ± 21 mg/dl) when compared with control animals (SC: 83 ± 8 mg/dl,

$p<0.001$ one-way ANOVA). However, after experimental protocol it was significantly reduced in the trained animals (TD: 270 ± 17 mg/dl, $p<0.05$ vs. SD Student's test) and significantly increased in sedentary animals (SD: 381 ± 41 mg/dl, $p<0.05$ Student's test).

The cardiovascular evaluations from all groups are presented in table 1. SD animals presented higher SBP and MAP levels when compared SC. RT was effective in preventing the changes in these parameters. The HR was not altered by the diabetes, however RT was able to produce bradycardia. As shown in figure 1, spontaneous BRS was lower in SD (0.38 ± 0.1 ms/mmHg) when compared to the SC animals (2.01 ± 0.3 ms/mmHg, $p<0.05$). This BRS impairment was restrained by RT (2.63 ± 0.5 ms/mmHg, $p<0.01$).

Figure 2 shows a schematic picture of pulse interval (upper panel) and spectra of pulse interval (lower panel) of an animal representative from each group: sedentary control (SC), sedentary diabetic (SD) and trained diabetic (TD).

Power band from LF and HF in normalized units and LF/HF ratio are presented in figure 3 A and B respectively. SD animals presented increase LF (from 41.2 ± 2.1 to 59.2 ± 4.4 n.u.) and decrease HF band (from 58.8 ± 2.1 to 40.8 ± 4.4 n.u.) when compared to the SC animals ($p<0.05$). Nevertheless, RT promoted a decrease LF (37.9 ± 3.4 n.u.) and increase HF band (62.1 ± 3.4 n.u.) when compared to the SD animals ($p<0.05$). On the CAB LF/HF ratio was higher in SD animals (from 0.71 ± 0.06 to 1.67 ± 0.31 ms^2, $p<0.01$), however the RT was able to prevent this increase (0.63 ± 0.11 ms^2, $p<0.01$).

As shown in table 2, the ECG analyses showed that there was a lengthening in QTc interval in DS when compared with SC animals ($p<0.001$). In DT animals, the lengthening in QTc interval was not observed.

DISCUSSION

The purpose of this study was to evaluate the hemodynamic and autonomic responses promoted by RT in diabetic rats. The current study showed, for the first time to our knowledge, that alloxan-induced diabetes produces BRS and CAB dysfunctions in rats and that RT was able to prevent them. Furthermore RT improved glucose control, prevented the increase of BP and improved QTc interval. The 1RM was used as an index of training efficiency and the maximal strength achieved by the rats was used to determine the training load and to demonstrate training adaptation. Increased workload demonstrated efficiency of this model of exercise training in diabetic rats. This same efficiency was observed in others studies by using normal animals (17, 18).

It is known that the poor glycemic control increases the risk of cardiovascular complications and mortality in patients with DM (1). In our study, the glycemia was reduced in diabetic animals submitted to RT. Similarly to other studies (19, 20), our results showed effectiveness of RT in improving glycemic control in DM. Despite this reduction in glucose levels was not enough to restore the normal glycemia, it appears have contributed to avoid the development of autonomic unbalance during our experimental period. Clinical studies have demonstrated that autonomic unbalance can be prevented or controlled by improving the glycemic control (21, 22).

The majority of animal studies that investigate the cardiovascular effects in diabetic rats uses streptozotocin (STZ) as diabetogenic agent (6, 9, 23), however in our study was used ALX since there are studies in the literature that reported the diabetogenic proprieties of this drug (24). The cardiovascular effects from STZ-induced diabetes

have been widely described in the literature (8, 25), and they are summarized as: hypotension, bradycardia, decreased baroreflex gain and heart rate variability characterized mainly by reduction in vagal and increase in sympathetic tonus (26). In the present study, BP was significantly higher in SD animals. These results are in agreement with findings of other studies that showed higher BP levels when normotensive rats are made diabetic with ALX (27, 28). This increase in BP seems to be due an increase of oxidative stress and consequently reduction of availability nitric oxide to vascular tissue (29). The association of DM and hypertension in humans is well documented (30). Therefore, this model produces cardiovascular changes more closely related to the human diabetes.

This study suggests that RT applied 40% of 1RM was able to reduce BP. Other investigations demonstrated similar results but with higher workloads as 65 and 75% in normal animals (17, 18). The literature is controversial regarding to the RT effect on hemodynamic parameters. Recent studies examined the effects of RT on BP showing that dynamic RT is associated with a decreasing in resting systolic and diastolic BP in hypertensive adults (31, 32). Conversely, others studies that evaluated the effect of RT on BP in hypertensive patients with type 2 DM showed that the RT has no effect on resting BP. The difference between these studies can be related to the variation in the duration of the RT or the workload applied (17, 18).

The bradycardia observed in the present study in TD animals corroborates the results of longitudinal studies involving resistance short-term training (33, 34) and is a reversible common adaptation mainly after ET.

The results obtained so far show that ALX was able of inducing diabetes associated with hypertension and the RT was able to induce better fitness, reduce hyperglycemia and prevent development of hypertension in ALX-diabetic animals.

A number of factors are involved in the pathogenesis of hypertension when it is associated with DM as sodium retention, extracellular fluid volume overload, altered activity of sympathetic nervous system, renin angiotensina system and increased vascular reactivity towards norepinephrine and angiotensin II (35). In order to evaluate the possible mechanisms involved in the cardiovascular changes induced by ALX and RT in diabetic animals were investigated the arterial baroreflex and autonomic nervous system participation by using BRS and HRV analysis.

The analysis of the simpatho-vagal balance by means of the LF/HF ratio showed a sympathetic predominance in DS animals while in trained animals the simpatho-vagal balance shifted to a parasympathetic tone. These results show that ALX induces cardiac autonomic neuropathy which is characterized by reduction of the baroreflex sensitivity, higher sympathetic activity and lower parasympathetic activity.

Other study demonstrated parasympathetic denervation of the heart with ALX (36). Several studies demonstrated impairment on BRS and HRV, however on diabetic rats induced with STZ (6, 23). The disturbance in sympatho-vagal balance associated with the diminished BRS is similar to the cardiac autonomic neuropathy described in human diabetes.

In addition, RT was able to improve arterial baroreflex gain associated with increased parasympathetic activity. Therefore, it is possible that autonomic modulation induced by RT plays a role in the hypotensive and bradycardic effects in ALX diabetic animals.

There are evidences that demonstrate improvement in BRS and HRV with ET in diabetes (12, 37), but the literature about RT effects on autonomic nervous system is poor.

There is experimental evidence of a protective effect of vagal stimulation on ventricular electrical vulnerability, correlating the parasympathetic underactivity the development of lethal arrhythmias (38). This protection appears to have been lost in our SD animals confirmed by their longer QT and QTc intervals. The calculation of the QTc interval allows a better analysis of cardiac repolarization independent of the duration of the RR interval. Depending on the magnitude of increase of QTc interval, it could mean higher duration of ventricular repolarization. Studies have demonstrated increased QTc interval in diabetic humans and it is considered a precursor of increased risk of sudden death (38, 39).

On the other hand, to check if the bradycardia induced possibly by higher parasympathetic activity observed in TD animals would potentially increase other eletrocardiographic variables besides R-R interval the PR, QRS, QT and QTc intervals were evaluated. Our results support that RT induces bradycardia without altering QTc interval in ALX diabetic rats. It means that RT did not bring any prejudice to the time of ventricle repolarization. No significant differences were observed in the other electrocardiographic variables studied (PR and QRS intervals).

Perspectives

The results of the present study provide evidence for the effectiveness of RT in reducing some of the cardiovascular complications associated with DM. Therefore RT can be used as a safe non-pharmacological therapy for the treatment of human diabetes associated to hypertension. However the possible mechanisms involved in these effects need to be more clarify. The maintenance in baroreflex sensitivity and heart rate variability caused by resistance training exercise training suggest

peripheral modulation of autonomic system. The next step will investigate possible participation of central autonomic modulation.

In conclusion, these results indicate that ALX induces diabetes associated to cardiac neuropathy and the RT was able to get better glycemic control and prevent cardiovascular complications from diabetes. In addition, they suggest that the effects of RT to prevent the increase in blood pressure have involvement of BRS and cardiac autonomic balance.

Acknowledgements

We thank Mr Osvaldo Andrade Santos for the technical support.

Sources of Fundings

This work was supported by grants from CNPq, CAPES, FAPITEC-SE, Ministério da Saúde, SES/SE, Brazil.

Conflict of interest : None

References

1. Klein R. Hyperglycemia and microvascular and macrovascular disease in diabetes. *Diabetes Care*.1995;18:258-268.

2. Wild S, Roglic G, Green A, Sicree R and King, H. Global prevalence of Diabetes. Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047-1053.

3. Vinik AI, Strotmeyer ES, Nakave AA and Patel CV. Diabetic neuropathy in older adults. *Clin in Geriatr Med*. 2008;24(3):407-435.

4. Vinik AI and Ziegler D. Diabetic cardiovascular autonomic neuropathy. *Circulation*. 2007;115:387-397.

5. Schroeder EB, Chambless LE, Liao D, Prineas RJ, Evans GW, Rosamond WD and Heiss G. Diabetes, glucose, insulin, and heart rate variability. *Diabetes Care*. 2005;28:668-674.

6. Dall'Ago P, Silva VO, De Angelis KL, Irigoyen MC, Fazan Jr R and Salgado HC. Reflex control of arterial pressure and heart rate in short-term streptozotocin diabetic rats. *Bra. J Med. Bio. Res*. 2002;35(7):843-849

7. Mancia G, Paleari F and Parati G. Early diagnosis of diabetic autonomic neuropathy: present and future approaches. *Diabetologia*. 1997;40:482-484.

8. Farah VMZ, De Angelis K, Joaquim LF, Candido GO, Bernardes N, Fazan R, Schaan BD and Irigoyen MC. Autonomic modulation of arterial pressure and heart rate varibility in hypertensive diabetic rats. *Clinics*. 2007;62(4):477-482.

9. Fazan R, Ballejo G, Salgado MC, Moraes M and Salgado HC. Heart rate variability and baroreceptor function in chronic diabetic rats. *Hypertension*. 1997;30(Part 2):632-635.

10. Balducci S, Zanuso S, Fernando F, Falluca S, Falluca F, Pugliese G and Intalian Diabetes Exercise Study (IDES) Group. Physical activity/exercise training in type 2 diabetes. The role of the Italian Diabetes and Exercise Study. *Diabetes Metab Res Rev*. 2009;25:29-33.

11. Castaneda C. Diabetes control with physical activity and exercise. *Nutr Clin Care*. 2003;6(2):89-96.

12. Figueroa A, Baynard T, Fernhall B and Carhart R. Endurance training improves post-exercise cardiac autonomic modulation in obese women with and without type 2 diabetes. *Eur J Appl Physiol*. 2007;100:437-444.

13. Koutroumopi M, Pitsavos C and Stefanadis C. The role exercise in cardiovascular rehabilitation: a review. *Acta Cardiol*. 2008;63(1): 73-79.

14. Tamaki T, Uchiyama S and Nakano S. A weight-lifiting exercise model for inducing hypertrophy in the hindlimb muscles of rats. *Med Sci Sports Exerc*. 1992;24:881-886.

15. Bertinieri G, Di RM, Cavallazzi A, Ferrari AV, Pedotti A, Mancia G. A new approach to analyses of the arterial baroreflex. *J Hypertens*. 1985;Suppl 3:S79-S81.

16. Oosting J, Struijiker-Boudier HAJ and Janssen BJA. Validation of a continuous baroreceptor reflex sensitivity index calculated from spontaneous fluctuations of blood pressure and pulse interval in rats. *J Hypertens*. 1997;15:391-399.

17. Barauna VG, Junior ML, Costa Rosa LF, Casarini DE, Krieger JE and Oliveira EM. Cardiovascular adaptations in rats submitted to a resistance-training model. *Clin Exp Pharmacol Physiol*. 2005;32:249-254.

18. Pinter RCCE, Padilha AS, Oliveira EM, Vassallo DV and Lizardo JHF. Cardiovascular adaptive responses in rats submitted to moderate resistance training. *Eur J Appl Physiol*, 2008;103:605-613.

19. Loimaala A, Groundstroem K, Rinne M, Nenonen A, Huhtala H, Parkkari J and Vuori I. Effect of long-term endurance and strength trainig on metabolic control and arterial elasticity in patients with type 2 Diabetes Mellitus. *Am J Cardiol*. 2009;103:972-977.

20. Castaneda C, Layne JE, Munoz-Orians L, Gordon PL, Walsmith J, Foldvari M, Roubenoff R, Tucker KL and Nelson ME. A randomized controlled trial of resistance training to improve glycemic control in older adults with type 2 diabetes. *Diabetes Care*. 2003;25(12):2335-2341.

21. Burger AJ, Weinrauch LA, D'Elia JA and Aronson D. Effect of glycemic control on heart rate variability in type I diabetic patients with cardiac autonomic neuropathy. *Am J Cardiol*. 1999;84(6):687-691.

22. Holder M, Holl RW, Bartz J, hecker W, Heinze E, Leichter HE and Teller W. Influence of long-term glycemic control on the development of cardiac autonomic neuropathy in pediadric patients with type I diabetes. *Diabetes Care*. 1997;20(6):1042-1043.

23. Fazan R Jr, Dias da Silva VJ, Ballejo G and Salgado HC. Power spectral of arterial pressure and heart rate in streptozotocin induced diabetes in rats. *Hypertension*. 1999;17:489-95.

24. Dunn JS, Sheehan HL and McLathie NGB. Necrosis of islets of Langerhans produced experimentally. *Lancet*. 1943;1:484-487.

25. De Angelis K, Schann BD, Dall'Ago P, Maeda CY, Wichi R and Irigoyen MC. Cardiovascular control in experimental diabetes. *Braz J Med Biol Res*. 2002;35(9):1091-1100.

26. Dowell RT, Atkins FL and Love S. Integrative nature and time course of cardiovascular alterations in the diabetic rat. *J Cardiovasc Pharmacol* . 1986;8:406-413.

27. Cavaliere TA, Taylor DG, Kerwin LJ and Antonaccio MJ. Cardiovascular Effects of Alloxan Diabetes in Normotensive and Spontaneously Hypertensive Rats. *Pharmacolgy*. 1980;20:211-223.

28. Kulkarni JS, Metha AA, SAntani DD and Goyal RK. Effects of chronic treatment with cromakalin and glibeclamide in alloxan-induced diabetic rats. *Pharmacol Res*. 2002;46(2):101-105.

29. Hadi HAR and Suwaidi JAL. Endothelial dysfunction in diabetes mellitus. *Vascular Health and Risk Management*. 2007;3(6):853-876.

30. Kannel WB and Mcgee DL. Diabetes and cardiovascular risk factors: The Framingham study. *Circulation*. 1979;59(1):8-13.

31. Braith WR and Stewart KJ. Resistance training, its role in the prevention of cardiovascular disease. *Circulation*. 2006;113:2642-2650.

32. Fagard RH and Cornelissen VA. Effect of exercise on blood pressure control in hypertensive patients. *European Journal of Cardiovascular Prevention and Rehabilitation*. 2007;14:12-17.

33. Goldberg L, Eliot DL and Kuehl KS. A comparison of the cardiovascular effects of running and weight training *Journal of Strength and Conditioning Research*. 1994;8:219-224.

34. Kanakis C and Hickson C. Left ventricular responses to a program of lower-limb strength training. *Chest*. 1980;78:618-621.

35. Stas SN, El-Atat FA, Sowers JR. Pathogenesis of hypertension in diabetes. *Reviews in Endocrine & Metabolic Disorders*. 2004;5:221-225.

36. Tomlinson DR and Yusof AP. Autonomic neuropathy in the alloxan-diabetic rat. *J Auton Pharmacol*. 1983;3(4):257-263.

37. De Angelis KLD, Oliveira AR, Dall'Ago P, Peixoto LRA, Gadonski G, Fernandes TG and Irigoyen MC. Effects of exercise training in autonomic and myocardial dysfunction in streptozotocin-diabetic rats. *Braz. J. Med. Biol. Res*. 2000;33:635-641.

38. Giunti S, Bruno G, Lillaz E, Gruden G, Lolli V, Chaturvedi N, Fuller JH, Veglio M and Perrin P. Incidence and risk factors of prolonged QTc interval in type 1 diabetes. The EURODIAB Prospective Complications Study. *Diabetes Care*. 2007;30:2057-2063.

39. Pourmoghaddas A and Hekmatnia A. The relationship between QTc interval and cardiac autonomic neuropathy in diabetes mellitus. *Molecular and Cellular Biochemistry*. 2003;249:125–128.

Figure legends

Figure 1. Arterial baroreflex gain after experimental protocol in Sedentary Control (SC) (n = 8), Sedentary Diabetic (SD) (n = 8) and Trained Diabetic (TD) (n = 8). Data are presented as means ± SEM. To evaluate difference between groups, it was used one-way ANOVA followed by Bonferroni post-test. *p<0.05 SD vs SC; ††p<0.01 TD *vs* SD.

Figure 2. Schematic picture of pulse interval (upper panel) and spectra of pulse interval (lower panel) of an animal representative from each group: Sedentary Control (SC), Sedentary Diabetic (SD) and Trained Diabetic (TD).

Figure 3: Analysis of spectral power in normalized units (n.u.)(A) and in ms^2 (B) of cardiac autonomic balance in Sedentary Control (SC)(n = 8), Sedentary (SD)(n = 8) and Trained (TD)(n = 8) diabetic rats. LF = Low frequence; HF = High frequence. Data are presented as means ± SEM. To evaluate difference between groups, it was used one-way ANOVA followed by Bonferroni post-test. *p<0.05 SD *vs* SC; †p<0.05 TD *vs* SD; **p<0.01 SD *vs* SC and ††p<0.01 TD *vs* SD.

Table legends

Table 1. Cardiovascular evaluation in Sedentary Control, Sedentary and Trained diabetic rats.

Table 2. PR, QRS, QT and QTc intervals from ECG in Sedentary Control, Sedentary and Trained Diabetic rats.

Figure 1

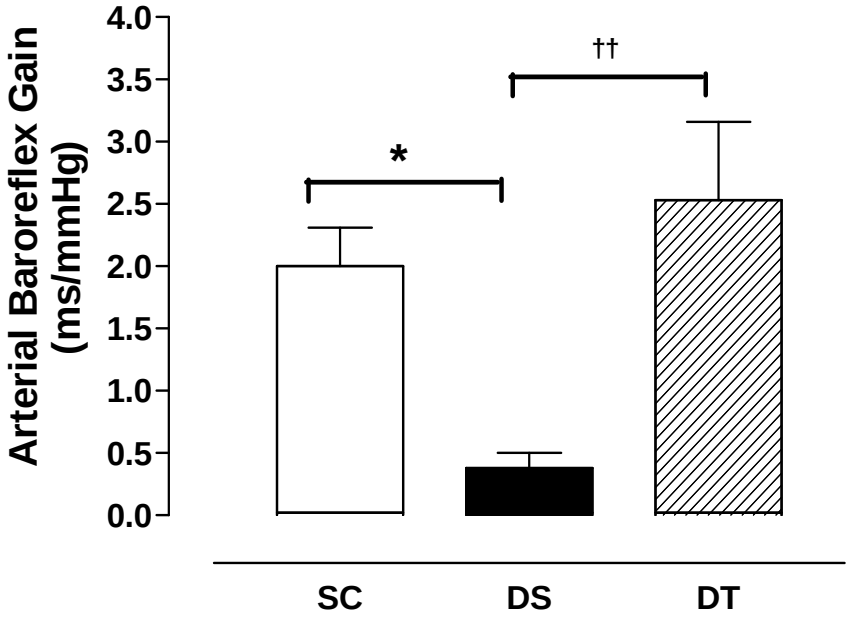

Arterial Baroreflex Gain
(ms/mmHg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
††
SC
DS
DT

Figure 2

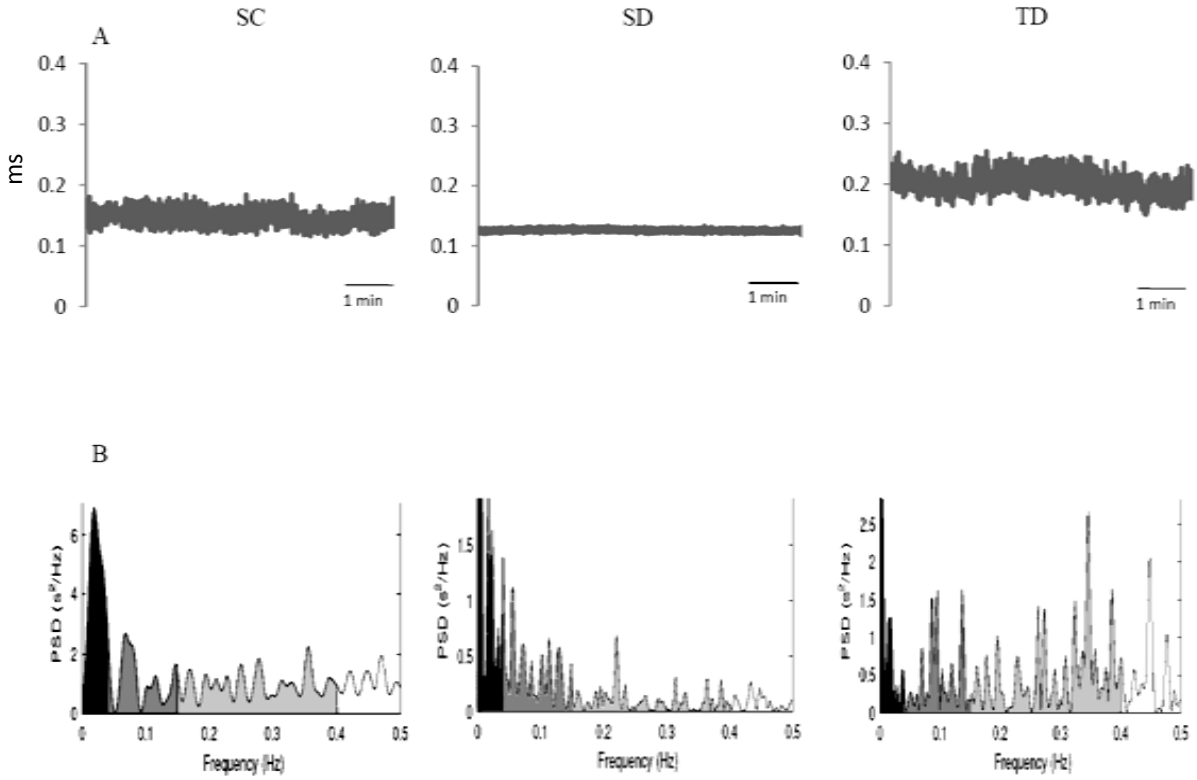

SC
SD
TD
A
ms
0.4
0.3
0.2
0.1
0
1 min
B
PSD (s²/Hz)
Frequency (Hz)
0
0.1
0.2
0.3
0.4
0.5

Figure 3

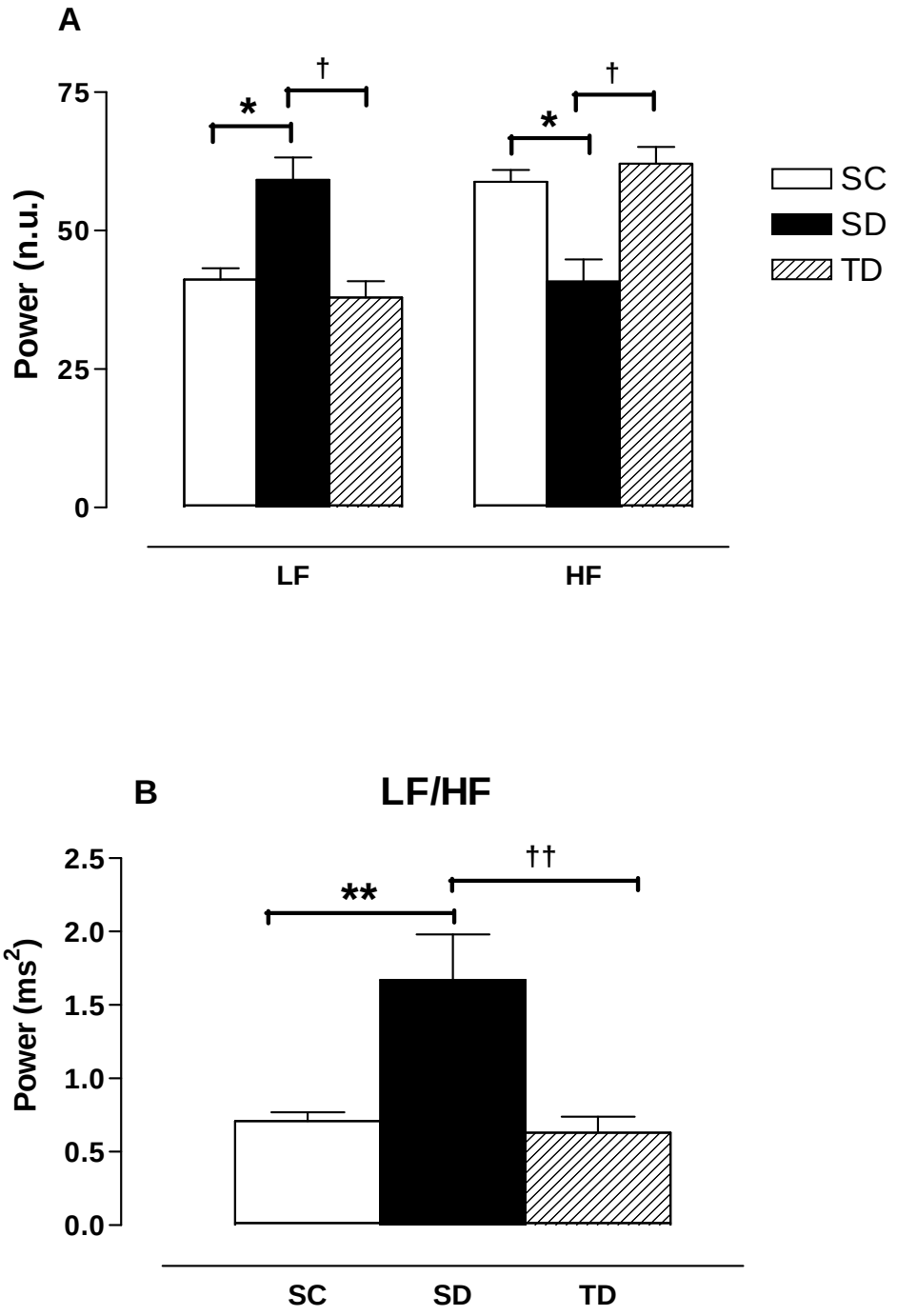

A
75
50
25
0
Power (n.u.)
*
†
*
†
LF
HF
SC
SD
TD
B
LF/HF
2.5
2.0
1.5
1.0
0.5
0.0
Power (ms2)
**
††
SC
SD
TD

Table 1

Parameters	**SC (n = 8)**	**SD (n = 8)**	**TD (n = 8)**
SBP (mmHg)	138.1 ± 1.7	171.6 ± 4.1 ***	132.7 ± 3.7†††
DBP (mmHg)	87.8 ± 2.7	102.4 ± 7.8	84.8 ± 5.4
MAP (mmHg)	104.7 ± 5.4	125.1 ± 5.4*	100.7 ± 4.2††
PP (mmHg)	50.1 ± 3.5	68.1 ± 7.7	47.8 ± 4.7
HR (bpm)	361.7 ± 11.3	386.1 ± 24.2	298.4 ± 12††

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; PP = pulse pressure; HR= heart rate; SC = Sedentary Control; SD = Sedentary Diabetic; TD = Trained Diabetic. Values are means ± SEM. To evaluate difference between groups, it was used one-way ANOVA followed by Bonferroni post-test. * $p<0.05$, *** $p<0.01$ *vs* SC,†† $p<0.01$ and ††† $p<0.001$ *vs* SD group.

.

Table 2

Intervals (ms)	**SC (n = 8)**	**SD (n = 8)**	**TD (n = 8)**
PR	39 ± 2.3	42 ± 0.6	35 ± 1.8
QRS	36 ± 1	33 ± 2	33 ± 2
QT	57 ± 2	70 ± 2**	62 ± 3
QTc	4.4 ± 0.1	5.1 ± 0.1***	4.6 ± 0.1††

SC = Sedentary Control; SD = Sedentary Diabetic; TD = Trained Diabetic. Values are means ± SEM. To evaluate difference between groups, it was used one-way ANOVA followed by Bonferroni post-test. **p<0.01, *** p<0.001 *vs* SC and ††p<0.01*vs* SD.

CONCLUSÃO

5 CONCLUSÃO

Este estudo demonstra pela primeira vez, ao nosso conhecimento, que as alterações cardiovasculares envolvidas no diabetes, induzido pela aloxana em ratos, possivelmente envolve comprometimento da sensibilidade barorreflexa e balanço autonômico cardíaco. Tal desbalanço é caracterizado pela retirada vagal a qual produz prolongamento no intervalo QTc. Além disso, demonstra que o exercício resistido é eficaz na melhora do controle glicêmico e reduz os níveis de pressão arterial no diabetes. Também evidencia, pela primeira vez nesse modelo, que as alterações cardiovasculares proporcionadas pelo treinamento físico resistido de intensidade leve no diabetes, possivelmente envolve melhora nos mecanismos de sensibilidade barorreflexa e balanço autonômico cardíaco, o qual caracteriza-se pela predominância parassimpática. Porfim que o treinamento foi capaz de impedir o prolongamento do intervalo QTc.

PERSPECTIVAS

6 PERSPECTIVAS

Investigar se há participação de modulação central autonômica cardíaca sobre a hemodinâmica de ratos diabéticos decorrente do treinamento físico resistido.

Averiguar o envolvimento de estresse oxidativo sobre a melhora da sensibilidade barorreflexa em animais diabéticos submetidos ao treinamento físico resistido.

REFERÊNCIAS

Referências

ALVARENGA, C. Hipertensão arterial na Diabetes *Mellitus* tipo 2, Evidência para a abordagem terapêutica. **Rev. Port. Clin. Geral**, v. 21, p. 597-604, 2005.

AMERICAN COLLEGE OF SPORTS MEDICINE (ACSM). Exercise and diabetes type 2. **Med Sci Sports Exerc**, p. 1345-1360, 2000a.

AMERICAN COLLEGE OF SPORTS MEDICINE (ACSM). **Guidelines for exercise testing and prescription**. 6ª ed. Baltimore: Lippincott Williams & Wilkins, 2000b.

AMERICAN DIABETES ASSOCIATION (ADA). Physical activity/Exercise and Diabetes. **Diabetes Care**, v. 27: S58-S62, 2004.

AMERICAN DIABETES ASSOCIATION (ADA). Diagnosis and Classification of Diabetes Mellitus. **Diabetes Care**, v. 29 (suppl.1), p. 43-48, 2006.

BARCELÓ, A. et al. The cost of diabetes in Latin America and the Caribean. **Bull World Organization Health Organ**, v. 81, n. 1, p. 19-27, 2003.

BARRETO E. O. et al. Thymus involution in alloxan diabetes: analysis of mast cells. **Mem. Inst. Oswaldo Cruz**, v. 100, n. 1, 2005.

BARZILAV J. I. et al. Cardiovascular disease in older adults with glucose disorders: comparisons of American.Diabetes Association of diabetes mellitus with WHO criteria. **Lancet**, v. 354, p. 622-625, 1999.

BATTELL, M. L. et al. Other models of type 1 diabetes. In: Experimental models of diabetes. JH McNeill(Ed). CRC Press. Boca Raton, Florida, p. 219-229, 1999.

BORGES, G. R. et al. Myocardial performance in conscious streptozotocin diabetic rats. **Cardiovascular Diabetology**, v. 5, n. 16, p. 1-8, 2006.

BRAITH, R. W. et al. Neuroendocrine activation in heart failure is modified by endurance exercise training. **J Am Coll Cardiol**, v. 34, p. 1170-5, 1999.

CAMACHO, R. C. et al. Glucoregulation During and After Exercise in Health and Insulin-Dependent Diabetes. **Exercise and Sport Sciences Reviews**, v. 33, n. 1, p. 17-23, 2005.

CASTANEDA, C. et al. A randomized controlled Trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes. **Diabetes Care**, v. 25, p. 2335-2341, 2002.

CASTANEDA, C. Type 2 diabetes mellitus and exercise. **Rev Nutr Clin Care**, v. 3, p. 349-358, 2001.

CEFALU, W. T. “Insulin Resistance: Cellular and Clinical concepts. Overview of Insulin action”. **Exp Biol Med**, v. 226, p. 13-26, 2001.

CIOLAC, E. G., GUIMARÃES G. V. Importância do exercício resistido para o idoso. **Rev Soc Cardiol Est São Paulo**, v. 12, p. 15-26, 2002.

CIOLAC, E. G.; GUIMARÃES G. V. Exercício físico e síndrome metabólica. **Rev Bras Med Esporte**, v. 10, n. 4, 2004.

COSTA, L. P. et al, Treinamento físico melhora a disfunção quimiorreflexa em ratos diabéticos por STZ. **Rev. bras. Educ. Fís. Esp**, v. 18, n. 3, p. 293-301, 2004.

DALL’AGO, P. et al, Baroreflex and chemorreflex dysfunction in STZ-diabetic rats. **Brazilian Journal of Medical and Biological Research**, v. 30, p.119-124, 1997.

De ANGELIS, K. et al. Effects of exercise training on autonomic and myocardial dysfunction in streptozotocin-diabetic rats. **Brazilian Journal of Medical and Biological Research**, v. 33, p. 635-641, 2000.

De ANGELIS, K. et al. Cardiovascular control in experimental diabetes. **Brazilian Journal of Medical and Biological Research**, v. 35, p. 1091-1100, 2002.

De ANGELIS, K.; SANTOS, M. S. B.; IRIGOYEN, M. C.. Sistema nervoso autonômo e doença cardiovascular. **Revista da Sociedade de Cardiologia do Rio Grande do Sul**. Ano XIII, n. 03, Set/Out/Nov/Dez, 2004a.

De ANGELIS, K. et al. Exercise training changes autonomic cardiovascular balance in mice. **J Appl Physiol**, v. 96, p.2174–2178, 2004b.

De FRONZO R. A. Pathogenesis of type 2 diabetes mellitus. **Med Clin North Am**, v. 88, p.787-835, 2004.

DEBUSK, R. F. et al. Cardiovascular responses to dynamic and static effort soon after myocardial infarction: implication to occupational work assessment. **Circulation**, v. 58, p. 368-375, 1978.

DORN J. et al. Correlates of compliance in a randomized exercise trial in myocardial infarction patients. **Med Sci Sports Exerc**, v. 33, p. 1976-8, 2001.

EDELSTEIN S. L. et al. Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. **Diabetes**, v.46, p. 701-10, 1997.

ERIKSSON, J. et al. Resistance training in the treatment of non-insulin-dependent diabetes mellitus. **International Journal of Sports Medicine**, v. 18, p. 242-246, 1997.

FACTOR S. M. et al. Hypertensive-diabetic cardiomyopathy in the rat: an experimental model of human disease. **Am J Pathol**, v. 102, n. 2, p. 219-228, 1981.

FACTOR S. M. et al. Coronary microvascular abnormalities in the hypertensive-diabetic rat. A primary cause of cardiomyopathy? **Am J Pathol**, v. 116, n. 1, p. 9-20, 1984.

FANG, Z. Y.; PRINS, J. B.; MARWICK, T. H. Diabetic Cardiomyophaty: Evidence, Mechanisms, and Therapeutic Implications. **Endocrine Reviews**, v. 25, n. 4, p. 543-567, 2004.

FEINGENBAUM, M.; POLLOCK, M. Prescription of resistance training for health and disease. **Med Sci Sports Exerc**, v. 31, n. 1, p. 38-45, 1999.

FEUVRAY, D. LOPASCHUK, G. D. Controversies on the sensitivy of the diabetic heart ischemic injury: the sensitivity of the diabetic heart to ischemic injury is decreased. **Cardiovasc Res**, v. 34, p. 113-120, 1997.

FLETCHER, G. F. et al. Exercise standards for testing and training: a statement for healthcare professionals from the American Heart Association. **Circulation**, v. 104, p. 1694-1740, 2001.

FREITAS-FOSS M. C.; FOSS M. C.. Diabetic ketoacidosis and hyperosmolar hyperglycemic state. **Medicina,Ribeirão Preto**, v. 36, p. 389-393, 2003.

GIULIANO, D. et al. Oxidative stress and diabetic vascular complications. **Diabetes Care**, v. 19, n. 3, p. 257-267, 1996.

GIUNT, S. et al. Electrocardiographic Left Ventricular Hypertrophy in Type 1 Diabetes **Diabetes Care**, v. 28, n. 9, p. 2255-2257, sept, 2005.

GOBEL, F.L. et al. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. **Circulation**, v. 57, p. 549-556, 1978.

GOODYEAR, L. J.; KAHN, B. B. Exercise, glucose transport, and insulin sensitivity. **Ann Rev Med**, v. 49, p. 235-261, 1998.

GRAVES, E. J.; FRANKLIN, B. A. **Treinamento Resistido na Saúde e Reabilitação**. Rio de Janeiro: Revinter. 2001.

GROSS, J. L. et al. Diabetes Melito: Diagnóstico, Classificação e Avaliação do Controle Glicêmico. **Arq Bras Endocrinol Metab**, v. 46, p. 16-26, 2002.

GROSSMAN, E. et al. Left ventricular mass in diabetes-hypertension. **Arch Intern Med**, v. 152, n. 5, p. 1001-1004, 1992.

GU, K. et al. Diabetes and decline in heart disease mortality in US adults. **JAMA**, v. 281, n. 14, 1999.

HARTHMANN, A. D. et al. Exercise training improves arterial baro-and chemoreflex in control and diabetic rats. **Autonomic Neuroscience: Basic and Clinicial**, v. 133, p. 115-120, 2007.

HENRIKSEN, E. J. Exercise effects of muscle insulin signaling and action. Invited review: Effects of acute exercise training on insulin resistance. **J Apply Physiol**, v. 93, p. 788-796, 2002.

HICKS, K. K. et al. Effects os streptozotocin-induced diabetes on heart rate, blood pressure and cardiac autonomic nervous control. **Journal of the Nervous System**, v. 69, p. 21-30, 1998.

HONKOLA, A. et al. Resistance training improves the metabolic profile in individuals with type 2 diabetes. **Acta Diabetologica**, v. 34, p. 245-248, 1997.

IRIGOYEN, M. C. et al. Exercise Training Improves Baroreflex Sensitivity Associated With Oxidative Stress Reduction in Ovariectomized Rats. **Hypertension**, v. 46, p. 998-1003, 2005.

JENNINGS, G. et al. Antihypertensive and hemodynamic effects of one year's regular exercise. **Journal of Hypertension**, v. 4, p. S659-661, 1986.

JESSEN, N.; GOODYEAR, L. J. Contracting signaling to glucose transport in skeletal muscle. **J Appl Physiol**, v. 99, p. 3330-337, 2005.

KANNEL, W. B.; HJORTLAND, M.; CASTELLI, W. P. Role of diabetes in congestive heart failure: The Framingham study. **Am j Cardiol**, v. 34, n. 1, p. 29-34, 1974.

KAWANO, H. et al. Resistance training in men is associated with increased arterial stiffness and blood pressure but does not adversely affect endothelial function as measured by arterial reactivity to the cold pressor test. **Exp Physiol**, v. 93, n.2, p. 296-302, 2007.

KLEIN, B. E. K. et al. Electrocardiographic Abnormalities in Individuals With Long-Duration Type 1 Diabetes. **Diabetes Care**, v. 28, n.1, Jan. 2005.

KROOK, A. et al. Sending the signal: molecular mechanisms regulating glucose uptake. **Med Sci Sports Exerc**, v. 36, n. 7, p. 1212-1217, 2004.

KULKARNI , J. S. et al. Effects of chronic treatment with cromakalim and glibenclamide in alloxan-induced diabetic rats. **Pharmacological Research**, v. 46, n. 2, 2002.

LaMONTE, M. J.; BLAIR, N. S.; CHURCH, T. S. Physical activity and diabetes prevention. **J Appl Physiol**, v. 99, p. 1205-1213, 2005.

LEE, D. P. et al. Clinical Utility of the Exercise ECG in Patients With Diabetes and Chest Pain. **Chest**, v. 119, p. 1576-1581, 2001.

LENZEN, S. et al. Alloxan derivatives as a tool for the elucidation of the mechanism of the diabetogenic action of alloxan. In: **Lessons from Animal Diabetes,** E SHAFRIR (ed), Birkhauser, Boston, p.113-122, 1996.

LENZEN, S.; PANTEN, U. Alloxan: history and mechanism of action. **Diabetologia**, v. 31, p. 337-342, 1988.

LERARIO, A.C. Avaliação da pressão arterial em diabetes tipo 2. **Rev. Hipertensão**, v. 5, n. 1, 2002.

LEVINE B. et al. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. **N Eng J Med**, v. 323, p. 236-241, 1990.

LIN, Y. D. et al. Autonomic neuropathy precedes cardiovascular dysfunction in rats with diabetes. **European Journal of Clinical Investigation**, v. 38, p. 607-614, 2008.

LOIMAALA, A. et al. Exercise Training Improves Baroreflex Sensitivity inType 2 Diabetes. **Diabetes**, v. 52, jul. 2003.

MAEDA, S. et al. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young man. **Life Sci**, v. 69, p. 1005-1016, 2001.

MACHADO J. L. M. et al. Caracterização de um modelo experimental de neuropatia em ratos diabéticos induzidos pela aloxana**. Acta Cirúrgica Brasileira**, v. 15, n. 2, 2000.

MIHL, C; DASSEN, W. R. M.; KUIPERS, H. Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. **Netherlands Heart Journal**, v. 16, n. 4, p. 129-133, 2008.

MIKI, K. et al. Lumbar sympathetic nerve activity and a hindquarter blood flow during REM sleep in rats. **J. Physiol**, v. 557, p. 261-271, 2004.

MINISTÉRIO DA SAÚDE. Casos de Hospitalização devido as complicações do Diabetes. **Sistema de Informação de Atenção Básica – SIAB**: Situação Saúde de Sergipe, 2007.

MUSI, N. et al. AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. **Diabetes**. v. 50, p. 621-627, 2001.

NASS, A. A. O. et al. QT and QTc dispersion are accurate predictors of cardiac death in newly diagnosed non-insulin dependent diabetes: cohort study. **BMJ**, v. 316, p. 745-746, 1998.

NEGRÃO, C. E.; BARRETTO, A. C. P. **Cardiologia do exercício: do Atleta ao Cardiopata**. 2ª Ed. São Paulo: Manole, 2006. 372p.

NEMOTO, O et al. Left Ventricular Dysfunction and Remodeling in Streptozotocin-Induced Diabetic Rats. **Circ J**, v. 70, p. 327-334, 2006.

NOTKINS A. L. The causes of diabetes. **Sci Am**, p. 241-62, 1979.

NYGREN, A. et al. Propagation of the cardiac impulse in the diabetic rat heart: reduced conduction reserve. **J Physiol**, v. 580, n. 2, p. 543-560, 2007.

OLIVEIRA, V. L. L. et al. Cardiopulmonary Reflex Impairment in Experimental Diabetes in Rats. **Hypertension**, part II, p. 813-817, out. 1999.

PAN, R. X. et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. **Diabetes Care**, v. 20, n. 4, p. 537-544, 1997.

PASSOS, V. M. A.; BARRETO, S. M.; DINIZ, L. M. Diabetes tipo 2: prevalência e fatores associados em uma comunidade brasileira: Projeto Bambuí de estudo de saúde e envelhecimento. **São Paulo Med. J**, v. 123, n. 2, p. 66-71, 2005.

POORNIMA, I. G.; PARIKH, P.; SHANNON, P. Diabetic cardiomyopathy: The search for unifying hypothesis. **Circulation**, v. 98, p.596-605, 2006.

POLITO, M. D.; FARINATTI, P. T. V. Respostas de freqüência cardíaca, pressão arterial e duplo-produto ao exercício contra-resistência: uma revisão de literatura. **Rev Port de Cien Desp**, v.3, n. 1, p. 79-91, 2003.

POLLOCK, M. et al. Exercise training and prescription for the elderly. **South Med J**, v.87, p. S88-95. 1994.

POLLOCK, M. L. et al. Resistance exercise in individuals with and without cardiovascular disease: benefits, rationale, safety, and prescription: an advisory from the committee on exercise, rehabilitation, and prevention, council on clinical cardiology, American Heart Association. **Circulation**, v. 101, p. 828-33, 2000.

POWERS, A. L. "Diabetes Mellitus". In: Braunwald, E. et al. **Harrison'sPrinciples of Internal Medicine**. Nova York McGraw Hill Companies, Inc. p. 2109-2138, 2001.

PYE, M. P.; COBBE, S. M. Mecahnism of bentricular arrhythmias in cardiac falirue and hyperthrophy. **Cardiovasc Res**, v. 26, p. 740-750, 1992.

ROITH, D. L.; ZICK, Y. "Recent advances in our understanding os insulin action and insulin resistance". **Diabetes Care**, v. 24, p. 588-597, 2001.

RODGERS, R. L. Depressor effect of diabetes in the spontaneously hypertensive rat: associated changes in heart performance. **Can J Physiol Pharmacol**, v. 64, n. 9, p. 1177-1184, 1986.

RODRIGUES, B. et al. Streptozotocin-induced diabetes: Induction, mechanism(s), and dose dependency. In: **Experimental models of diabetes**. JH McNeill(Ed). CRC Press. Boca Raton, Florida, p.3-17, 1999.

RODRIGUES, B.; McNEILL, J. H. Cardiac function in spontaneously hypertensive diabetic rats. **Am J Physiol**, v. 251, n. 3, p.571-580, 1986.

ROSSING, P. et al. Predictors of mortality in insulin dependent diabetes: 10 year observational follow up study. **BMJ**, v. 313, n. 7060, p. 779-784, 1996.

RUSSELL, C. et al. Hypertension control: results from the Diabetes Care Program of Nova Scotia registry and impact of changing clinical practice guidelines. **Cardiovascular Diabetology**, v. 4, n. 11, 2005.

RYDER, J. W. et al. Intracellular mechanisms underlying increases in glucose uptake in response to insulin or exercise in skeletal muscle. **Acta Physiol Scand**, v. 171, p. 249-257, 2001.

SAKAMOTO, K.; GOODYEAR, L. J. Exercise Effects on Muscle Insulin Signaling and Action. Invited Review: Intracellular signaling in contracting skeletal muscle. **J Appl Physiol.** v.93, p. 369–383, 2002.

SANCHEZ, O. A.; LEON, A. S. **Treinamento Resistido para Pacientes com Diabetes *Mellitus***. In: James Graves, Barry Franklin. Treinamento Resistido na Saúde e Reabilitação. Editora Revinter: Rio de Janeiro, p.297-320, 2006.

SASAKI J. E.; SANTOS M. J. O Papel do Exercício Aeróbico sobre a Função Endotelial e sobre os Fatores de Risco Cardiovasculares. **Arq Bras Cardiol**, v. 87, p. 227-233, 2006.

SCHAAN, B. D.; HARZHEIM, E.; GUS, I. Cardiac risk profile in diabetes mellitus and impaired fasting glucose**. Rev Saúde Pública**, v. 38, n. 4, p. 529-536, 2004.

SKYLER J. S. Diabetes Mellitus: pathogenesis and treatment strategies. **J Med Chem**, v. 47, p. 4113-7, 2004.

SOCIEDADE BRASILEIRA DE DIABETES (SBD). Diretrizes da Sociedade Brasileira de Diabetes para o tratamento e acompanhamento do Diabetes Mellitus, 2007.

SURWIT, R. S.; WILLIAMS, P. G. Animal models provide insight into psychosomatic factors in diabetes. **Psycosom Med**, v. 58, p. 582-589, 1996.

SZKUDELSKI, T ,et al. Alloxan in vivo does not exert deleterious effects on pancreatic B cells. **Physiol. Res**, v. 47, p. 343-346, 1998.

SZKUDELSKI, T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. **Physiol. Res**, v. 50, p. 536-546, 2001.

TANCREDE, G.; ROUSSEAU-MIGNERON, S.; NADEAU, A. Benefical effects of physical training inrats with a mild streptozotin-induced diabetes mellitus. **Diabetes**, v.31, p. 406-409, 1982.

TIPTON, C. M. Exercise training and hypertension, an update. **Exercise and Sport Sciences Reviews**, v. 4, p. 447-505, 1991.

TOMASELLI, G. F. et al. Sudden cardiac death in heart failure: the role of abnormal repolarization. **Circulation**, v. 90, p. 2534-2539, 1994.

TORQUATO, M. T. C. G. et al. Prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30-69 years. **Diabetes Care**, v. 15, n. 11, p. 1509-1516, 1992.

TOSCANO, C. M. As campanhas nacionais para detecção das doenças crônicas não-transmissíveis: diabetes e hipertensão arterial. **Rev. Ciênc. Saúde Coletiva**, v. 9, n. 4, 2004.

TUOMILEHTO J. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. **N Engl J Med**, v. 344, p. 1343-1350, 2001.

USTINOVA, E. E. et al. Oxidative Stress impairs cardiac chemorreflexes in diabetic rats. **Am J Physiol Heart Circ Physiol**, v. 279, p. 2176-2187, 2000.

VADLAMUDI, R. V. S. V.; McNEILL, J. H. Effect of experimental diabetes on rat cardiac cyclic AMP phosphodiesetrase and inotropy. **Am J Physiol**, v. 244, p. 844–851,1983.

VENCO, A. et al. Echocardiographic features of hypertensive-diabetic heart muscle disease. **Cardiology**, v. 74, n. 1, p. 28-34, 1987.

VINIK, A. I. et al. Diabetic Autonomic Neuropathy. **Diabetes Care**, v. 26, n. 5, p. 1553-1579, 2003.

VINIK, A. I.; ZIEGLER, D. Diabetic Cardiovascular Autonomic Neuropathy. **Circulation,** v. 115, p. 387-397, 2007.

WALLBERG-HENRIKSSON, H.; RINCON, J.; ZIERATH, J. R. Exercise in the management of non-insulin-dependent diabetes mellitus. **Sports Med**, v. 25, n. 1, p. 25-35, 1998.

WICHI, R et al. Noninvasive and invasive evaluation of cardiac dysfunction in experimental diabetes in rodents. **Cardiovascular Diabetology.** v. 6, n. 14, 2007.

WILD, S.; ROGLIC, G.; GREEN, A.; SICREE, R.; KING, H. Global Prevalence of Diabetes. **Diabetes Care,** v. 27, p. 1047-1053, 2004.

WORLD HEALTH ORGANIZATION. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes *mellitus*. **Report of a WHO Consultation,** Geneva, 1999.

ANEXO A

UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado "Avaliação dos efeitos do treinamento físico aeróbio e resistido sobre os parâmetros hemodinâmicos, bioquímicos e eletrocardiográficos em ratos diabéticos", sob coordenação do Prof. Dr. Márcio Roberto Viana Santos, foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 13/03/2009.

São Cristóvão, 14 de março de 2009

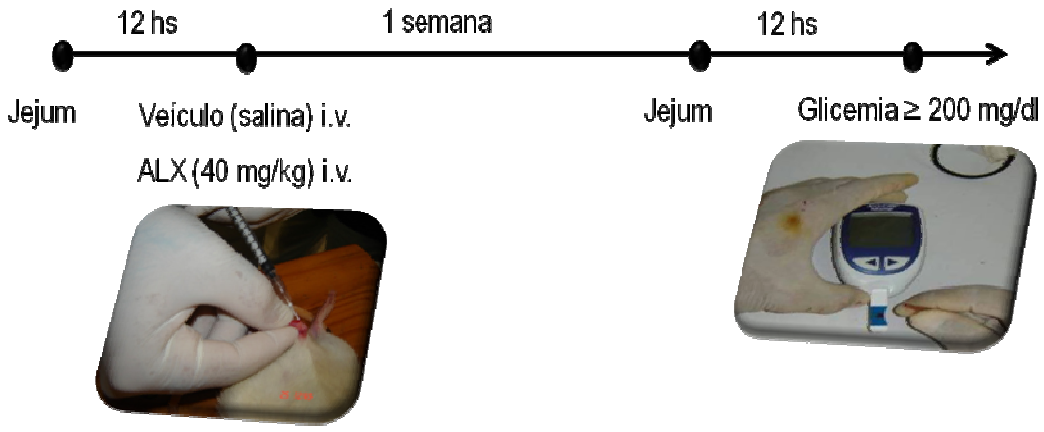
Profª. Drª. Flavia Teixeira Silva
Presidente do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
49100-000
Fones: 2105 6661/6606

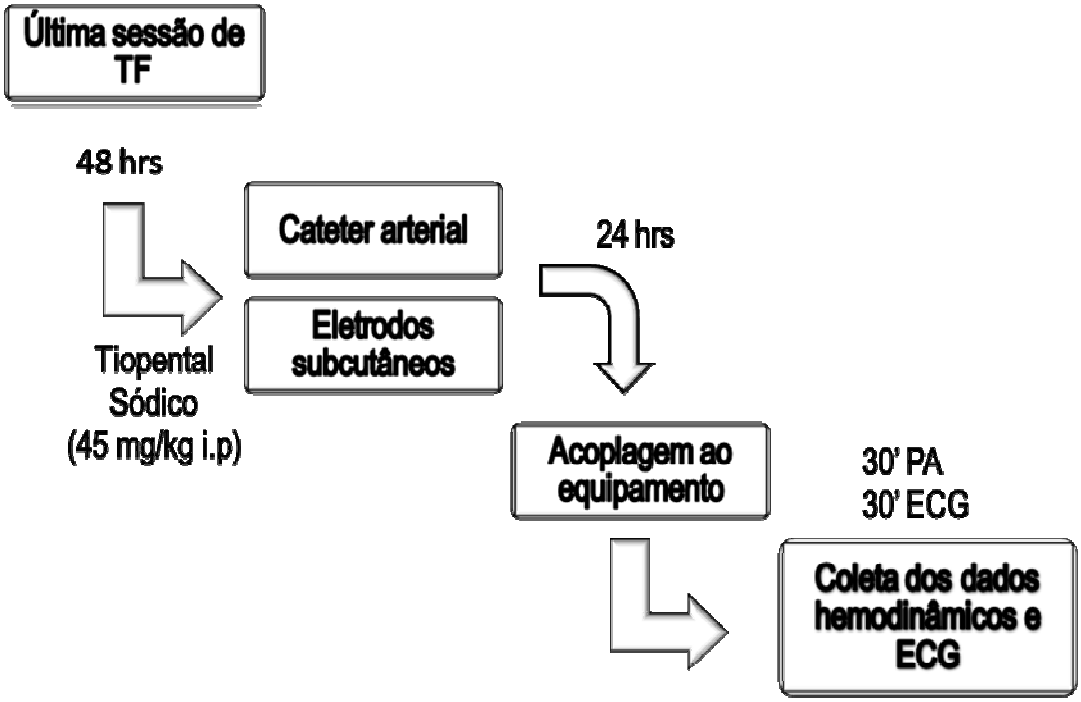
ANEXO B

CRONOLOGIA DOS EXPERIMENTOS

Indução do diabetes:



Coleta dos dados hemodinâmicos:



ANEXO C

Instructions to Authors

Online Manuscript Submission

Hypertension publishes scientific investigation of the highest quality in the broad field of blood pressure regulation and pathophysiology, clinical treatment, and prevention of hypertension. The editors encourage submission of original articles that deal with basic, clinical, and population studies of hypertension and related fields such as nephrology, endocrinology, neuroscience, vascular biology, physiology, pharmacology, cellular and molecular biology, and genetics.

Submitted manuscripts must not contain material previously published, except as an abstract, and must not be under consideration for publication elsewhere, in whole or in part. Manuscripts should conform to "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (*N Engl J Med.* 1991; 324:424-428). Manuscripts are examined by the editors and are usually sent to expert reviewers. Decisions will generally be communicated within 3 weeks after receipt of the manuscript. Acceptance is based on originality, scientific excellence, and topical balance of the journal.

Address new and revised manuscripts and correspondence to the editorial office:

John E. Hall, PhD
Hypertension Editor-in-Chief
University of Mississippi Medical Center
2500 N. State St.
Jackson, MS 39216-4505
Phone: 601-815-1667
Fax: 601-815-1675
Email: hypertension@physiology.umsmed.edu
Online Submission: http://submit-hyper.ahajournals.org

Editorial Conflict-of-Interest Policy. Original manuscripts authored or coauthored by the Editor in Chief and/or any of the Associate Editors are handled by either a Consulting Editor or an Associate Editor at a different institution, who makes all decisions about the manuscript, (including choice of referees and ultimate acceptance or rejection). The entire process is handled confidentially. All manuscripts submitted from the Editor's home institutions are also handled entirely by either a Consulting Editor or an Associate Editor at a different institution. The Editor in Chief and/or Associate Editors may additionally, from time to time, refer a manuscript to a Consulting Editor to avoid a perceived or real conflict of interest. AHA Scientific Publishing Ethical Conduct Policy

Journal Categories/Article Types	Revised Manuscripts	Costs to Authors
Online Submissions	Tips for Submitting Online	Policies

General Instructions for Preparing Manuscripts	Guidelines for Digital Images	Forms
Guidelines for Clinical Trials	How to Format Digital Images	Permission to Reprint
New Manuscripts	Online/Data Supplements	Author Rights & Permissions

Journal Categories

Original Scientific Communications. These are regular (original manuscripts) scientific contributions. Manuscripts should **not exceed 6000 words** including title page, abstract, references, legends, tables, and figures. (Please note that a single bar graph is approximately 150 words, and table with 3 columns and 10 rows is approximately 100 words.) In exceptional circumstances, the editors may consider manuscripts longer than 6000 words when design complexity or research requires a greater length. For preparation, see "General Instructions."

Brief Reviews. Brief Reviews are generally invited by the Editors and are definitive summaries of areas that are important to research in hypertension and related areas. Reviews should briefly, but comprehensively, review the literature on a specified topic. The topic should be timely and sufficiently focused to allow a comprehensive review **without exceeding 6000 words**, including title page, abstract, references, legends, and tables, and figures. (Please note that a single bar graph is approximately 150 words, and table with 3 columns and 10 rows is approximately 100 words.) In special circumstances, reviews may exceed these page limits and specific guidelines will be provided at the time that the review is invited. The author should discuss controversies that have been resolved or that remain, the future of the field both technically and conceptually, and define major unresolved questions. All Brief Reviews, whether invited or not, will undergo peer review.

Hypertension Grand Rounds. These articles are invited reviews that take a case-based approach, including topics related to diagnosis, prevention, and treatment of hypertension. The case presentation will be followed by a discussion of scientific and practical clinical issues related to the case. Where applicable, a discussion of future research necessary for resolving unanswered questions will be included. The topic should be timely and sufficiently focused to allow a comprehensive review **without exceeding 6000 words**, including title page, case presentation, references, legends, tables, and figures . (Please note that a single bar graph is approximately 150 words, and table with

3 columns and 10 rows is approximately 100 words.) In special circumstances, reviews may exceed these page limits and specific guidelines will be provided at the time that the review is invited. Hypertension Grand Rounds, whether invited or not, will undergo peer review.

Hypertension Highlights. These articles are intended to highlight, provide further perspective, and enhance the overall significance of recent studies published in *Hypertension* that contribute to our understanding of hypertension and related areas. Hypertension Highlights should be **approximately 3000 words** and review progress that has occurred during the past 2-3 years in a focused area of hypertension research. Figures that effectively illustrate and summarize key points are encouraged. References should generally be restricted to those published in *Hypertension* during the last 2-3 years.

Editorial Commentaries. These articles are invited brief commentaries on articles that appear in *Hypertension* or on topics of particular importance to the readers of the journal. They should be approximately **1500 words**, describe controversial issues, and review questions that remain to be addressed. Use of a figure to illustrate key points is encouraged. References are limited to 10 and generally should be restricted to recent years. Editorial Commentaries are generally invited by the editors, but whether invited or not, they will undergo peer review.

Tutorials. These are similar to Brief Reviews but are presented at a level commensurate with their use as a teaching tool. Schematic diagrams and figures are encouraged wherever possible to illustrate important scientific or technical points. Elements of Editorial Commentaries can also be integrated into the tutorial at the author's discretion. The topic should be timely and sufficiently focused to allow a comprehensive review **without exceeding 6000 words**, including title page, abstract, references, legends, and tables, and figures. (Please note that a single bar graph is approximately 150 words, and table with 3 columns and 10 rows is approximately 100 words.) In special circumstances, Tutorials may exceed these page limits and specific guidelines will be provided at the time that the review is invited. The author should discuss controversies that have been resolved or that remain, the future of the field both technically and conceptually, and define major unresolved questions. Tutorials, whether invited or not, will undergo peer review.

Letters to the Editor. Letters to the Editor express views about articles published in *Hypertension*. Letters should be no longer than **500 words** and should relate to an article published in *Hypertension,* within the preceding three months. References are limited to 5. Authors of the article cited in the letter will be invited to reply, as appropriate, and the reply must be signed by all authors of the original publication.

Preliminary or circumscribed novel findings of unusual interest may also be submitted as a Letter to the Editor and should be no longer than **1000 words**. Authors must double-space text and references, provide a brief title, and obtain signatures from all authors on a copyright transfer agreement for all Letters to the Editor. Letters and replies are reviewed by the Editors and may be edited, and they will appear online only.

Manuscript Submissions

Online Submissions: A formal online submission module is available and is the preferred mode of submission to *Hypertension*. To submit your **original or revised** manuscript online, please go to http://submit-hyper.ahajournals.org and follow the detailed instructions located on our submission website. If you have any questions about the online submission process, please feel free to contact the Editorial Office at hypertension@physiology.umsmed.edu . If you do not receive confirmation of submission within three days, please contact the Editorial Office. The following items should be uploaded at the time of submission:

1. A cover letter that includes a statement of submission: "All authors have read and approved the submission of the manuscript; the manuscript has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract." The cover letter may include the names of six to eight potential reviewers, with address, email address, and fax number, who are experts in the area of research and who do not have a conflict of interest. It is especially helpful to suggest three to four members of the Editorial Board.
2. Authorship Responsibility and Copyright Transfer Agreement
3. Acknowledgment release signatures, if applicable
4. One copy of any potentially overlapping work that is in preparation, has been previously submitted or published, or is in-press, if applicable
5. One copy of any article currently in-press, which is cited in the References, if applicable
6. One copy of any abstracts published or submitted for publication, if applicable
7. All sources of support must be cited
8. All potential conflicts of interest related to the manuscript must be stated Conflict of Interest Disclosure Questionnaire

If you are unable to upload the items above, please fax them to the *Hypertension* Editorial Office within 48 hours after completion of online manuscript submission.

General Instructions for New Submissions

- Manuscripts must be typed, double-spaced using a 12-point font, including references, figure legends, and tables, on one side of the page only.
- Leave 1-inch margins on all sides. Do not use proportional spacing or justified margins.
- Number every page except the title page, including figures, tables, and references. Cite each figure and table in text in numerical order.
- Assemble manuscripts in this order:

1. Title page
2. Abstract
3. Text, including Introduction, Methods, Results, Discussion, and Perspectives
4. Acknowledgments
5. Source(s) of Funding
6. Conflict(s) of Interest/Disclosure(s)
7. References
8. Figure Legends
9. Tables

10. Figures

- Cite each reference in text in numerical order and list in the References section. In-text reference numbers may be repeated but not omitted.
- Use SI units of measure in all manuscripts. For example, molar (M) should be changed to mol/L; mg/dL to mmol/L; and cm to mm. Units of measure previously reported as percentages (ie, hematocrit) are expressed as a decimal fraction. Measurements currently not converted to SI units in biomedical applications are blood and oxygen pressures, enzyme activity, H+ concentration, temperature, and volume. The SI unit should be used in text, followed by the conventionally used measurement in parentheses.
- For style, consult the < ed 9th Style, of Manual Association Medical American>, Baltimore, MD, Williams & Wilkins, 1998. (NOTE: The use of et al. in the author listing of references is not allowed.)
- Please provide sex-specific and/or racial/ethnic-specific data, when appropriate, in describing outcomes of epidemiologic analyses or clinical trials; or specifically state that no sex-based or racial/ethnic-based differences were present. See the Uniform Requirements for more details.
- Consult current issues of *Hypertension* for examples of format.

Guidelines for Clinical Trials

- In accordance with the Clinical Trial Registration Statement from the International Committee of Medical Journal Editors (ICMJE) (*Circulation*. 2005;111:1337-1338.), all clinical trials submitted for publication in Hypertension must be registered in a public trials registry at or before the onset of participant enrollment. This requirement applies to all clinical trials that begin enrollment after July 1, 2005.
- Research is considered to be a clinical trial if it involves prospective assignment of human subjects to an intervention or comparison group to study the relation between a medical intervention and a health outcome. Studies that are designed for other purposes, such as to study pharmacokinetics or major toxicity studies (e.g., phase 1 trials) are exempt.
- The registry must be accessible to the public at no charge, searchable, open to all prospective registrants, and managed by a not-for-profit organization. The registry must include the following information: a unique identifying number, a statement of the intervention(s), study hypothesis, definition of primary and secondary outcome measurements, eligibility criteria, target number of subjects, funding source, contact information for the principal investigator, and key dates (registration date, start date, and completion date). The registries listed below are approved by the ICMJE:

1. United States National Library of Medicine
2. International Standard Randomized Controlled Trial Number (ISRCTN)
3. University Hospital Medical Information Network (UMIN)
4. Australian Clinical Trials Registry (ACTR)
5. Netherlands Trial Register

Clinical trials maybe listed with Other registries, but these registries must meet the above-mentioned requirements.

- The authors will be requested to provide the exact URL and unique identification number for the trial registration at the time of submission. This information will be published in a footnote on the first page of the article.

- Clinical trial reports should also comply with the Consolidated Standards of Reporting Trials (CONSORT) and include a flow diagram presenting the enrollment, intervention allocation, follow-up, and data analysis with number of subjects for each. Please also refer specifically to the CONSORT Checklist of items to include when reporting a randomized clinical trial.

General Instructions for Revised Manuscripts

- In the top right-hand corner, indicate the manuscript number followed by R1 to denote a first revision.
- In your written response to the reviewers' comments, give the page number(s), paragraph(s), and line number(s) where each revision was made.
- Revisions not received within three months will be administratively withdrawn. For further consideration the manuscript must be resubmitted de novo. At the editors' discretion, and in cases where substantial new data are required, extensions may be granted for revisions. In such cases, every effort will be made to retain the original reviewers.
- Respond to each referee's comments, indicating precisely the changes made in response to the critiques. Also give reasons for suggested changes that were not implemented, and identify additional changes made.

General Instructions for Preparing a Manuscript

5. **Title Page (Page 1, but do not number)**
   - Full title of manuscript, in capital letters, limited to 120 characters total.
   - Authors' full names and affiliations
   - A short title (total characters must not exceed 50, including spaces) to be typeset at the top of the journal page
   - Word count of manuscript, including references, figures, legends, word count of abstract, and total number of figures
   - The full name, title, and complete address for corresponding author, including street and post office box as well as telephone and fax numbers, and email address
6. **Abstract**
   - Maximum abstract length is 250 words
   - Do not use acronyms or abbreviations
   - Do not use subheadings
   - Do not cite references
   - The abstract should include the rationale for the study, a brief description of methods and presentation of significant results, and a succinct interpretation of the data.
   - Provide five to seven key words for your manuscript, using Index Medicus as a guide
7. **Text**

Abbreviations. Abbreviations should be defined at the first mention in the text.

Methods section. The methods section should provide sufficient detail for the experiments to be reproduced.

- **Materials and Data Availability:** To allow others to replicate and build on work published in Hypertension, authors should make materials, data, and associated protocols available to readers or list the primary source of materials. Authors must

disclose upon submission of the manuscript any restrictions on the availability of materials or information.

Authors should make Unique Materials (e.g., cloned DNAs; antibodies; bacterial or animal cells; viruses; and computer programs) promptly available on request by qualified researchers for their own use. It is reasonable for authors to charge a modest amount to cover the cost of preparing and shipping the requested material and some materials may require a Materials Transfer Agreement between institutions.

- **Studies in Experimental Animals:** Indicate that the study was approved by an institutional review committee. All studies in animals should be conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, or the equivalent. The species, strain, number used, and other relevant characteristics of the animals should be stated. When describing surgical procedures, identify the pre-anesthetic and anesthetic agents used and state the amount or concentration and the route and frequency of administration for each. The use of paralytic agents, such as curare or succinylcholine, is not an acceptable substitute for anesthetics. For other invasive procedures, report the analgesic or tranquilizing drugs used. If none was used, provide justification for such exclusion. Generic names of drugs must be given.
- **Studies in Humans:** Indicate that the study was approved by an institutional review committee and that the subjects gave informed consent. All studies that involve the use of humans must adhere to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, Revised November 13, 2001, effective December 13, 2001. Describe the characteristics of human subjects or patients and indicate that the procedures followed were in accordance with institutional guidelines.
- **Blood Pressure Methods for Human Studies:** Blood pressure measurement methods should be described in detail in the text or by reference. Information should include number of readings, instrument type(s), cuff size(s), arm position, posture, and observer training.
- **Genomic and Proteomic Studies:** All papers reporting gene expression profiling data must comply with the Minimum Information About Microarray Data Experiments (MIAME) standard. Authors of papers that include genomic, proteomic, or other high-throughput data are required to make their data easily accessible for the reviewers and the editors during the review process. You may submit your data to the NCBI gene expression and hybridization array data repository (GEO) and provide the GEO accession number, or you may provide a link to a secure or publicly accessible website which hosts the data. Prior to publication, the data must be submitted and an accession number obtained. Access to the information in the database must be available at the time of publication. GEO has a web-based submission route, suitable for a small number of samples, or a batch submission tool (called SOFT). Submission FAQ.
- **Guidelines for Protein and Nucleic Acid Sequences:** Newly reported nucleotide or protein sequences must be deposited in GenBank or EMBL databases, and an accession number must be obtained. Access to the information in the database must be available at the time of publication. Authors are responsible for arranging release of data at the time of publication. The authors must also provide a statement in the manuscript that this sequence has been scanned against the database and all

sequences with significant relatedness to the new sequence identified (and their accession numbers included in the text of the manuscript).

1. GenBank

   GenBank Submissions, National Center for Biotechnology Information, 8600 Rockville Pike, Building 38A, Room 8N-805, Bethesda, MD 20894, Tel: (301) 496-2475

2. EMBL Nucleotide Sequence Submissions

   European Bioinformatics Institute, Hinxton Hall, Hinxton, Cambridge CB10 1SD, UK, Tel.: 44-1223-494401; Fax: 44-1223-494472; e-mail: support@ebi.ac.uk

3. DNA Data Bank of Japan

   Center for Information Biology, National Institute of Genetics, Mishima, Shizuoka, 411, Japan, Tel.: 81-559-81-6853; Fax: 81-559-81-6849

- Submission to any data bank is sufficient to ensure entry in all.
- **Guidelines for Human Phenotype-Genotype Association or Linkage Studies:**

A. Reporting issues.
1. Report process for selecting genes and SNPs.
2. Report Hardy-Weinberg statistics or p-values and method of calculating same.
3. Refer to existing public domain websites for the Human Gene Ontology name and the rs number for SNPs.

- http://www.gene.ucl.ac.uk/nomenclature
- http://www.ncbi.nlm.nih.gov/projects/SNP
- http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Snp

4. Describe genotyping methods. If numerous primers have been used, please include them in an online supplement.

B. False positive and false negative concerns. Given well-described problems with both false positive and false negative associations, phenotype-genotype association studies should meet some or all of the criteria below:

1. Phenotype is clearly defined, is heritable, and if a quantitative phenotype is reported, reproducibility data are provided or referenced.
2. The sample size is adequate to detect a SNP or haplotype with a modest effect. For genotype-trait associations, provide an estimate of the effect size that could be detected with power 0.80 or higher with the allele frequency and sample size reported.
3. Since multiple statistical testing methods are frequently used in genotyping-phenotyping studies, please include specifics of the primary model(s) tested. Non-essential secondary models may be published as electronic data supplements. Clinically relevant confounders should be included in multivariable models or residuals.

C. Review criteria for human linkage studies. Manuscripts should include the following:

1. Identifying plausible candidate genes under the linkage peak.
2. Follow-up fine mapping to narrow the region of linkage, and/or genotyping some of the candidate genes under the linkage peak.
3. Replication data from another sample.

Guidelines for Studies on Diagnostic Tests: For information regarding the Standards for Reporting of Diagnostic Accuracy (STARD) go to *Clinical Chemistry*.2003;49:7-18. or *Ann of Intern Med.* 2003;138:W1-W12.

Drugs and Reagents: Give generic rather than trademark names of drugs. The generic chemical identification of all investigational drugs must be provided. The complete name and location of the manufacturer must be supplied for all reagents, equipment, and devices used in the Methods.

Statistics: A subsection on statistics should be included in the Methods section and the measures of variance, such as standard deviation or standard error, should be indicated.

Methods should be limited to essential new information. To save space for the authors and the journal, if methods have been previously published, the author may refer to that paper and submit copies of that paper as reference material.

The following information should be included as an **Online Data Supplement**:

For animals used in experiments, state the species, strain, number used, and other pertinent descriptive characteristics.

For human subjects or patients, describe their characteristics.

When describing surgical procedures on animals, identify the pre-anesthetic and anesthetic agents used and state the amount or concentration and the route and frequency of administration for each. The use of paralytic agents, such as curare or succinylcholine, is not an acceptable substitute for anesthetics. For other invasive procedures on animals, report the analgesic or tranquilizing drugs used. If none was used, provide justification for such exclusion. Generic names of drugs must be given.

Manuscripts that describe studies on humans must indicate that the study was approved by an institutional review committee and that the subjects gave informed consent. Reports of studies on both animals and humans must indicate that the procedures followed were in accordance with institutional guidelines. To save space for the authors and the journal, if methods have been previously published, the author may refer to that paper and submit copies of that paper as reference material.

Discussion. This section should not be used to restate the results but rather to illuminate and place into perspective the results. Excessive discussion and reiteration of points that are obvious from the results are discouraged.

"Perspectives". Authors should include a brief (<250) "Perspectives" section at the end of the Discussion Section. The "Perspectives" section should be clearly labeled with a separate heading. The purpose of "Perspectives" is to indicate the broad implications of the study, and to permit reasonable speculation on the overall importance and future directions of the work. Such perspectives should not replace the conclusions drawn from the study and should be limited to one paragraph. This section should, however, replace the "In summary..." paragraph that is often placed at the end of the discussion.

8. **Acknowledgments**

The Acknowledgments section lists substantive contributions of individuals. The Editorial Office must receive written, signed consent from each person recognized in the Acknowledgments to be mentioned in the article, because acknowledgment can imply endorsement of data and conclusions.

9. **Sources of Funding**
   Authors **must** list all sources of support for research in this section.
10. **Conflict(s) of Interest/Disclosure(s) Statement**
    Authors **must** disclose any and all relationships that could be perceived as real or apparent conflict(s) of interest as a **FOOTNOTE** after the Sources of Funding section. Conflict-of-interest/disclosure will be published as a footnote to the accepted article. This pertains to relationships with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of the article. Such relationships include, but are not limited to, employment by an industrial concern, ownership of stock, membership on a standing advisory council or committee, being on the board of directors, or being publicly associated with the company or its products. Other areas of real or perceived conflict of interest related to the subject of the article could include receiving honoraria or consulting fees or receiving grants or funds from such corporations or individuals representing such corporations.

    If no author has anything to disclose, please list "None".

11. **References**
    References must conform to the journal's style -- consult the American Medical Association Manual of Style, 9th ed, Baltimore, MD, Williams & Wilkins, 1998.(NOTE: All authors must be listed in the author listing.)
    Accuracy of reference data is the author's responsibility. Verify all entries against original sources, especially journal titles, inclusive page numbers, publication dates, accents, diacritical marks, and spelling in languages other than English.
    All references must be double-spaced.
    All authors must be listed in references. Shortened lists of author names followed by "et al" must be replaced with complete information.
    Cite references in numerical order according to first mention in text.
    Personal communications, unpublished observations, and submitted manuscripts must be cited in the text as "(authors' full names, unpublished data, year)."
    Abstracts may be cited only if they are the sole source and must be identified in the reference as "Abstract."
    "In press" citations must have been accepted for publication and the name of the journal or book publisher included.
12. **Tables**
    Each table must begin on a separate page, double-spaced. The table number must be in Arabic numerals followed by a period and a brief informative title.
    Use same size type as in text.
    Supply a brief heading for each column.
    Indicate footnotes in tables by symbols in this order: *, †, ‡, §, ||, ¶, #, **.
    Do not use vertical lines in tables. Use horizontal lines above and below the column headings and at the bottom of the table only. Use extra space to delineate sections within the table.
    Do not duplicate data in figures and tables.
    Define acronyms and abbreviations in a separate listing.

(Please note that a table with 3 columns and 10 rows is approximately 100 words.)

13. **Figures and Legends**

Figures may be black and white line drawings, graphs, color illustrations, or halftones (gel blots/stains).

Authors are responsible for the cost of printing color illustrations.

Flaws will not be corrected.

Figure parts should be clearly labeled. Letters, symbols, arrows, etc must be uniform in size and style within each figure, and when possible between figures. We recommend that you use either 12 pt Arial Bold font or 14 pt Times New Roman Bold font.

Avoid headings on the figure when possible. Heading information should appear in the figure legend.

Line art should not contain very thin lines, which are hard to reproduce.

Supply a scale bar with photomicrographs.

Provide figure legends on a separate page, double-spaced.

If there are abbreviations or symbols in the figures, they must be defined in the figure or the figure legend.

Limit white space between all panels and between panels and panel labels.

Electronic source files of figures may be submitted in a pdf format for initial submission of a manuscript. Electronic source files of figures for subsequent submissions must follow the guidelines set by the publishers.

Guidelines for digital images

The use of digital media for image acquisition and processing introduces the potential for inadvertent distortion of data. To prevent such distortion, the following guiding principles should be used:

0. Data should neither be added to, nor removed from, an image by digital manipulation. Images gathered at different times or from different locations should not be combined into a single image, unless it is stated that the resultant image is a product of time-averaged data or a time-lapse sequence. Figures assembled from multiple images must indicate the separation of the parts by lines and described in the legend.
1. The use of touch-up tools, such as cloning and healing tools in Photoshop, or any feature that deliberately obscures manipulations, is unacceptable.
2. Linear adjustment of contrast, brightness or color must be applied equally to controls and all parts of an entire image. Contrast should not be adjusted so that data disappear. Excessive manipulations, such as processing to emphasize one region in the image at the expense of others (e.g. through the use of a biased choice of threshold settings), is unacceptable, as is emphasizing experimental data relative to the control.
3. When submitting revised final figures upon conditional acceptance, authors may be asked to submit original, unprocessed images.
4. All image acquisition tools and image processing software packages used should be listed. Deviations from the above, including nonlinear adjustments, must be indicated in the figure legend along with a description of the processing software used.

How to format digital images is available at **Instructions on Formatting Digital Images**

If you are unable to size your digital art appropriately or provide the correct resolution, we encourage you to send a **PDF** created from the original source file of the figure (i.e. In the program that created the figure, 1) go to your print menu, 2) change printer to Adobe PDF, and 3) save file as the MS #-Fig 1, etc.).

(Please note that a single bar graph is approximately 150 words.)

14. **Cover Figures**

The editors encourage submission of color images for consideration as potential cover figures. These may be uploaded along with a figure legend as a supplemental file when a revised manuscript is submitted. Please make sure to clearly label this as a cover figure.

Cover figures should illustrate a major finding or concept and be associated with the general topic of the article, or they may be altered/enhanced versions of an original figure within the manuscript.

A photograph or diagram is appropriate, but complex flow charts are not suitable.

A very brief caption should be included.

Cover figure submissions must follow the same guidelines as original figure submissions attached to manuscripts (see "Figures and Legends").

15. **Online Supplements**

This optional section provides an opportunity for authors to present supporting materials to the manuscript. The manuscript appears both in the print version and online, whereas Online Supplements are independent from the manuscript and appear only online. Online Supplements undergo peer review and therefore must be submitted simultaneously with original submissions.

Online Supplements may consist of any of the following, in any combination: the expanded materials and methods; additional figures and supporting information; additional tables and supporting information; and, video files.

The guidelines below should be used for online supplements:

Material to be published as an online only supplement should be uploaded online as either a word doc or pdf, if possible. An exception to this would be if the online supplement is a video file.

The online supplement should have a title page with the label of ONLINE SUPPLEMENT above the title.

The online supplement should be single-spaced.

If citations are made in the Online Supplement, the Online Supplement must contain its own independent Reference Section with references numbered sequentially, beginning with reference 1, even if some of these references duplicate those in the print version.

Number supplementary figures and tables as S1, S2, etc.

Place the supplemental figure legend underneath the corresponding figure.

When referring to online-only material in the print version of the manuscript, use the phrase "please see http://hyper.ahajournals.org."

Data Supplements appear only online and will not appear in reprints of the article. The Editorial Office is not responsible for converting files to a suitable format.

The print version of the Table of Contents of *Hypertension* highlights articles that contain Online Supplements by having "Data Supplement Online" typed in a box underneath the author listing.

Compliance With NIH and Other Research Funding Agency Accessibility Requirements

Several research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. Within medical research, 3 funding agencies in particular have announced such policies:

The US National Institutes of Health (NIH) requires authors to deposit post-prints of articles, which have received NIH funding, in its repository PubMed Central (PMC). This deposit should be done within the 12 months after publication of the final article in the journal.

The Howard Hughes Medical Institute (HHMI) requires, as a condition of research grants, deposit in PMC, but within 6 months after publication of the final article.

The Wellcome Trust requires, as a condition of research grants, deposit in UK PMC within 6 months after publication of the final article.

As a service to authors, the Publisher (Wolters Kluwer Health/Lippincott Williams & Wilkins) of the AHA journals will identify to PMC articles that require depositing. The Copyright Transfer Agreement provides the primary mechanism for identifying such articles. The AHA also requests that, during the submission process in Bench>Press, funding is indicated on the Manuscript Metadata Page (i.e., first screen of submission process).

WKH/LWW will transmit the post-print of an article, which is based on research funded in whole or in part by 1 or more of these 3 agencies, to PMC.

On NIH request, it remains the legal responsibility of the author(s) to confirm with the NIH the provenance of their manuscript for purposes of deposit.

Author(s) will not deposit their articles themselves.

Author(s) will not alter the post-print already transmitted to NIH.

Author(s) will not authorize the display of the post-print prior to:

m. 12 months after publication of the final article, in the case of NIH,
n. 6 months after publication of the final article, in the case of HHMI and the Wellcome Trust

For more information about authors' rights and responsibilities, please visit the Authorship Responsibility and Copyright Transfer Agreement

For more information, please visit PMC

Author Permissions and Rights Questions and Answers

Permissions

- Requests for permission to reproduce figures, tables, or portions of articles originally published in ***Hypertension*** can be obtained via Rightslink (a service of the Copyright Clearance Center), not the Editorial Office. **All permission requests are now processed via the Rightslink online system.** Steps for obtaining permission include:

0. On ***Hypertension's*** home page, either search for the article using the Search feature or locate a copy of the article in the online archives for which you are requesting permission.
1. Next, select the Full Text or PDF version of the article.
2. Then, locate the "Request Permissions" link in the menu on the right side of the Web page (under "Services"). A new Web browser will open, which is Rightslink.
3. Follow the step-by-step instructions in Rightslink for requesting permission by:

- selecting the way the content will be used.
- creating an account, if one does not exist already.
- accepting the terms and conditions for reuse.
- determining method of payment.

- Further information can be found in the Permissions and Rights Instructions.
- **Note:** For AHA Scientific Statements and Guidelines, permission to reprint, modify, alter, enhance, copy, or distribute this content must be obtained from the American Heart Association. Instructions are located at http://www.americanheart.org/presenter.jhtml?identifier=4431. A link to the "Permission Request Form" appears on the right side of the Web page, in the "Related Items" box.

Costs to Authors

Authors are charged:

- $70 per printed page of an article to defray costs of publication (information is sent with author's proof) or $35 per page in the case of copyedited online-only material.

Authors of papers that are accepted for publication and that exceed the 6,000 word limit will be charged for the overage. Authors will be charged an additional $425 for each additional 1,000 words over 6,000. The usual $70 page charge will also apply. Word count will be calculated by the editorial office and will include references, figures, and tables.

- Expense for color reproduction of figures are based on the number of figures. Charges are $495 for 1 figure, $990 for 2, $1350 for 3, and $1710 for 4. Each additional figure after 4 color figures is $90. Color figures that are online only are half the print cost.
- Expense for replacing poor-quality art
- Expense for reprints (price lists are sent with the author's proof)
- $50 per printed page for excessive author alterations
- $100 per page for printing a correction (erratum) resulting from an author's error, after publication of the article

Policies</FONT< STRONG>

AHA Scientific Publishing Conflict of Interest Policy

AHA Scientific Publishing Ethical Conduct Policy

Forms

Authorship Responsibility and Copyright Transfer Agreement

Conflict(s) of Interest/Disclosure(s) Questionnaire

Acknowledgment Permission Form

Change of Authorship Form

Artwork Guidelines (PDF)

Journal Subject Heads for Article Collections Feature on Journals Web Site

Key Word List

ANEXO D

----- Mensagem encaminhada de hypertension@physiology.umsmed.edu -----
Data: Thu, 18 Feb 2010 16:40:50 -0800 (PST)
De: hypertension@physiology.umsmed.edu
Endereço para Resposta (Reply-To): hypertension@physiology.umsmed.edu
Assunto: HYPERTENSION -- Manuscript Submission
Para: Marcio Santos <marcio@infonet.com.br>

18 February 2010

PhD Marcio R V Santos
Universidade Federal de Sergipe
Department of Physiology
Departamento de Fisiologia, Av. Marechal Rondon, S/N, Rosa Elze,
Universidade Federal de Sergipe
São Cristóvão, SE 49100000 Brazil
marcio@infonet.com.br

MS ID#: HYPERTENSION/2010/151738
MS TITLE: RESISTANCE TRAINING PREVENTS HYPERTENSION AND IMPROVES CARDIAC AUTONOMIC MODULATION IN ALLOXAN DIABETIC RATS

Dear Dr. Santos:
You have successfully approved the pdf of your manuscript entitled "RESISTANCE TRAINING PREVENTS HYPERTENSION AND IMPROVES CARDIAC AUTONOMIC MODULATION IN ALLOXAN DIABETIC RATS".
To follow the status of your manuscript please go to http://submit-hyper.ahajournals.org, enter the author area and click on the "Under Review-Check Status" queue.

Yours sincerely,

Gerry McAlpin
Hypertension
University of Mississippi Medical Center
2500 N. State St.
Jackson, MS 39216-4505
USA
Hypertension@physiology.umsmed.edu