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TREINAMENTO AERÓBIO E DIABETES EXPERIMENTAL EM RATOS: PERFIL ENDÓCRINO-METABÓLICO NO CEREBELO.

Luciana Mendonça Arantes

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências da Motricidade- Área de Biodinâmica da Motricidade Humana.

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Orientadora: Profa. Dra. Eliete Luciano

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DEDICATÓRIA

Aos meus avós paternos e maternos (*in memorian*) por terem sido meus mestres e doutores, me ensinando o verdadeiro valor da vida.

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RESUMO

O presente estudo foi delineado para avaliar os efeitos do treinamento físico aeróbio no perfil endócrino-metabólico de ratos diabéticos, com maior ênfase no tecido cerebelar. Para tanto, ratos adultos da linhagem Wistar foram submetidos à aplicação com aloxana monoidratada Sigma (32 mg/kg de peso corporal). Cinco dias após a administração da droga, será realizado um teste de glicemia para comprovação do estado diabético dos animais, sendo considerados diabéticos apenas aqueles ratos que apresentarem glicemia igual ou superior a 250 mg por 100 ml de sangue. Após a constatação do diabetes, os ratos foram distribuídos, aleatoriamente, em quatro grupos: Controle Sedentário (CS) - ratos controles que não realizarão exercícios físicos; Controle Treinado (CT)- ratos controles que serão submetidos ao protocolo de exercícios físicos; Diabéticos Sedentários (DS) - ratos diabéticos aloxânicos que não serão submetidos ao protocolo de exercícios físicos; Diabéticos Treinados (DT) - ratos diabéticos aloxânicos que serão submetidos ao mesmo protocolo de exercícios físicos do grupo controle treinado (CT). O protocolo de treinamento físico consistirá de natação por 1 hora/dia, 5 vezes/semana, durante 8 semanas consecutivas e com sobrecarga, atada ao tórax, correspondente à máxima fase estável de lactato, em % do peso corporal. Os valores de glicemia e trigliceridemia foram aumentados nos animais diabéticos (DS e DT) e ambos foram reduzidos no grupo diabético treinado quando comparados aos valores do grupo diabético sedentário. A indução do diabetes reduziu os valores de insulinemia nos animais de ambos os grupos (DS e DT) e o treinamento físico não alterou os valores deste parâmetro. O treinamento aeróbio não modifica as concentrações de IGF-1 e Insulina no cerebelo. Foi observado diferenças no equilíbrio corporal de ratos diabéticos e controles. O grupo diabético apresentou menor desempenho que o grupo controle, mostrando maior dificuldade nas atividades que requerem equilíbrio. Com relação à histologia, observamos diferenças qualitativas nos diferentes grupos estudados.

Palavras chave: diabetes mellitus, treinamento aeróbio, insulina, equilíbrio.

ABSTRACT

This study had been designed to evaluate the aerobic exercise training effects on endocrine- metabolic profile of diabetic rats, with greater emphasis in the cerebellum. Therefore, adult *Wistar* rats had been submitted to induction by injecting alloxan monohydrate. Five days after the drug administration, a blood glucose test was performed for proving the glucose diabetic state of the animals, being considered diabetic only those rats which had shown blood glucose equal or higher than 250 mg per 100 ml of blood. After the finding of diabetes, the rats were divided randomly into four groups: Sedentary Control (SC) - control rats which did not engage in physical exercise; Trained Control (TC) - control rats which were submitted to the physical exercise protocol; Sedentary Diabetic (SD) - alloxan diabetic rats which were not submitted to the physical exercise protocol; Trained Diabetic (TD) - alloxan diabetic rats that were submitted to the same physical exercise protocol group of the trained control group (TC). The physical exercise training protocol had been consisted of swimming for 1 hour / day, 5 times / week during 8 consecutive weeks and with an overload, attached to the chest, corresponding to the maximum lactate steady state, in % of the body weight. The values of the blood glucose and triglycerides were increased in diabetic animals (SD and TD) and both were reduced in the trained diabetic group when compared to the sedentary diabetic group values. The inducing of diabetes reduced the insulin values in the animals of both groups (SD and TD), and the physical exercise training has not changed the values of this parameter. The aerobic training did not affect the concentrations of IGF-1 and insulin in the cerebellum. Differences had been observed in the body balance of the diabetic rats and control group. The diabetic group had shown lower performance than the control group, presenting greater difficulty in the activities that require equilibrium. Related to histology, qualitative differences in the different groups had been observed.

Keywords: diabetes mellitus, exercise training, insulin, equilibrium.

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INTRODUÇÃO

Durante o último século o estilo de vida da sociedade se alterou de modo acelerado com redução da atividade física que, juntamente com uma alimentação mais calórica, favoreceram a ocorrência de obesidade, estresse, hipertensão arterial e diabetes (MILECH; OLIVEIRA, 2004).

O *Diabetes Mellitus* (DM) é um grupo de doenças metabólicas, resultante de defeitos na secreção e/ou ação da insulina que levam à hiperglicemia (ADA, 2006) e a uma variedade de alterações metabólicas em diversos tecidos, incluindo o renal, cardiovascular, nervoso e ósseo.

Alguns estudos tem demonstrado que a prática do exercício físico regular promove importantes adaptações endócrinas e metabólicas que são fundamentais para o controle metabólico da doença em humanos e animais diabéticos. Tem sido demonstrado que o treinamento físico aumenta a expressão do receptor de insulina em algumas regiões cerebrais frente ao exercício físico (PARK et al., 2000; ZHAO et al., 2002).

Outro dado pertinente é que o treinamento físico aumenta as concentrações séricas e teciduais de IGF-1 (fator de crescimento semelhante a insulina) em organismos saudáveis, além de recuperar os níveis séricos desse peptídeo em animais diabéticos (ZANCONATO et al., 1994; BORST et al., 2001; GOMES et al., 2006). Os IGFs são peptídeos encontrados na forma de IGF-1 e IGF-2, sintetizados pelo fígado e pela maioria das células orgânicas, em resposta a ativação promovida pelo hormônio de crescimento (GH) ou de forma GH-independente (ZANCONATO et al., 1994).

Sabe-se que os fatores neurotróficos derivados do cérebro, IGF-1 e fator de crescimento derivado do endotélio vascular são os principais fatores de crescimento conhecidos para mediar os efeitos do exercício sobre o cérebro (VAN PRAAG et al., 2005; COTMAN et al., 2007). Gomes et al (2009) analisaram os efeitos do treinamento físico aeróbio sobre as concentrações de insulina e IGF-1 no hipocampo de ratos diabéticos e observaram que o treinamento físico promoveu alterações metabólicas e hormonais que foram associadas com uma melhora na homeostase da glicemia e uma maior atividade do IGF-1 no hipocampo.

Alguns estudos tem observado participação de neurônios dopaminérgicos na ação do hormônio insulina no mesencéfalo, mais especificamente na substância

negra (FIGLEWICZ et al., 2003). Para Gallego et al (2003) o diabetes reduz a atividade do sistema dopaminérgico. Porém, há uma carência de estudos que analisem os efeitos do treinamento físico nas varáveis metabólicas e funcionais no cerebelo em indivíduos diabéticos.

Considerando que o cerebelo regula o movimento e o equilíbrio, ajustando informações e recebendo aferências dos sistemas motores descendentes do cérebro, lesões nesta região podem provocar distúrbio da coordenação dos movimentos dos olhos e dos membros e déficit de equilíbrio e redução do tônus muscular (GHEZ, 1991).

Joyal et al (1996) lesaram regiões mediais e hemisférios laterais do cerebelo de ratos e submeteram-nos a provas de equilíbrio e à prova de memória espacial do labirinto aquático de Morris. As lesões resultaram inicialmente em alterações do equilíbrio, mas os animais evoluíram ao longo das tentativas. Ratos com lesões de estruturas mediais do cerebelo (vermis e núcleo fastígio) apresentaram déficits numa prova de plataforma visível, enquanto animais com lesões em hemisférios cerebelares mostraram alterações somente na condição da plataforma submersa, mostrando seletividade desta região na memória espacial. A hipótese conclusiva destes autores é de que o cerebelo atue na modulação destas funções em integração com outras regiões do sistema nervoso central, como hipocampo e neocórtex.

Tendo em vista que o cerebelo constitui somente 10% do volume total do cérebro e, no entanto, contém mais da metade de todos os neurônios do sistema nervoso central (GHEZ, 1991), fazem-se necessárias pesquisas e estudos laboratoriais que analisem e investiguem o comportamento metabólico, funcional e neuronal do cerebelo de ratos diabéticos.

Do ponto de vista social e econômico há grande importância em se estudar DM devido às altas taxas de morbidade, mortalidade, incapacitação para o trabalho e desencadeamento de outras patologias, dentre elas, as doenças neurodegenerativas. Tal patologia exige uma atenção e cuidados especiais no sentido de uma detecção precoce dos indivíduos susceptíveis para que haja possibilidade de intervenção profilática (BALDA; PACHECO, 1999) e prevenção não farmacológica nos mesmos.

Considerando a importância de tais abordagens na doença em discussão e os efeitos benéficos do exercício físico regular em indivíduos diabéticos, faz-se

necessário investigar os possíveis efeitos do treinamento aeróbio sobre o metabolismo cerebral em ratos diabéticos.

1. OBJETIVO GERAL

Analisar os efeitos do treinamento físico aeróbio no perfil endócrino-metabólico de ratos diabéticos, com maior ênfase no tecido cerebelar.

2.1 OBJETIVOS ESPECÍFICOS

Investigar os efeitos do treinamento físico sobre:

- concentrações séricas de glicose, insulina e triglicérides.
- concentrações de insulina e IGF-1 no cerebelo.
- equilíbrio corporal dos ratos.
- aspectos histológicos no cerebelo de ratos diabéticos.

2. REVISÃO DA LITERATURA

3.1 Diabetes Mellitus

O *Diabetes Mellitus* (DM) é classificado como um grupo de moléstias metabólicas caracterizadas por hiperglicemia, provocada por defeitos na secreção e/ou ação da insulina, polipeptídio anabólico produzido nas células beta do pâncreas (ADA, 2006).

Os sintomas decorrentes da hiperglicemia acentuada incluem perda de peso, poliúria, polidipsia, infecções, visão turva e complicações agudas que podem levar ao risco de vida, como cetoacidose diabética e síndrome hiperosmolar hiperglicêmica não cetótica (SBD, 2003; GROSS et al., 2002). Em longo prazo, pode causar retinopatia com perda crescente da visão, nefropatia, neuropatia, amputações, disfunções gastrintestinais, geniturinárias, cardiovasculares e sexuais. Pacientes com diabetes também apresentam aumento na incidência de doenças cardio e cérebro-vasculares (BROTMAN; GIROD, 2002).

As formas de DM são classificadas de acordo com a sua etiologia e não mais como “insulino dependente” ou “não-insulino dependente” (SBD, 2003).

O diabetes mellitus tipo 1 (DMT1) e o diabetes mellitus tipo 2 (DMT2) são as formas mais prevalentes e se destacam pela diferença na forma de apresentação clínica (SBD, 2003).

O DMT1 caracteriza-se por resposta imunológica auto-imune, fatores genéticos predisponentes e influência do meio ambiente na destruição das células-beta produtoras de insulina. Os principais genes predispostos ao DMT1 são identificados pela tipagem do antígeno leucocitário humano (HLA) no cromossomo 6, contudo outros genes podem estar envolvidos. Dentre os fatores ambientais que podem estar envolvidos no desenvolvimento da doença estão micróbios (LAMMI et al, 2005), dieta e toxina (COSGROVE, 2004). Algumas formas de DMT1 são idiopáticas, encontrando-se casos em que há insulinopenia e predisposição à cetoacidose, não havendo evidências de autoimunidade, não sendo associada a nenhum tipo particular de HLA. A DMT1 ocorre mais freqüentemente em pessoas jovens e a instalação clínica é abrupta.

O DMT2 age na resistência à ação da insulina e está associado à obesidade, levando à perda progressiva das células beta pancreáticas. Fatores de predisposição genética e adquiridos influenciam na redução da sensibilidade à

insulina, podendo levar a diminuição da concentração e da atividade quinase do receptor de insulina, da concentração e da fosforilação dos IRS-1 e –2, da atividade da PI 3-quinase, da translocacão dos GLUTs, da atividade de enzimas intracelulares (PESSIN; SATIEL, 2000). Manifesta-se predominantemente após os 40 anos e na grande maioria dos casos não é necessária à utilização de insulina exógena (MILECH; OLIVEIRA, 2004).

A deficiência de insulina causa, ainda, outra alteração metabólica significativa: o aumento da degradação e oxidação de gordura, resultando em excessiva produção de cetonas (KELLY et al., 2003).

Para prevenção e controle do DM são indicados medicamentos orais, injeção de insulina, balanço nutricional e atividade física (ADA, 2005).

3.2 Diabetes e Exercício

O exercício físico promove uma melhora na ação insulínica, especialmente no músculo esquelético. Naturalmente, como o DMT2 caracteriza-se predominantemente por resistência à insulina, nesses pacientes observa-se mais facilmente o efeito benéfico dos exercícios sobre o controle glicêmico.

Tanto indivíduos com DMT1 como indivíduos não diabéticos apresentam também melhor sensibilidade à insulina induzida pelo exercício. Estes efeitos são induzidos pelo exercício físico dinâmico em decorrência de várias adaptações: aumento da densidade capilar, aumento da expressão e translocação de GLUT4 para a membrana plasmática, aumento das fibras musculares mais sensíveis à ação insulínica, possíveis alterações na composição de fosfolípides do sarcolema, aumento na atividade de enzimas glicolíticas e oxidativas e aumento na atividade da glicogênio-sintetase (JESSEN; GOODYEAR, 2005).

O exercício físico realizado regularmente tem efeitos benéficos sobre o metabolismo, crescimento corporal e reparação tecidual em organismos sadios e diabéticos.

Como a musculatura representa 40% da massa corporal total, a contração muscular é muito significativa para o controle da glicemia e para prevenção do diabetes (WEINECK, 1999). O controle glicêmico é o principal fator que interfere sobre a concentração lipídica dos pacientes com DM.

Sabe-se que a insulina pode interferir também no metabolismo protéico e que organismos hipoinsulinêmicos podem apresentar redução na taxa de síntese protéica muscular (FEDELE et al., 2001; WOJTASZEWSKI et al., 2002).

Souza & Luciano (1996) demonstraram que as baixas concentrações séricas de hormônio de crescimento (GH) em ratos diabéticos (induzidos por aloxana) não são modificadas pelo treinamento físico, embora tenha ocorrido melhora no desenvolvimento ósseo desses animais. Isto pode estar relacionado com o aumento do fator de crescimento semelhante a insulina (IGF-1), visto que alguns autores têm encontrado aumento das concentrações séricas desse polipeptídio em ratos submetidos ao exercício físico (FEDELE et al., 2001; GOMES et al., 2003).

No entanto, o efeito benéfico do exercício depende da doença estar ou não razoavelmente “controlada” antes do início da atividade (ZINMAN et al., 2003). Controle significa que a glicemia deve encontrar-se próxima dos valores normais de referência. O diabético controlado possui insulina suficiente, de modo que a glicose pode ser captada pelos músculos durante o exercício e neutralizar o aumento normal da liberação de glicose hepática decorrente da ação das catecolaminas e do glucagon (POWERS; HOWLEY, 2000).

Contudo, é importante ressaltar que um metabolismo correto é, portanto, pré-requisito para o trabalho muscular, como forma de terapia do diabetes (WEINECK, 1999).

3.3 Diabetes, Sistema Nervoso Central e Exercício Físico

Alguns estudos têm relatado grandes alterações anatômicas, funcionais e bioquímicas no sistema nervoso central (SNC) de animais diabéticos (TOMLINSON et al., 1992; OZTURK et al., 1996). Esta variedade de alterações (geralmente nomeada como neuropatia diabética) afeta o cérebro, medula espinhal e nervos periféricos. Estas alterações foram reportadas como mudanças degenerativas no sistema nervoso de ratos diabéticos, com degeneração do tecido ganglionar, redução de calibre axonal e desmielinização (MONCKTON; PEHOWICH, 1980; TOMLINSON; YUSOF, 1983; SCHMIDT; PULARD, 1986; KNIEL et al.,1986).

No SNC, o diabetes reduz o peso do cérebro e o volume neocortical, que está associado com uma redução do número de neurônios corticais (JAKOBSEN et al., 1987). Todas estas alterações centrais e periféricas são consistentes com a diminuição da atividade neuronal (GALLEGO et al., 2003). Esta diminuição da

atividade neuronal pode estar relacionada ao prejuízo nos aspectos cognitivos que ocorre em modelos animais de DMT1 e DMT2 (GRZEDA; WISNIEWSKA, 2008).

Análises em nível molecular indicam que a insulina está presente em diferentes regiões do sistema nervoso central e atua por meio de receptores específicos como um agente neuromodulador (SCHWARTZ et al., 2000; PARK, 2001).

Estudos recentes sugerem que a insulina pode agir como um sinalizador metabólico aferente para o SNC. Resistências iniciais em se aceitar tal conceito se deve ao fato das células neuronais não dependerem da insulina para a captação de glicose. Com a descrição da presença de insulina no cérebro (HAVRANCOVA et al., 1978a) sugeriu-se que esta era produzida no próprio SNC (HAVRANCOVA et al., 1978a), entretanto, a presença do hormônio no liquido cefalo-raquidiano em concentrações proporcionais a dos níveis do plasma circulante (WOODS, PORTER, 1978) e a inexistência de transcritos de insulina na maior parte do cérebro provam que a insulina detectada no SNC era produzida pelas células β-pancreáticas, e que esta atravessava a barreira hemato-encefálica (BHE) através de um sistema de transporte especializado (PARDRIGE, 1986; BAURA et al., 1993). A alta densidade de receptores de insulina no plexo coróide sugere que o mesmo esteja envolvido no transporte da glicose através da BHE (ZHAO et al., 1999).

Algumas funções relacionadas a ação da insulina no SNC são sugeridas, como ações no crescimento, sobrevivência e regulação de genes envolvidos na diferenciação celular (BOTHWELL, 1982; NAKAMURA, SHITARA, TAKAKURA, 1988; COKER et al., 1990), no metabolismo das células neuronais e da glia (NAKAMURA et al, 1988), nas sinapses do SNC, através do seu receptor (ABBOT, WELLES, FALLON, 1999); e regulação central da função autonômica, da termogênese, do controle do apetite e consequentemente do peso corporal (SCHWARTZ et al., 1992; PORTE JR et al., 1998; SCHWARTZ et al., 2000).

Alterações na secreção ou diminuição das concentrações cerebrais de insulina e de seus receptores foram relacionadas com diabetes, obesidade, e com sérias desordens cognitivas em humanos e em animais (ZAIA; PIANTANELLI, 2000; ZHAO et al., 2002). Alguns estudos mostram que há síntese de insulina no sistema nervoso central de ratos adultos (ZHAO et al., 2002) apesar de ainda não haver um consenso entre os pesquisadores.

Associado a insulina, o IGF 1 é a chave reguladora do metabolismo celular e do crescimento. Vários estudos tem sugerido que o IGF 1 tem uma capacidade direta para modular a eficácia sináptica via regulação da formação da sinapse, liberação de neurotransmissores e excitabilidade neuronal (ARAUJO et al., 1989; KAR et al., 1997).

O exercício físico aumenta as concentrações de IGF-1 periférico e à sensibilidade a insulina melhorando a saúde cerebral e as funções cognitivas (CARRO et al., 2001; CHEN; RUSSO-NEUSTADT, 2007; YUEN; DUNGER, 2007). Estudos recentes realizados em nossos laboratórios têm demonstrado que o treinamento aeróbio aumenta as concentrações de IGF 1 circulantes, hepáticas e musculares em ratos diabéticos (GOMES et al., 2005; GOMES et al., 2006; LEME et al., in press).

O exercício físico aumenta a expressão do receptor de insulina em regiões do hipocampo (PARK et al., 2000; ZHAO et al., 2002), aumentando, também, as concentrações séricas e teciduais de IGF-1 em organismo saudáveis, além de recuperar os níveis séricos desse peptídeo em animais diabéticos (ZANCONATO et al., 1994; BORST et al., 2001; GOMES et al., 2006).

Gomes et al (2009) analisaram os efeitos do treinamento físico aeróbio sobre as concentrações de insulina e IGF-1 no hipocampo de ratos diabéticos. Concluiu-se no estudo que o treinamento físico promove alterações metabólicas e hormonais que são associadas a uma melhora na homeostase da glicemia e maior atividade do IGF-1 sistêmico e no hipocampo dos ratos diabéticos.

Contudo, mesmo sabendo que exercícios regulares promovem um aumento no controle metabólico dos animais e indivíduos diabéticos, sendo um importante componente no tratamento do DM, pouco se conhece a respeito dos efeitos do treinamento físico sobre a modulação das concentrações cerebrais de insulina e de IGF-1 no cerebelo de ratos diabéticos.

4. MATERIAIS E MÉTODOS

Para desenvolver este estudo, foram utilizados cerca de 50 ratos adultos da linhagem Wistar (Rattus Norvegicus Albinus Wistar) com aproximadamente 75 dias de idade, provenientes do Biotério Central da Universidade Estadual Paulista (UNESP) de Botucatu e mantidos no biotério do laboratório de Biodinâmica do Departamento de Educação Física, Instituto de Biociências da UNESP-Rio Claro-SP. Os animais foram alojados em gaiolas de polietileno (5 ratos por gaiola), mantidos à temperatura ambiente de 25º C e fotoperíodo de 12 horas de claro/12 horas de escuro e alimentados com ração balanceada padrão Purina® e água "*ad libitum"*. O projeto de pesquisa foi aprovado pelo Comitê de Ética no Uso de Animal (Protocolo n° 6024) (Anexo 1).

4.1 INDUÇÃO DO DIABETES E DELINEAMENTO EXPERIMENTAL

Para indução do diabetes experimental, os ratos, com aproximadamente 300 g, foram anestesiados moderadamente com éter etílico e em seguida receberam uma dose única de aloxana monoidratada Sigma (32 mg/kg de peso corporal) dissolvida em tampão citrato 0,01M, pH 4,5. Após este procedimento, os ratos foram recolocados nas gaiolas recebendo no primeiro dia pós-aloxana, uma solução de água e glicose (15%), além de ração (LUCIANO; LIMA, 1997). Cinco dias após a administração da droga, foi realizado um teste de glicemia para comprovação do estado diabético dos animais, sendo considerados diabéticos apenas aqueles ratos que apresentaram glicemia igual ou superior a 250 mg por 100 ml de sangue.

Após estes procedimentos, foi registrada a massa corporal dos animais para serem distribuídos nos grupos sedentários e treinados conforme a massa corporal para animais controles e glicemia e massa corporal para animais diabéticos, com objetivo de obter igual distribuição dos animais nos grupos baseada nestes parâmetros.

Desta forma, os animais foram distribuídos nos grupos:

Controle Sedentário (CS) - ratos controles que não realizaram exercícios físicos;

Controle Treinado (CT)- ratos controles que foram submetidos ao protocolo de exercícios físicos;

Diabéticos Sedentários (DS) - ratos diabéticos aloxânicos que não foram submetidos ao protocolo de exercícios físicos;

Diabéticos Treinados (DT) - ratos diabéticos aloxânicos que foram submetidos ao mesmo protocolo de exercícios físicos do grupo controle treinado (CT).

4.2 PROTOCOLO DE TREINAMENTO FÍSICO

Período de adaptação: Inicialmente os animais realizaram uma adaptação ao meio líquido em água rasa durante 5 dias. Posteriormente, com a finalidade de respeitar o princípio fisiológico da sobrecarga, os animais realizaram uma adaptação ao exercício com aumento progressivo da sobrecarga.

Todos os ratos que participaram do treinamento físico foram submetidos a testes de esforço para a identificação da máxima fase estável de lactato (MFEL). A MFEL equivale a mais alta concentração de lactato sanguíneo onde sua entrada na circulação é compensada pela remoção durante exercícios com cargas constantes. Segundo Heck e colaboradores (1985) sua determinação tem se mostrado útil na prescrição de exercícios e na avaliação do condicionamento aeróbio. Gobatto seus colaboradores (2001) descreveram um protocolo para a determinação da MFEL para ratos durante exercício de natação, que foi utilizado no presente estudo. Em síntese, os animais foram submetidos a uma série de testes de natação suportando sobrecargas constantes em relação ao peso corporal. Estas cargas foram crescentes entre os testes até que não fosse mais possível estabilização das concentrações de lactato sanguíneo durante a sessão de exercício. No presente estudo, cada teste consistiu de 30 minutos de natação contínua suportando uma carga, com coleta de sangue, por corte na extremidade da cauda a cada 5 minutos para a determinação das concentrações de lactato. Uma única incisão foi suficiente para obter as amostras, nas quais a concentração de lactato foi determinada em analisador de lactato. Houve intervalo de 48 horas entre os testes. O critério de estabilização empregado foi a diferença igual ou inferior a 1,0mM de lactato sanguíneo entre 10 e 25 minutos de exercício.

O protocolo de treinamento físico consistiu de natação por 1 hora/dia, 5 vezes/semana, durante 8 semanas consecutivas e com sobrecarga, atada ao tórax, correspondente à máxima fase estável de lactato, em % do peso corporal (GOBATTO et al., 2001; OLIVEIRA et al, 2007).

As sessões de natação foram realizadas em tanques de amianto com 100 cm de comprimento, 70 cm de largura e 60 cm de altura. A água foi mantida numa

profundidade de 40cm, para que os animais não apoiassem a cauda no fundo do tanque. A temperatura da água foi controlada, sendo mantida em 31º C ± 1º C, durante a realização do exercício, por ser considerada termicamente neutra em relação à temperatura do ratos (AZEVEDO,1994).

4.3 PROTOCOLO DE TREINAMENTO DE EQUILÍBRIO

Para o treinamento de equilíbrio foi adotada a metodologia empregada por Rodrigues Alves (2003). Primeiramente, todos os ratos foram treinados a caminhar sobre uma trave elevada, durante oito dias seguidos. O equipamento foi composto de uma trave de madeira (18mm de largura e espessura, por 2m de comprimento), com uma plataforma de 10cm x 10cm, em cada uma das extremidades, apoiadas em suportes com 20cm de altura. Na lateral da trave foram realizadas duas marcas verticais, indicando o percurso de 1m, situado na porção central da trave, onde o equilíbrio corporal foi avaliado (Figura 1).

O treinamento consistiu, inicialmente, em colocar o rato sobre a trave elevada. No primeiro momento o rato foi colocado sobre a plataforma para se adaptar ao ambiente. Nos dias subsequentes, foi colocado cada vez mais distante da plataforma, até que apresentasse condições de atravessar a trave integralmente, atingindo a plataforma oposta. O teste foi realizado individualmente, em sessões de 5 minutos para cada rato. O treinamento foi encerrado quando o rato estava apto a caminhar sobre a trave quatro vezes, isto é, ir e vir duas vezes. Os ratos que não conseguiram caminhar sobre a trave, após o período de treinamento, foram desqualificados para a pesquisa. Contudo, foram mantidos na gaiola para manter o mesmo número de ratos em cada gaiola até a finalização do experimento.

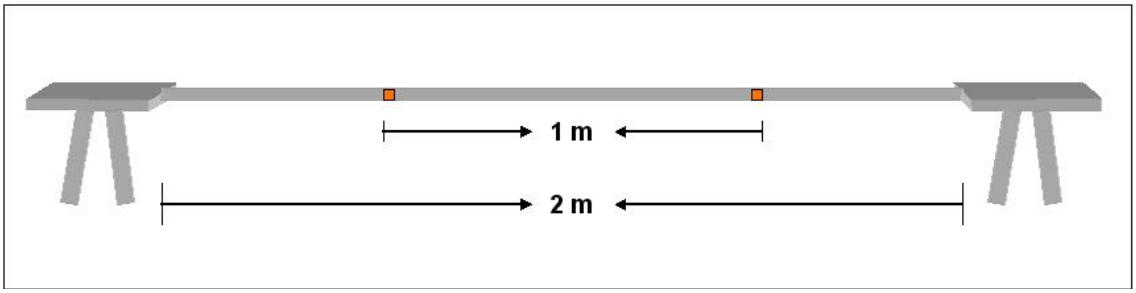

Figura 1 – Desenho esquemático da trave elevada (Rodrigues Alves, 2003)

4.4 AVALIAÇÕES PRÉVIAS AO SACRIFÍCIO DOS ANIMAIS

Durante o período experimental, os ratos foram avaliados semanalmente com relação ao peso corporal, ingestão alimentar e hídrica para fins de registros e posterior análise.

4.5 AVALIAÇÕES APÓS O SACRIFÍCIO DOS ANIMAIS

Ao final do período experimental, os animais foram mantidos em repouso por 48 horas em relação à última sessão de exercícios, com alimentação e água “ad libitum”. Amostras de sangue foram coletadas em capilares e centrifugadas para determinação do hematócrito. O sacrifício foi realizado por decapitação em guilhotina e amostras teciduais e de sangue coletadas. O sangue foi centrifugado à 3000 rpm por 10 minutos e a partir do soro foram realizadas as seguintes análises:

•Glicemia: A glicose foi determinada pelo método enzimático colorimétrico da glicose oxidase-peroxidase (HENRY, 1974).

•Insulinemia: Determinada pelo método de radioimunoensaio (RIA) - Kit Coat-A-Count da Diagnostic Products Corporation (DPC) de fase sólida.

• Hematócrito: Foi obtido em centrífuga de micro-hematócrito.

•Triglicerídeos séricos: Foram determinados pelo método enzimático colorimétrico, com kit comercial (Laborlab).

Análises do Cerebelo:

Insulina: O cerebelo foi removido cuidadosamente, pesado e homogeneizado em 5 ml de líquido extrator para insulina. Em seguida foi realizada uma centrifugação para posterior retirada de 2 ml do sobrenadante que foi neutralizado para pH 8,5. Após nova centrifugação 100 microlitros do sobrenadante, foi realizada a dosagem cerebral de insulina por meio da técnica de radioimunoensaio (por precipitação com carvão ativado).

IGF-1: O cerebelo foi extraído, pesado e homogeneizado em 5 ml de líquido extrator para IGF-1. Em seguida foi realizada uma centrifugação para posterior retirada de 2 ml do sobrenadante que foi neutralizado para pH 8,5. Após nova centrifugação 100 microlitros do sobrenadante, foi realizada a dosagem cerebral de IGF-1 por meio da técnica de radioimunoensaio (por precipitação com carvão ativado).

Análises histopatológicas

Após os cérebros serem retirados da caixa craniana, foram processados e embebidos em parafina. Posteriormente, as peças foram trimadas em um fixador de madeira e encaminhadas para o micrótomo, regulado para fornecer fitas com espessura de 12µm. Os cortes foram realizados no plano coronal, seriados de 4.80 – 5.30 mm em relação ao Bregma, em seguida, as fitas processadas passaram para o banho histológico, onde foram montadas as lâminas com 6 cortes em cada em média, e que já haviam sido anteriormente gelatinizadas. Todos os protocolos experimentais apresentados abaixo foram desenvolvidos segundo SPICER (1987).

Corante azul de toluidina:

Após 24 horas as lâminas foram coradas pela técnica de coloração dupla de fucsina ácida e azul de toluidina semelhante à descrita por AUER, OLSSON, SIESJÖ (1984). Este método de coloração dupla é extremamente sensível para a detecção de neurônios necróticos, já que o azul de toluidina cora somente núcleo de neurônios íntegros, enquanto que a fucsina ácida cora de vermelho o citoplasma de neurônios necróticos ou em processo de degeneração, tornando essa técnica muito fidedigna na análise de neurônios mortos pelo contraste evidente que determina.

Os cortes foram analisados com o auxílio da descrição de PAXINOS et al. (1982) com uma média de 6 cortes por lâmina.

Tricômero de Van de Grift

Foi realizado para determinar as fibras elásticas e colágenas presentes no tecido analisado. O procedimento do mesmo foi feito da seguinte forma:

- quatro minutos de hematoxilina;
- quinze minutos de água corrente;
- passado na solução hidrocloridrico 1%;
- passado na solução amônia 1%;
- cinco minutos na solução Ponceau Fucsina;
- lavado no ácido fosfomolibidico 1%
- dois minutos em light Green;
- lavado em ácido acético 1%;
- desidratado e montado

Hematoxilina Eosina (HE)

Foi realizado para analisar os aspectos gerais do tecido. O procedimento do mesmo foi realizado da seguinte forma:

- hematoxilina – cerca de 8 minutos

- água corrente com várias trocas
- limpeza das lâminas com auxílio de um gaze
- diferenciador da hematoxilina – 10 segundos (5ml de ácido clorídrico em 100ml de álcool 75%)
- água corrente – 2 minutos com uma troca
- eosina – 30 segundos a 3 minutos
- água corrente – 1 minuto
- diferenciador de eosina – álcool 75% - 1 minuto

5. ANÁLISE ESTATÍSTICA

Inicialmente os dados foram tratados a partir de procedimentos descritivos. A normalidade da distribuição dos dados foi analisada pelo teste de Shapiro-Wilk. Os dados que apresentaram distribuição “normal” foram aplicados análise de variância (ANOVA) one way, com aplicação do teste “*post-hoc*” de Tukey, com nível de significância estabelecido em 5% ($p<0,05$). Os resultados foram expressos como média ± desvio padrão.

6. RESULTADOS E DISCUSSÃO

6.1 ARTIGO 1 - INSULIN CONCENTRATIONS IN CEREBELLUM AND BODY BALANCE IN DIABETIC MALES RATS: AEROBIC TRAINING EFFECTS

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INSULIN CONCENTRATIONS IN CEREBELLUM AND BODY BALANCE IN DIABETIC MALES RATS: AEROBIC TRAINING EFFECTS

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ABSTRACT

Brain insulin has had widespread metabolic, neurotrophic, and neuromodulatory functions and has been involved in the central regulation of food intake and body weight, learning and memory, neuronal development, neuronal apoptosis. **PURPOSE:** The present study investigated the role of swimming training on cerebral metabolism on insulin concentrations in cerebellum and the body balance performance of diabetic rats. **METHODS:** Forty Male Wistar rats were divided in four groups: sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). Diabetes was induced by Alloxan (32 mg kg b.w.), single dose injection. The mean blood glucose of diabetic groups was 367±40 mg/dl. Training program consisted in swimming 5 days/week, 1 h/day, 8 weeks, supporting a workload corresponding to 90% of maximal lactate steady state (MLSS). For the body balance testing rats were trained to traverse for 5 min daily for 5–7 days. All dependent variables were analyzed by one-way analysis of variance (ANOVA) and a significance level of $P < 0.05$ was used for all comparisons. **RESULTS:** The body balance testing scores were different between groups. Insulin concentrations in cerebellum were not different between groups. **CONCLUSION:** It was concluded that in diabetic rats, aerobic training does not induce alterations on cerebellum insulin but induces important metabolic, hormonal and behavioral alterations which are associated with an improvement in glucose homeostasis, serum insulin concentrations and body balance.

Introduction

Insulin is a hormone / protein produced and secreted by the beta cells of pancreatic islets, and whose main function is the stimulation of glucose uptake by target tissues [1].

Recent studies suggest that insulin may act as a marker for metabolic afferent central nervous system. It was suggested that insulin was produced in the CNS itself [2], however, the presence of the hormone in cerebrospinal fluid concentrations proportional to the levels of circulating plasma and the absence of insulin transcripts in any region of the brain that comes from all insulin was detected in the CNS produced by the pancreatic beta cells, crossing the blood-brain barrier (BBB) by a specialized transport system [3,4].

The impact of diabetes mellitus in the CNS has been demonstrated based on neurochemical changes, electrophysiological, structural and behavioral [5,6,7,8,9,10,11,12]. Insulin resistance has played a larger role in the type 1 diabetes mellitus (T1DM) disease process than commonly recognized. Some authors indicated that insulin has been in several regions of the central nervous system (CNS) acting as a neuromodulator, inhibiting food intake, stimulating fat oxidation, in dopamine reward [13], learning and memory, aging [14], and neurological disorders such as Alzheimer's (AD) and Parkinson's disease (PD) [15] and depression.

Metabolic disturbances present in Type I diabetes because disturbances in different parts of vestibular organ mainly in its central part, affecting also the individual body balance [16]. Thus, elucidation of the risk factors and patho-physiologic mechanisms caused by diabetic complications on the central nervous system await much importance.

Several studies have been shown interest in the use of physical training as a therapy to treat a variety of metabolic and CNS disorders, including diabetes mellitus, hyperlipidemia, obesity, AD, PD, depression, among other issues [17]. The aerobic training effects on insulin concentrations in brain have also been observed in recent studies [18]. Some authors [19] examined the aerobic exercise effects on concentrations of insulin and IGF-1 in the diabetic rat hippocampus and found that physical training promoted metabolic and hormonal disorders which were associated with an improvement in glucose homeostasis and higher activity of IGF-1 in the hippocampus.

However, there is a lack of studies analyzing the aerobic training effects on metabolic and functional variables in the cerebellum in diabetic rats. Whereas the cerebellum plays a great role in postural and motor adjustments as well as in conditioning and motor learning [18], this study aims to analyze the aerobic training effects on insulin concentrations of cerebellum and body balance in diabetic rats.

Materials and Methods

Animals

Forty-eight male Wistar rats (75-days-old) from Sao Paulo State University—UNESP—Botucatu Campus were used in all experiments. Every experiment involving animals had been conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose (Council of Europe N° 123, Strasbourg, 1985). The rats were housed in room at 25°C under 12-h light and 12-h dark periods. Rats had free access to standard food and water *ad libitum*.

Induction of Diabetes

Diabetes was induced by an intravenous injection of alloxan (32mg kg^{-1} b.w.; Sigma). After 72h of alloxan injection, fasting blood estimated, and levels above 250 mg/dL confirmed diabetes resulting from a damaged pancreas. We considered only the diabetic animals with blood glucose between (glucose < 264.6mg/dL) and (glucose > 639mg/dL).

Aerobic training

The rats were randomly allocated to one of four groups (n = 10 per group): sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). The training had been developed in a swimming session, 5 times/week, 1h with a load corresponding to 90% of the maximal lactate steady state (MLSS), during 8 weeks. For MLSS determination the rats had been submitted to three swimming sessions were submitted to three swimming sessions in a 100 cm x 80 cm x 80 cm tank with water at the temperature of 31±1°C performed with 25 min intervals of 1 week between them, supporting loads of 4, 5, or 6% of body wt with intervals of 1 week between them. Blood samples (25 µl) had been collected every 5 min from a

cut in the tip of the tail for lactate determination (adapted from Gobatto et al. 2001) [20]. The highest concentration during exercise which lactate appearance in blood equals its removal, has been known as ‘‘maximal lactate steady state’’ (MLSS) and has been considered an important endurance exercise capacity indicator. Figure 1 shows the values of the weight percentages of found loads equivalent to MLSS of control and diabetic groups.

Body Balance Testing

First, all the rats will be trained to walk on a wooden beam, for eight days. The equipment will consist of a wooden beam (18mm width and 2m thickness) with a 10cm x 10cm platform in each of the ends, supported on 20 cm high platform. On the side of the beam there will be two vertical marks indicating the distance of 1m, located on the beam central portion, where the body balance will be measured (Figure2).

The training will consist, initially, to place the rat over the wooden beam, as well as a food supplement (chocolate) on the platforms, to attract it. The rat is placed on the platform to adapt to the environment and the food supplement. On subsequent days, it will be placed increasingly farther from the platform, until it gets conditions to go across the full beam, reaching the opposite platform. It should return to the starting platform, receiving food supplement at the end of each passage. This will be performed individually in sessions of five minutes for each rat. The training will be ended when the rat is able to walk on the beam four times, come and go twice. Rats which can not walk on the beam, after the training period, will be disqualified for the research. However, they will be kept in a cage to maintain the same number of rats in each cage until the end of the experiment [21].

Serum and tissue analyses

A standard type of extraction procedure was used [22]. At the end of experimental period, the rats were sacrificed 48hr after their last exercise bout by decapitation. Trunk blood was collected in a glass tube, centrifuged for 5 min at 3000 rpm for glucose, insulin and hematocrit determinations. Blood samples for hematocrit were

collected before sacrifice trough a small cut at the top of the tail. These evaluations aimed the alloxan efficiency verification in inducing a diabetic picture in the animals in the conditions of the present study and also evaluating the aerobic training protocol efficiency.

Analytical methods

Serum glucose concentration was measured by a colorimetric method. Serum insulin concentration was determined by radioimmunoassay (RIA Kit Coat-A-Count-USA). The cerebellum was weighted and homogenized in 5ml of insulin extractor liquid, centrifugued (3000 rpm) and 2 ml of the supernatant were taken for insulin determination by radioimmunoassay.

Statistical Analyses

Results are expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using SPSS (version 10.0) and student's '*t*'-test using Sigma Plot (version 8.0). The Bonferroni test was used for post hoc comparisons. The values of $P < 0.05$ were considered as statistically significant. The scores obtained in the body balance test were analyzed by median.

Results and Discussion

Figure 3 shows the difference between the initial values (first week) and final (last week) of body weight. In this parameter, the reduction caused diabetes and physical training did not alter these values in controls or in diabetic animals.

The brain and other tissues require glucose in order to function properly [12]. Initially, it was observed in this study that blood glucose level of all rats before aloxana administration was within the normal range. Aloxana administration led to a significant increase ($p<0.001$) in blood glucose level of diabetic rats compared to control rats (Table 1). As expected, blood insulin was decreased in both diabetic groups (Table 1). These data corroborate the findings of Leme et al. (2008) [19] who analyzed blood glucose after aloxan administration and serum insulin concentrations after 4 weeks of aerobic training in diabetic rats. Some studies justify the increased responsiveness to insulin induced by swimming exercise in rat skeletal muscle may result partially from modulation of insulin signally pathway at different molecular level [19].

The values of hematocrit and no difference observed among the groups in the present study, suggests that alterations observed in blood glucose probably were not influenced by dehydratation [19]. (Table 1).

Differences have not been found on insulin concentrations in cerebellum of diabetic rats after 8 weeks of aerobic training (Figure 4). It is unclear in the literature, such as exercise acts in the insulin concentrations in some brain regions, specifically in the cerebellum. Leme et al (2008) [19] found significant increase in brain insulin concentrations in trained groups (control and diabetic) of rats after 4 weeks of aerobic training. Some authors have shown that there are differences in regional cerebral metabolic ability, suggesting different roles between receptor and insulin receptor intracerebral peripheral.

As we know, insulin is readily transported into the CNS across the blood–brain barrier by a saturable, insulin receptor-mediated transport process [23]. The raising of the peripheral insulin concentration acutely increases the concentration in the brain, whereas prolonged peripheral hyperinsulinaemia downregulates blood–brain barrier insulin receptors and reduces insulin transport into the brain [24]. Although insulin and insulin receptors are abundant in the brain, they are selectively distributed. Some authors suggest that the cerebellum has been known to be resistant to hypoglycaemia, and selective cerebellar dysfunction caused by hypoglycaemia has not been reported. However, any consensus among researches about insulin biosynthesis in the brain had been found [25,26,27,28]. Some authors considering that brain tissue in poorly controlled diabetes is not shielded from the potential adverse cellular effects of chronic hyperglycemia and may be exposed to excessive levels of alternative metabolic fuels (lactate and ketones), as well as glucose.

In relation to the whole brain, the cerebellum has an average insulin and insulin receptor content [29], and has localized tyrosine phosphorylation of the insulin receptor in response to insulin administration [30]. In addition, an important connection between insulin and gammaaminobutyric acid (GABA) signaling has been described in the cerebellum [31].

Alterations in brain insulin concentrations have been associated with the development of cognitive and behavioral disturbances in age-associated diseases, such as Parkinson’s disease (PD) [32]. Several studies suggest a high prevalence of insulin resistance in patients with PD. However, some authors think that the Diabetes Mellitus (DM) and PD share some common underlying causes as functional

disorders, as balance, coordination, among other factors [33,34,35,36]. Therefore, further studies are needed to clarify and understand why DM is related to increased risks of PD and if there is any relationship in the insulin concentrations in the brain, specifically, a relationship with cerebellum that plays any functions affected by the PD.

On the other hand, regular exercise improves metabolic control in diabetic animals and humans and is an important component of treatment in diabetes mellitus. Over the last several years numerous studies have shown that sedentary individuals are characterized by insulin resistance, glucose intolerance, and hyperlipidemia. Conversely, physical training has been shown to reverse all of these metabolic disorders [17].

Considering that cerebellum is a region of the brain that plays an important role in motor control, it does not initiate the movement, but it contributes to coordination, precision, accurate timing and, any damage to the cerebellum does not cause paralysis, but instead produces disorders in fine movement, balance, posture, and motor learning, we apply the test of body balance in diabetic rats and we found difference between diabetic and control groups (Figure 5). Diabetic group had a performance less than the control group test, showing it more difficult to perform activities that require body balance. Some studies [16] showed that metabolic disturbances present in Type I diabetes cause disturbances in different parts of the vestibular organ but mostly in its central part. The range of vestibular organ impairment in Type I diabetes may depend on the presence and character of hypoglycaemic incidents and the duration of the disease mainly and on the compensation of diabetes to some extent. Though the disturbances are present both in central vestibular and hearing organs in diabetes the evaluation of the first one seems to be more specific in detecting early disturbances present within central nervous system.

Comparing disturbances in the vestibular organ with clinical and biochemical parameters characterising diabetes, the range of vestibular organ impairment in diabetes mellitus type 1 seems to depend mainly on the presence and character of hypoglycaemic incidents and the duration of the disease and to some extent on the compensation of diabetes [16].

In summary, the results showed significant differences in blood glucose level and blood insulin in diabetic rats compared to control rats. No significant differences

had been found on insulin cerebellum concentrations of diabetic rats, but significant differences in body balance test between control and diabetic rats of aerobic training had been found.

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Authors' contributions

Arantes, L.M. conceived the study, developed the study protocol, reviewed the references, collected and analyzed the data, and wrote the paper. Bertolini, N.O., Moura, R.F., Mello, M.A.R., participated in the design of the study, reviewed the manuscript, collected the data, and collaborated on the biochemical dosages. Luciano, E. conceived the study, participated in its design and coordination and helped in the drafting of the manuscript. All authors read and approved the submission of the final manuscript.

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Table 1. Blood glucose (mg/dL), serum insulin (pmol L^{-1}) and hematocrit (%) after 8 weeks of training

Parameters	SC	TC	SD	TD
Blood glucose (mg/dL)	129.81±38.4	113.4±11.5	374.3±13.1[a,b]	347.1±83.5[a,b]
Serum insulin (pmol L^{-1})	0.38±0.18	0.39±0.19	0.04[a,b]±0.2	0.04[a,b]±0.01
Hematocrit (%)	58±1.2	56±2	57±4.4	57±4.9

SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic. ANOVA; $P<0.001$; a≠SC; b≠TC.

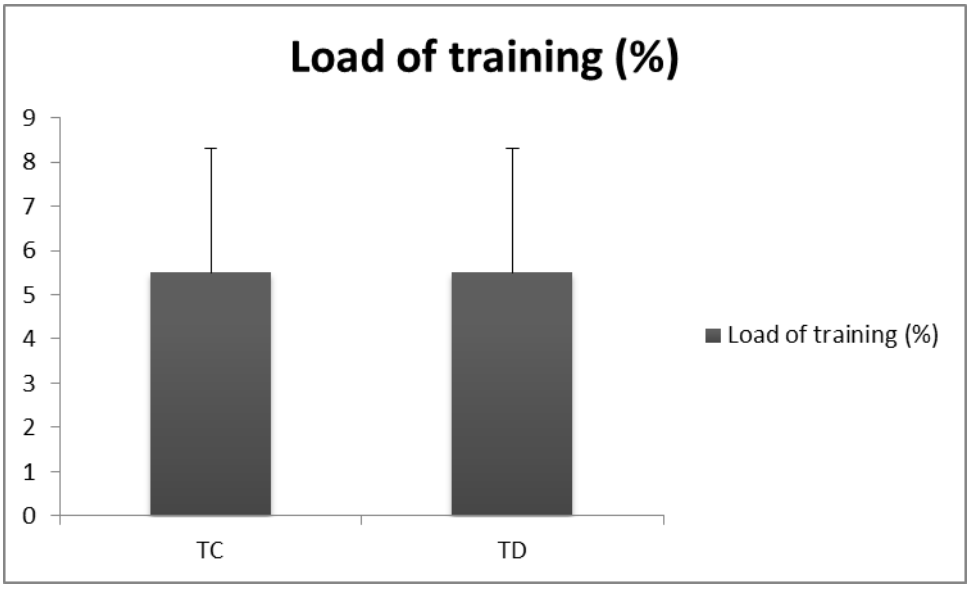

Figure 1. Loads in percentage of body weight corresponding to MLSS animals and diabetic controls. [The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$].

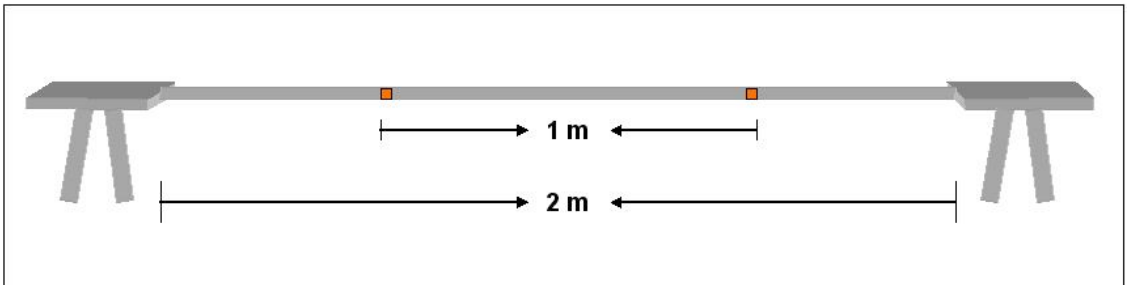

Figure 2 – Schematic drawing of the wooden beam (Jeffery et al., 1997)

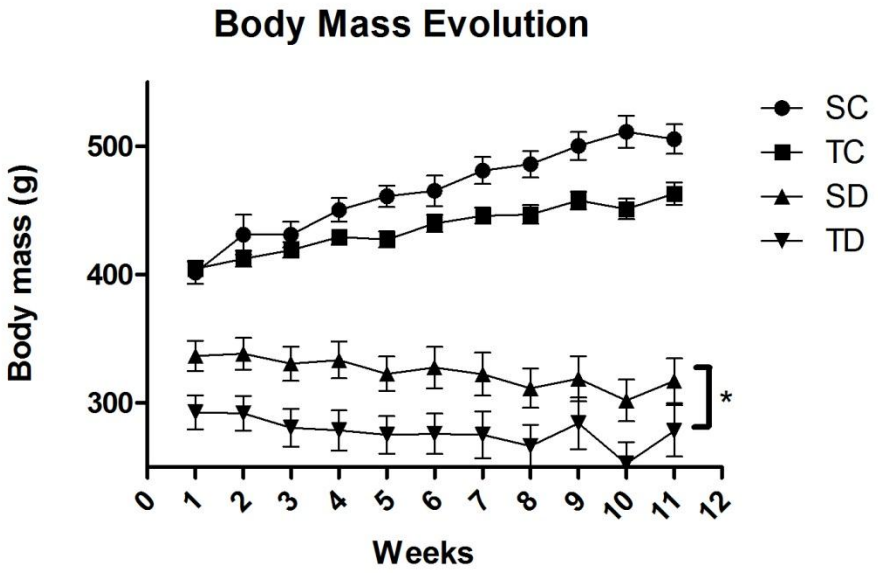

Figure 3. Difference between the initial values (first week) and final (lastweek) of body weight. [The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic. *TD≠SD

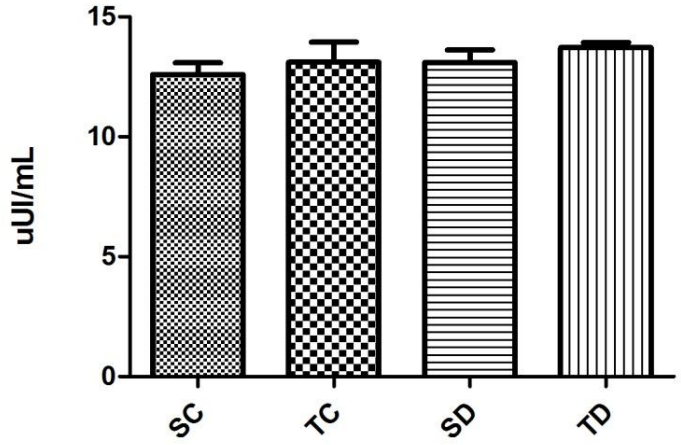

Figure 4. Insulin concentrations of cerebellum (uUl/mL) after 8 weeks of aerobic training. [The values are the mean ± SD of 10 rats pergroup. ANOVA $p<0.05$]. SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic.

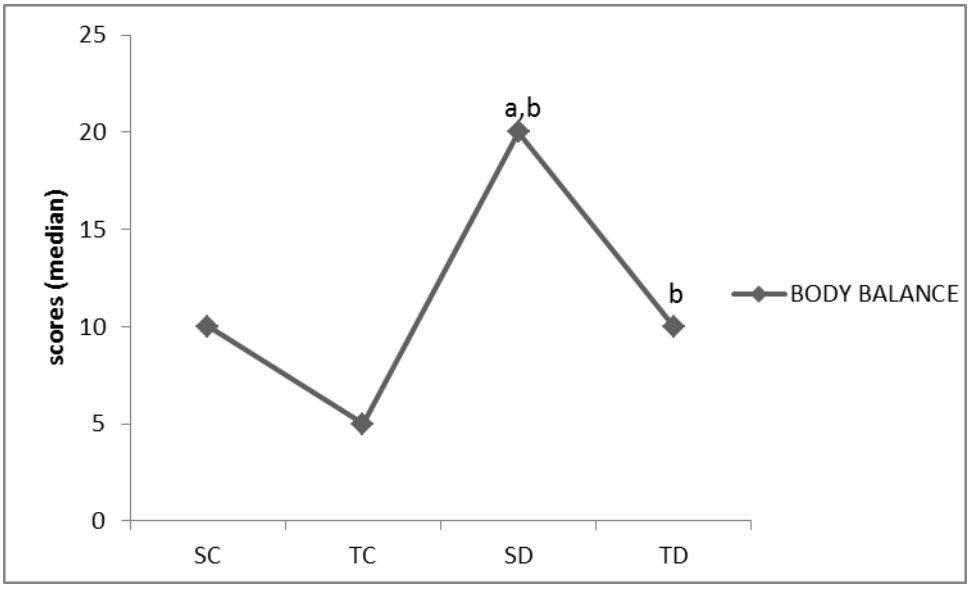

Figure 5. Body balance testing (scores) after 8 weeks of physical training. a≠SC and b≠TC ($p<0.05$). SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic.

6.2 ARTIGO 2 - PHYSICAL TRAINNING EFFECTS IN THE CEREBELLUM OF DIABETIC RATS

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PHYSICAL TRAINING EFFECTS IN THE CEREBELLUM OF DIABETIC RATS

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Running title: Aerobic training effects in diabetic rats.
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ABSTRACT

The impact of diabetes mellitus on Central Nervous System (CNS) function has been demonstrated at neurochemical, electrophysiological, structural and behavioral levels. **PURPOSE:** The present study investigated the role of swimming training on cerebral metabolism on IGF-1 concentrations in cerebellum and to analyze qualitatively the cerebellar tissue of trained diabetic rats and controls by the HE method (hematoxylin and eosin). **METHODS:** Forty Male Wistar rats were divided in four groups: sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). Diabetes was induced by Alloxan (32 mg kg b.w.), single dose injection. The mean blood glucose of diabetic groups was 367±40 mg/dl. Training program consisted in swimming 5 days/week, 1 h/day, 8 weeks, supporting a workload corresponding to 90% of maximal lactate steady state (MLSS). For the body balance testing rats were trained to traverse for 5 min daily for 5–7 days. All dependent variables were analyzed by one-way analysis of variance (ANOVA) and a significance level of $P < 0.05$ was used for all comparisons. **RESULTS:** IGF-1 concentrations in cerebellum were not different between groups. We found some differences in the cerebellar tissue of trained diabetic rats and controls when analyzed qualitatively by the HE method. **CONCLUSION:** It was concluded that in diabetic rats, aerobic training does not induce alterations on cerebellum IGF-1 but changed some metabolic functions. After 8 weeks of aerobic training was possible to observe qualitative differences in cerebellar tissue of diabetic rats.

Introduction

The impact of diabetes mellitus on Central Nervous System (CNS) function has been demonstrated at neurochemical, electrophysiological, structural and behavioral levels[1,2,3,4].

For many years, it had believed that insulin has not exerted important role in metabolism, growth and brain development[5]. As neuronal cells are not dependent on insulin for glucose uptake, scientists directed their studies for the traditional sites of insulin action. Havrankova et al[6] described the presence of insulin and insulin receptors in the brain and, since then, a growing number of studies have been conducted with the objective of advancing the knowledge and characterizing the actions of insulin in the CNS.

Insulin and Insulin-like Growth Factor-1 (IGF-1) belong to the same protein family, having biological action wide variety in CNS development[7,8,9,10].

Insulin-like growth factor-I (IGF-1), a member of the insulin super family and a well characterized mitogen, is expressed in the cerebellum on its development[11,12], exhibiting either in mouse or in rat brain IGF-I mRNA peak expression, during the first two postnatal weeks of life[13,14], when the cerebellum grows rapidly and the generation of cerebellar granule cells take place[15].

While type I IGF receptors are highly expressed in all types of cerebellar cells[16], a high density of IGF-I binding has been observed in external granular layer cells of the rat cerebellum during early development[17], once its spatiotemporal expression correlates with the timing of cerebellar development, IGF-I, may play an important role in the cerebellum development.

The cerebellum has been known as a critical brain structure for the coordination and control of voluntary movements[18,19]. However, recent evidence indicates that the cerebellum also plays a role in cognitive, behavioral and emotional functions[20,21,22,23].

It has been stated that moderate exercises increase cognition. Moreover, it will be demonstrated that the brain is responsive to physical activity[24,25], showing that physical activity presents potential in the prevention and treatment of cerebral traumatic damage[26] as well as in neurodegenerative diseases such as Parkinson disease[27] and Alzheimer's disease[28,29]. Studies support that many of these alterations occur in specific areas of important brain functions e.g. long-run memory[30]

and prevention of cognitive decline during the aging process[44]. Some studies also demonstrate evidence on neurogenesis and brain plasticity[31] specifically induced by families of neurothrophic molecules[32,33]. However, the mechanisms of these alterations have still been unknown.

Other studies showed that several types of human cerebellar degeneration are accompanied by changes in the peripheral IGF-I system which are similar, although not identical, to those found in diabetes[34,35]. However, there are few studies assessing the cerebellum of diabetic organisms, particularly of trained animals. In this present work, the variables of the IGF-1 growth system in cerebellum of sedentary and trained diabetic rats and the cerebellar granule cells quality in these different groups have been studied.

Materials and Methods

Animals

Forty-eight male Wistar rats (75-days-old) from Sao Paulo State University—UNESP—Botucatu Campus were used in all experiments. Every experiment involving animals had been conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose (Council of Europe N° 123, Strasbourg, 1985). The rats were housed in room at 25°C under 12-h light and 12-h dark periods. Rats had free access to standard food and water *ad libitum*.

Induction of Diabetes

Diabetes was induced by an intravenous injection of alloxan (32mg kg^{-1} b.w.; Sigma). After 72h of alloxan injection, fasting blood estimated, and levels above 250 mg/dL confirmed diabetes resulting from a damaged pancreas. We considered only the diabetic animals with blood glucose between (glucose < 264.6mg/dL) and (glucose > 639mg/dL).

Aerobic training

The rats were randomly allocated to one of four groups (n = 10 per group): sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). The training had been developed in a swimming session, 5 times/week, 1h with a load corresponding to 90% of the maximal lactate steady state (MLSS), during 8 weeks. For MLSS determination the rats had been submitted to three swimming sessions were submitted to three swimming sessions in a 100 cm x 80 cm x 80 cm tank with water at the temperature of 31±1°C performed with 25 min intervals of 1 week between them, supporting loads of 4, 5, or 6% of body wt with intervals of 1 week between them. Blood samples (25 µl) had been collected every 5 min from a cut in the tip of the tail for lactate determination (adapted from Gobatto et al. 2001)[36]. The highest concentration during exercise which lactate appearance in blood equals its removal, has been known as ‘‘maximal lactate steady state’’ (MLSS) and has been considered an important endurance exercise capacity indicator. Figure 1 shows the values of the weight percentages of found loads equivalent to MLSS of control and diabetic groups.

Serum and tissue analyses

A standard type of extraction procedure was used [22]. At the end of experimental period, the rats were sacrificed 48hr after their last exercise bout by decapitation. Trunk blood was collected in a glass tube, centrifuged for 5 min at 3000 rpm for glucose, insulin and hematocrit determinations. Blood samples for hematocrit were collected before sacrifice trough a small cut at the top of the tail. These evaluations aimed the alloxan efficiency verification in inducing a diabetic picture in the animals in the conditions of the present study and also evaluating the aerobic training protocol efficiency.

Analytical methods

Serum glucose concentration was measured by a colorimetric method. Serum insulin concentration was determined by radioimmunoassay (RIA Kit Coat-A-Count-USA). The cerebellum was weighted and homogenized in 5ml of insulin extractor liquid, centrifugued (3000 rpm) and 2 ml of the supernatant were taken for insulin determination by radioimmunoassay. The IGF-1 peptide supernatant concentration

was measured by Immunoradiometric assay (IRMA) using a commercially available kit (DSL-5600, Diagnostic System Laboratories Kits, Webster, TX).

Histological Analyses

The brains will be processed and embedded in paraffin after being removed from the cranial cavity. Subsequently, the pieces will be trimmed and set in a wooden holder and forwarded to the microtome, to provide tapes with thickness of 12mm. The cuts will be made in the coronal plane, 4.80 - 5.30 mm in relation to Bregma, and then the processed tapes will pass through histological bath, and where the slides will be mounted with 6 cuts on average which had already been previously gelatinized. The experimental protocol was Hematoxylin Eosin, developed according to SPICER (1987)[37].

Statistical Analyses

Results are expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using SPSS (version 10.0) and student's't'-test using Sigma Plot (version 8.0). The Bonferroni test was used for post hoc comparisons. The values of $P < 0.05$ were considered as statistically significant.

Results and Discussion

Figure 1 shows the difference between the body weight initial values (first week) and final ones (last week). In this parameter, the reduction caused diabetes and, physical training did not alter these values in controls or in diabetic animals.

The moderate intensity of exercise was selected. Thus the physical training had been conducted under the aerobic / anaerobic metabolic transition. Recent studies[38] have also used intensity of 90% of MLSS, showing that moderate exercise is effective metabolic variables in diabetic rats. Gobatto et al.[36] showed that swimming training at the MLSS was as efficient as treadmill running training in improving the physical condition of rats.

Table 1 shows that diabetes resulted in hyperglycemia and hypoinsulinemia in both SD and TD groups ($p<0.05$). However the training induced a reduction in blood glucose in TD. Physical training induced an increase in muscle glycogen stores

(gastrocnemius) in TC and TD (TC≠SC; TC≠SD; TD≠SC; TD≠SD) (Figure 2). Hematocrit was similar among all groups (Table 1).

Recent study[39] has also shown that chronic exercise decreases serum glucose in diabetic animals and humans because skeletal muscle responds to chronic exercise through a series of structural and functional adaptations.

Figure 3 shows that differences have not been found on IGF-1 concentrations in cerebellum of diabetic rats after 8 weeks of aerobic training. Recent studies[40] showed that Physical training recovered liver glycogen and increased serum and cerebellum IGF-1 peptide in diabetic rats. However, the sample was composed for Male Wistar rats (175–200 g; 38 days old), different from the present study in which the sample was composed of Male Wistar rats (75-days-old). Thus, non difference in the levels of IGF-1 in the cerebellum of the trained groups in this study may be related to the different factors that interfere on the levels of GH and IGF-1, among them the age or stage of the organism development can be highlighted[41].

IGF-I is involved in different processes in the nervous system development, such as neurogenesis, myelination and synaptogenesis[42]. In particular, this hormone plays important roles in the control of cellular processes like proliferation, survival and differentiation[43]. The effects of the absence of IGF-I on the nervous system are extremely serious. Mice that do not express IGF-I show a decrease in length of the dendrites as well as changes in axonal growth and dendritic arborization[44]. Mutations that lead to IGF-1 receptor loss of expression induces the appearance of microencephaly brain and mental retardation in humans brains[45].

Popken and co-workers[46] showed that IGF-I is widely expressed in the SNC, during a time dependency and coincidentally with neuronal development. In mice the expression peak of the IGF-I occurs during embryonic between E11 and E17, in which period the proliferation of neural progenitors also occurs[47].

Gomes et al[48], found that after six weeks of aerobic training, the diabetic group had significantly lower concentrations of serum GH, compared with the control group, but there was no effect of training for that hormone. This study results confirm that the production of IGF-1 is not necessarily dependent neither on serum levels of GH nor insulin and the moderate physical activity is important for diabetic organisms, improving some endocrine aspects.

Another objective of the study was to analyze qualitatively the cerebellar tissue of trained diabetic rats and controls by the HE method (hematoxylin and eosin).

Figures 4-7 show the sedentary control groups, trained control, sedentary diabetic and trained diabetic, respectively. It can be seen in Figures cell bodies; fat cells, Purkinje cells and basket cells. In the control groups (Figures 4 and 6) may be noted higher amounts of fat cells and minor amounts of cell bodies compared to the trained groups (Figures 5 and 7). The large Purkinje cells correspond to the effector pathway or the cerebellar cortex output and are most abundant in the trained groups (Figures 5 and 7).

As known, the molecular layer contains stellate cells located superficially and dendrites of Purkinje cells. The Purkinje cell layer contains cells in basket shape, called the cerebellum piriform cells or Purkinje cells, which occur exclusively in the cerebellum.

The Purkinje cells are the dominant elements in cerebellar information processing[49] being the only neurons that send their axons out of the cerebellar cortex[50] using GABA as the neurotransmitter[51]. The granular layer contains small glomeruli and granular cells in adult rats. The granule neurons represent approximately 95% of mature cells of the cerebellum. Purkinje cells are the main forming of synapses with other cells in the cerebellar cortex in the molecular layer[52]. The establishment of several synaptic connections avoids apoptosis of neurons which have a higher number of connections, once these cells are produced in excess throughout the neural system[53]. In Figures 2 and 4 it can be seen that the Purkinje cells stand out when analyzed by HE method, and apparently, there are greater quantities of granular cells, which may be indicative of the protective effects of physical exercise. Recent studies[54] have evaluated the induction of c-fos (a marker of neuronal activation) and expression of BDNF (brain derived neurotrophic factor) in rat hippocampus in response to forced acute exercise (seven days running) in different intensities and observed that the RNAm to the c-fos had been increasing even in rats which was submitted in low intensity running (30min/day to 15min/dia). Processes such as neurogenesis are increased after exercise[55.] Some studies have shown, though, physical exercise can affect hippocampal neurochemistry[56], neuronal activity, the expression of trophic factors and the proliferation and the survival of granule cells.

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Authors' contributions

Arantes, L.M. conceived the study, developed the study protocol, reviewed the references, collected and analyzed the data, and wrote the paper. Bertolini, N.O., Moura, R.F., Mello, M.A.R., participated in the design of the study, reviewed the manuscript, collected the data, and collaborated on the biochemical dosages. Luciano, E. conceived the study, participated in its design and coordination and helped in the drafting of the manuscript. All authors read and approved the submission of the final manuscript.

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Table 1. Blood glucose (mg/dL), serum insulin (pmol L^{-1}) and hematocrit (%) after 8 weeks of training

Parameters	SC	TC	SD	TD
Blood glucose (mg/dL)	129.81±38.4	113.4±11.5	374.3±13.1[a,b]	347.1±83.5[a,b]
Serum insulin (pmol L^{-1})	0.38±0.18	0.39±0.19	0.04[a,b]±0.2	0.04[a,b]±0.01
Hematocrit (%)	58±1.2	56±2	57±4.4	57±4.9

SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic. ANOVA; $P<0.001$; a≠SC; b≠TC.

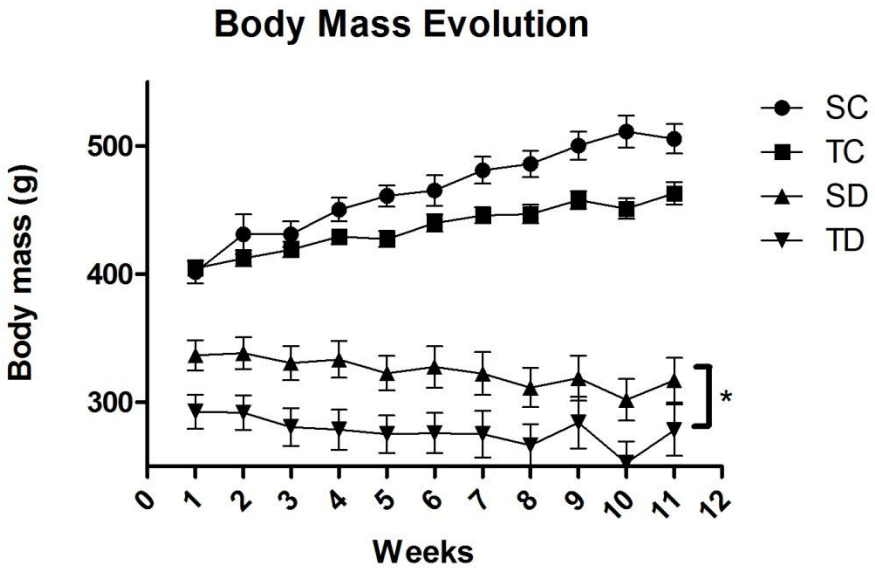

Figure 1. Difference between the initial values (first week) and final (last week)

of body weight. [The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic. *TD≠SD

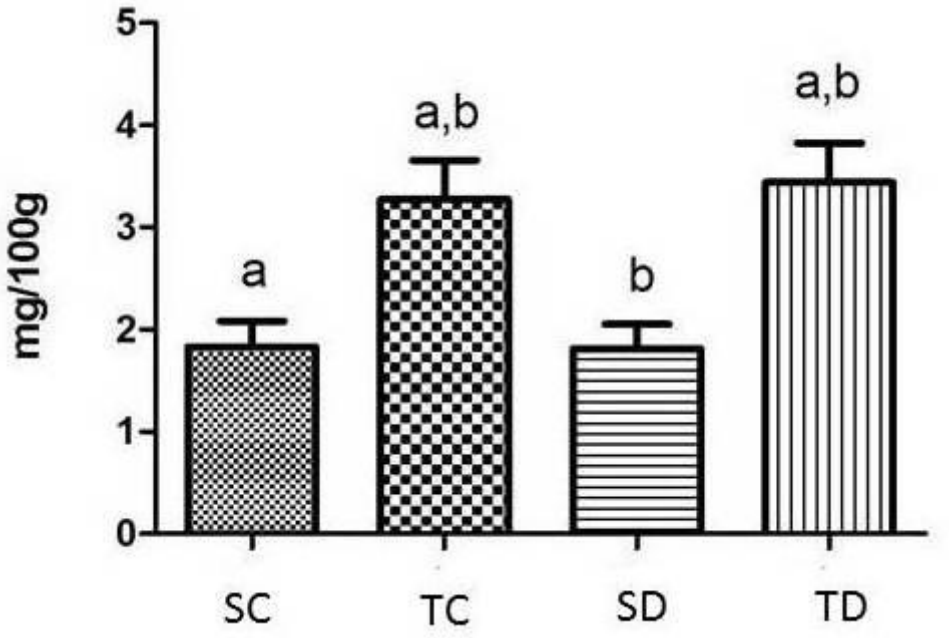

Figure 2. Glycogen stores (gastrocnemius) after 8 weeks of aerobic training. [The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control, TC – trained control, SD – sedentary diabetic, TD – trained diabetic. a≠SC; b≠SD.

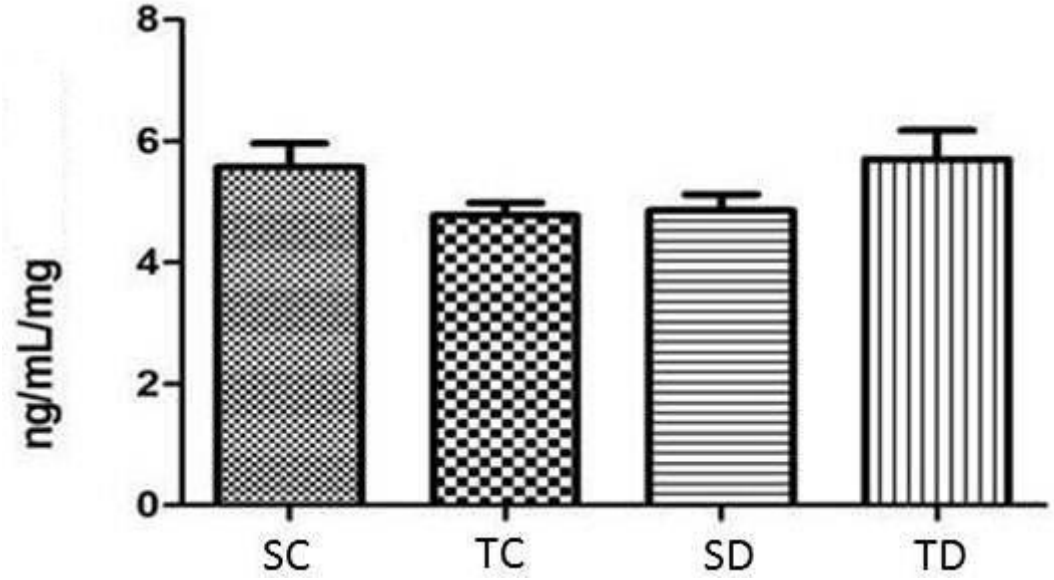

Figure 3. IGF-1 concentrations of cerebellum (ng/mL/mg) after 8 weeks of aerobic training.

[The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control,

TC – trained control, SD – sedentary diabetic, TD – trained diabetic.

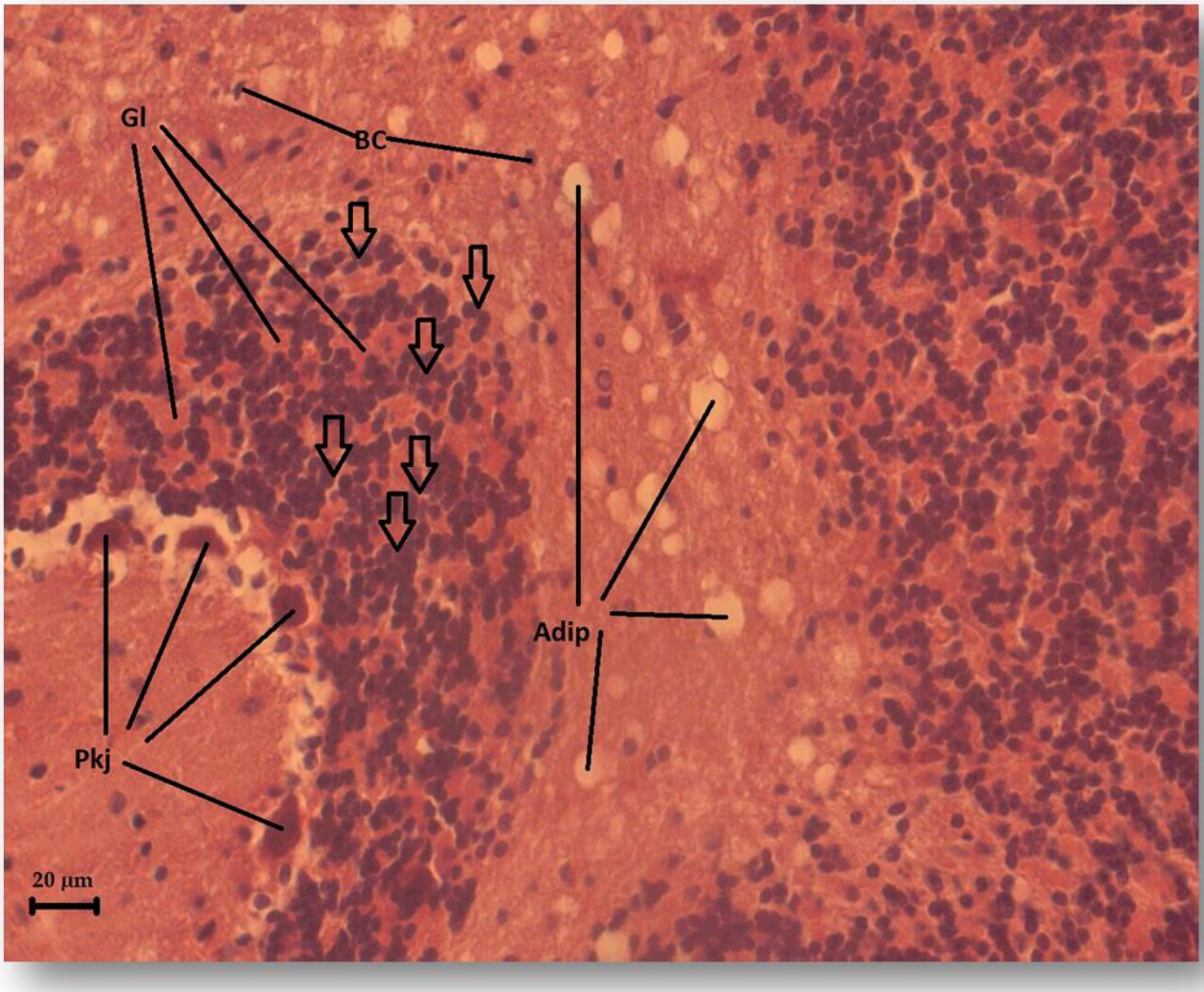

Figure 4. Photomicrograph of layer from the cerebellum, stained with HE (hematoxylin - eosin) from the sedentary control group. Arrows represent cell bodies; ADIP - fat cells; PKJ - Purkinje cells, BC - basket cells, Gal - glomeruli. Bar: 20µm.

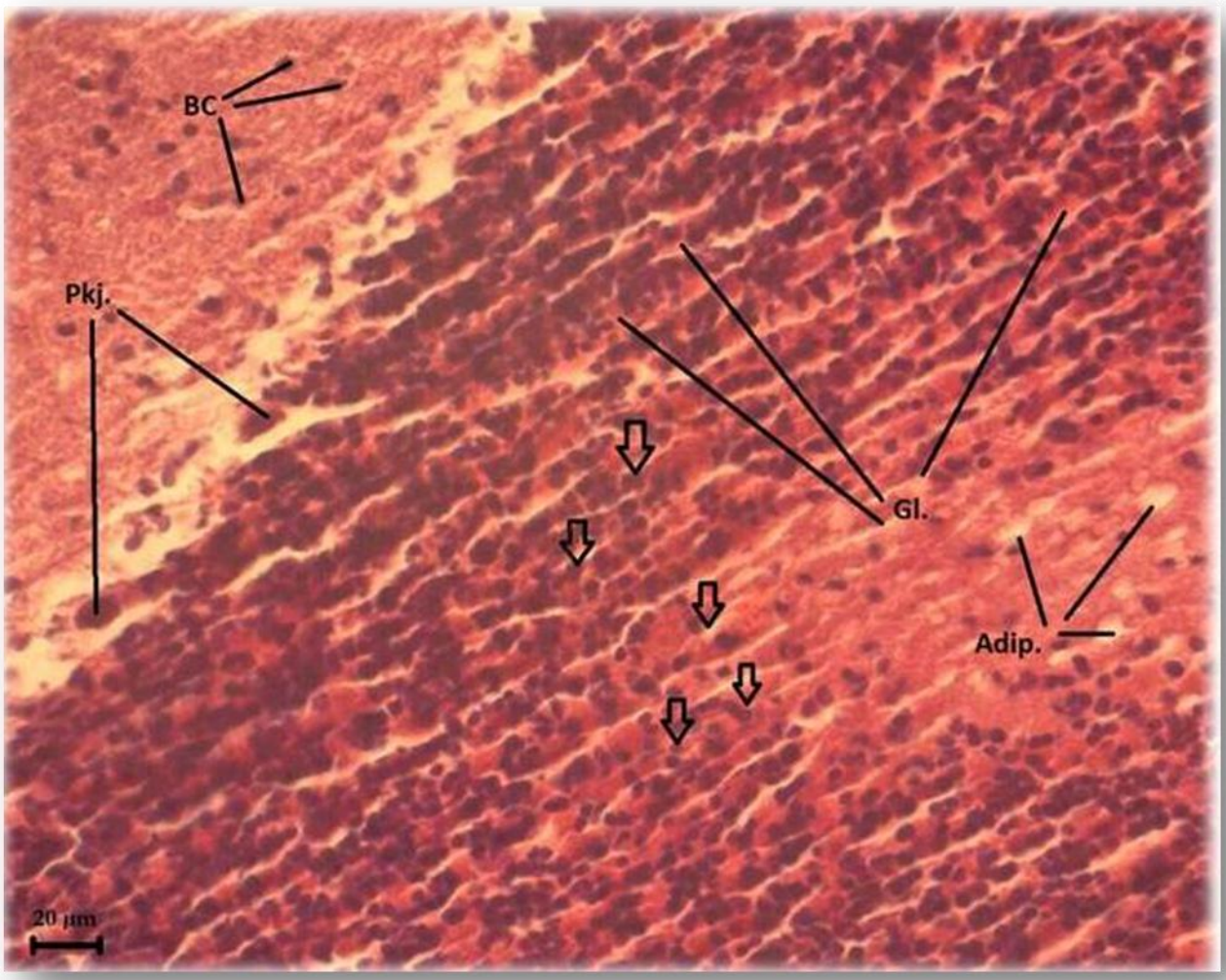

Figure 5. Photomicrograph of layer from the cerebellum, stained with HE (hematoxylin - eosin) from the training control group. Arrows represent cell bodies; ADIP - fat cells; PKJ - Purkinje cells, BC - basket cells, Gal - glomeruli. Bar: 20µm.

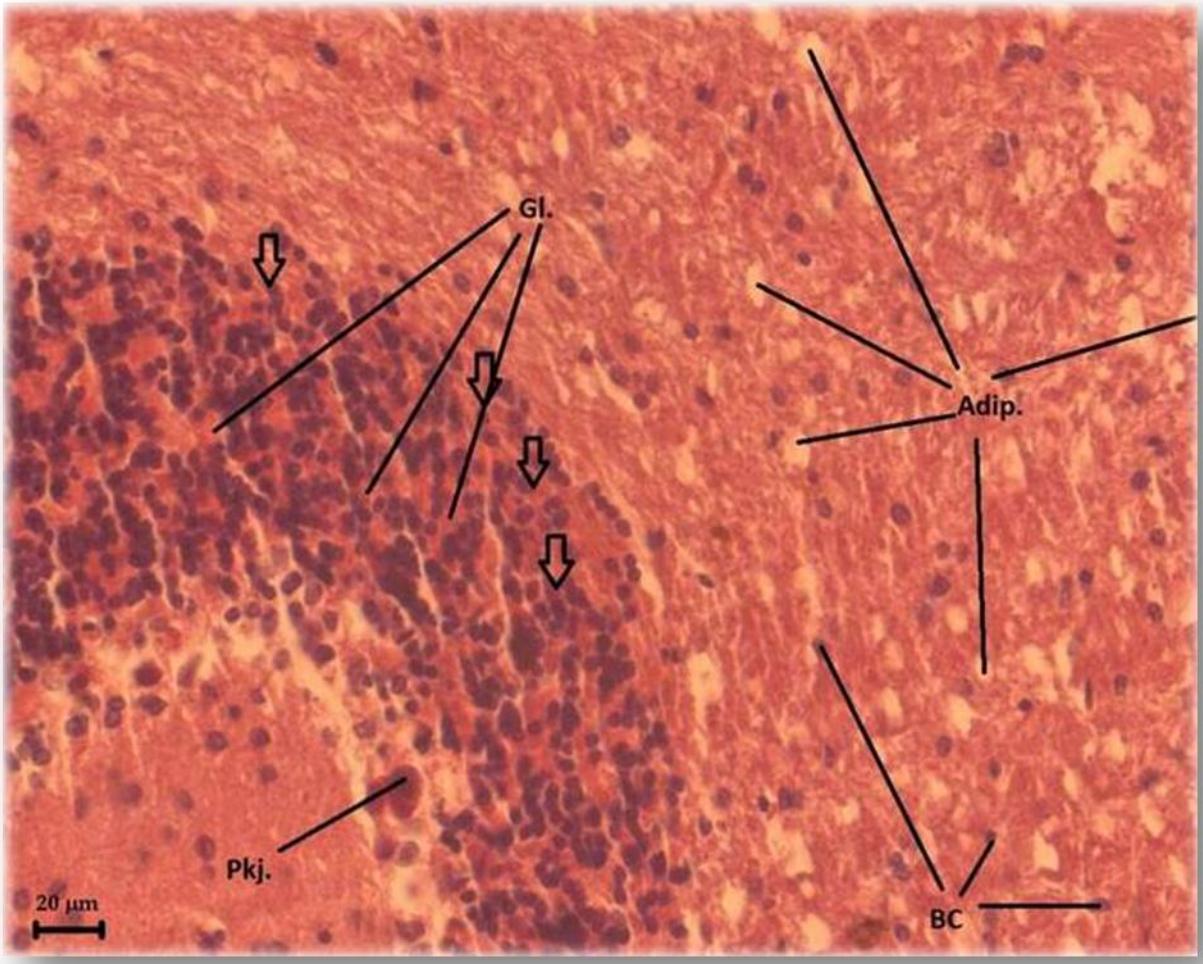

Figure 6. Photomicrograph of layer from the cerebellum, stained with HE (hematoxylin - eosin) from the sedentary diabetic group. Arrows represent cell bodies; ADIP - fat cells; PKJ - Purkinje cells, BC - basket cells, Gal - glomeruli. Bar: 20µm.

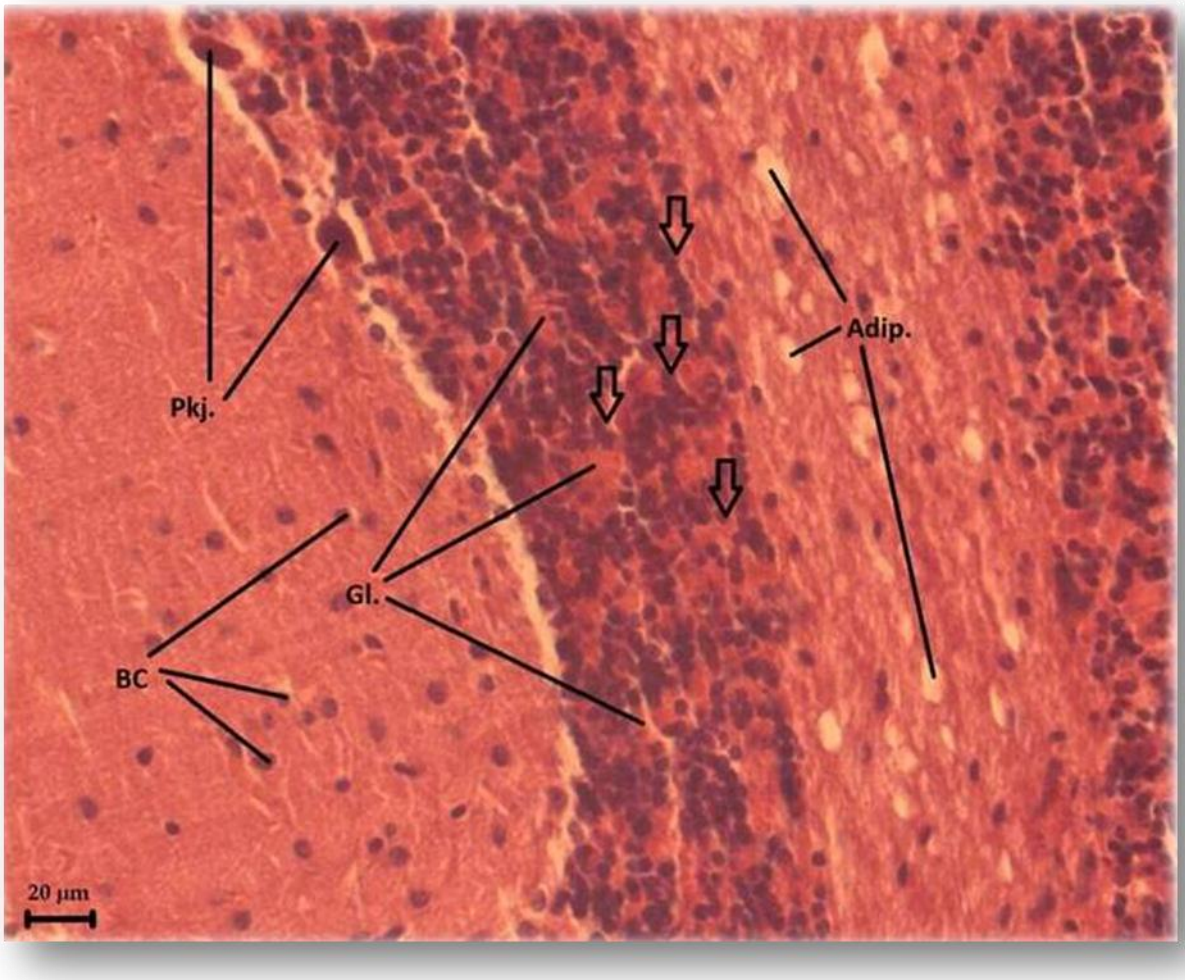

Figure 7. Photomicrograph of layer from the cerebellum, stained with HE (hematoxylin - eosin) from the training diabetic group. Arrows represent cell bodies; ADIP - fat cells; PKJ - Purkinje cells, BC - basket cells, Gal - glomeruli. Bar: 20µm.

6.3 ARTIGO 3 - MORPHOLOGICAL ASPECTS OF GRANULAR CELLS IN CEREBELLUM OF DIABETIC RATS IN RESPONSE TO AEROBIC TRAINING

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MORPHOLOGICAL ASPECTS OF GRANULAR CELLS IN CEREBELLUM OF DIABETIC RATS IN RESPONSE TO AEROBIC TRAINING

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Running title: Aerobic training effects in diabetic rats.
Key words: Morphological aspects; cerebellum; aerobic training
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Competing interests: The authors declare they have no competing interests.

ABSTRACT

There is a large anatomical, functional and biochemical changes in the central nervous system (CNS) of diabetic animals. **PURPOSE:** The purpose of study was to analyze the effects of exercise training on the morphological aspects of granular cells. **METHODS:** Forty Male Wistar rats were divided in four groups: sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). Diabetes was induced by Alloxan (32 mg kg b.w.), single dose injection. The mean blood glucose of diabetic groups was 367±40 mg/dl. Training program consisted in swimming 5 days/week, 1 h/day, 8 weeks, supporting a workload corresponding to 90% of maximal lactate steady state (MLSS). For the body balance testing rats were trained to traverse for 5 min daily for 5–7 days. All dependent variables were analyzed by one-way analysis of variance (ANOVA) and a significance level of $P < 0.05$ was used for all comparisons. **RESULTS:** Trained animals obtained a reduction in serum triglycerides. We found some differences in the cerebellar tissue of trained diabetic rats and controls when analyzed qualitatively by the Touluidine Blue method. **CONCLUSION:** It was concluded that in diabetic rats, aerobic training changed some metabolic functions. After 8 weeks of aerobic training was possible to observe qualitative differences in cerebellar tissue of diabetic rats.

Introduction

Diabetes Mellitus (DM) is classified as a group of metabolic diseases characterized by hyperglycemia caused by defects in the secretion and / or in the insulin action, anabolic polypeptide mainly produced in the beta cells of the pancreas[1].

Some studies have reported large anatomical, functional and biochemical changes in the central nervous system (CNS) of diabetic animals[2,3]. This variety of changes (usually named as diabetic neuropathy) affects the brain, spinal cord and peripheral nerves. These changes had been reported as degenerative changes in the diabetic rat nervous system with ganglionic tissue degeneration, reduction of demyelization and axonal caliber[4,5,6,7].

The central nervous system consists of neurons and glial cells, beginning its development still in the embryonic period and ending in postnatal life, either in rodents or in humans. During its development, some temporal windows occur, which are crucial to its full development. Any disturbance occurrence in these time intervals can cause cellular changes such as neural migration events, cell death by apoptosis or necrosis, among others. Such events could compromise neural function throughout life[8].

In the CNS, the diabetes reduces the brain weight and the neocortical volume, which has been associated with a reduction in the cortical neuron number[9]. All these central and peripheral changes are consistent with the decrease in the neuronal activity[10]. This decrease in neuronal activity may be related to the cognitive impairment aspects that occur in animal models of DMT1 and DMT2[11].

Whereas the cerebellum regulates the movement and the balance, adjusting and getting information, receiving afferents of the brain descending motor systems, lesions in this region can cause coordination disturbance of the eye movements and the limbs and balance deficits and decreased muscle tone[12]. From the histological point of view, the adult rat cerebellar cortex has three layers: the molecular layer (outer); Purkinje cells (lymph node) and the granular layer (internal) and contains six neuronal types[13], being Purkinje cells the main neuronal type. Granule cells are the most abundant type in the normal adult rat cerebellum: 80% of total neuronal cells, 85% of these cells are granular.

Moreover, some studies in rats have shown the benefits of exercise training in diabetes-induced metabolic alterations. Also, there is no data currently available on the effects of exercise training on the morphological aspects of granular cells.

Materials and Methods

Animals

Forty-eight male Wistar rats (75-days-old) from Sao Paulo State University—UNESP—Botucatu Campus were used in all experiments. Every experiment involving animals had been conducted according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose (Council of Europe N° 123, Strasbourg, 1985). The rats were housed in room at 25°C under 12-h light and 12-h dark periods. Rats had free access to standard food and water *ad libitum*.

Induction of Diabetes

Diabetes was induced by an intravenous injection of alloxan (32mg kg $^{-1}$ b.w.; Sigma). After 72h of alloxan injection, fasting blood estimated, and levels above 250 mg/dL confirmed diabetes resulting from a damaged pancreas. We considered only the diabetic animals with blood glucose between (glucose < 264.6mg/dL) and (glucose > 639mg/dL).

Aerobic training

The rats were randomly allocated to one of four groups (n = 10 per group): sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). The training had been developed in a swimming session, 5 times/week, 1h with a load corresponding to 90% of the maximal lactate steady state (MLSS), during 8 weeks. For MLSS determination the rats had been submitted to three swimming sessions were submitted to three swimming sessions in a 100 cm x 80 cm x 80 cm tank with water at the temperature of 31±1°C performed with 25 min intervals of 1 week between them, supporting loads of 4, 5, or 6% of body wt with intervals of 1 week between them. Blood samples (25 µl) had been collected every 5 min from a cut in the tip of the tail for lactate determination (adapted from Gobatto et al. 2001)[14]. The highest concentration during exercise which lactate appearance in blood equals its removal, has been known as ‘‘maximal lactate steady state’’ (MLSS) and has been

considered an important endurance exercise capacity indicator. Figure 1 shows the values of the weight percentages of found loads equivalent to MLSS of control and diabetic groups.

Serum and tissue analyses

At the end of experimental period, the rats were sacrificed 48hr after their last exercise bout by decapitation. Trunk blood was collected in a glass tube, centrifuged for 5 min at 3000 rpm for glucose and hematocrit determinations. Blood samples for hematocrit were collected before sacrifice trough a small cut at the top of the tail. These evaluations aimed the alloxan efficiency verification in inducing a diabetic picture in the animals in the conditions of the present study and also evaluating the aerobic training protocol efficiency.

Histological Analyses

The brains will be processed and embedded in paraffin after being removed from the cranial cavity. Subsequently, the pieces will be trimmed and set in a wooden holder and forwarded to the microtome, to provide tapes with thickness of 12mm. The cuts will be made in the coronal plane, 4.80 - 5.30 mm in relation to Bregma, and then the processed tapes will pass through histological bath, and where the slides will be mounted with 6 cuts on average which had already been previously gelatinized. The experimental protocol was Touluidine Blue, developed according to SPICER (1987)[15].

Statistical Analyses

Results are expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) using SPSS (version 10.0) and student's 't'-test using Sigma Plot (version 8.0). The Bonferroni test was used for post hoc comparisons. The values of $P < 0.05$ were considered as statistically significant.

Results and Discussion

Tracking the animals throughout the experimental period, it was found that the body weight gain was significantly lower among diabetics, but with a partial recovery among the diabetics and the trained ones (Figure 1). In this parameter, the reduction

caused the diabetes and the physical training did not alter these values in controls or in diabetic animals.

Glycemia in sedentary diabetic rats was significantly elevated when compared to the controls, but decreasing among the diabetic trained ones (Figure 2). These data corroborate the findings of Leme et al[16] who analyzed blood glucose after aloxan administration and serum insulin concentrations after 4 weeks of aerobic training in diabetic rats.

In figure 3, it can be seen that trained animals obtained a reduction in serum triglycerides. In a study by Tanaka et al[17], diabetic non-insulin-dependent patients who had undergone eight weeks of regular physical activity had a decrease in serum triglycerides. The authors related these adaptations in diabetic patients to an improvement in the peripheral insulin sensitivity caused by physical training. It also promotes benefits in other mechanisms such as decreased blood pressure, lower levels of LDL and triglycerides in the blood plasma and in the inhibition of platelet aggregation[18].

Therefore, in general, the chronic physical exercise plays an important role in the intervention of glucose and lipid metabolism of the body subjected to experimental diabetes.

Representative images of histological preparations are presented in Figures 4-7. Touluidine blue sections of cerebellum were evaluated under light microscopy. (Fig. 4-7).

According to Welz[19] the double coloring used is able to staining living neurons characterized by the presence of cytoplasm with a quite evidence and bluish nucleolus (toluidine blue), and either dead or degenerating neurons characterized by the absence of natural way as matted reddish (acid fuchsin). The aim of this study was to analyze qualitatively possible differences in the four groups (SC, TC, SD, TD), represented by figures 4-7, respectively.

TC and TD groups (Figures 5 and 7), it can be seen that the cell bodies (structural zone of the neuron, the major volume of the nerve cell local) are more abundant and more bluish stain when compared with the sedentary groups (Figure 4 and 6), which can be characteristic of living neurons.

The Central Nervous System has highly specialized cells that make multiple connections and are responsible for several functions. The natural aging process and some diseases that affect the brain and lead to loss of neurons (specialized cells)

interfere in the cognitive function, the memory and negatively affect the life quality of people. As known the physical training improves the functioning and metabolism, triggering neuronal health benefits and cognitive brain, by increasing cerebral blood flow and consequently increase in brain oxygenation[20].

According to Coelho et al[21], studies show that physical exercise induces an increasing in the neurotrophic factor levels in the hippocampus, frontal cortex, and cerebellum; promoting the cell survival (neurons) and neural plasticity. Aerobic exercises preserve the volume of the hippocampus, improving the spatial memory performance.

In groups SC and SD (Figures 3 and 5), it is possible to observe higher amounts of fat cells and larger pink spaces between the granulosa cells which are complex synapses called cerebellar glomeruli when compared to the trained groups (Figure 4 and 6). In the glomerulus, mossy fiber called an axon ends in a small bulb, which makes synapses with dendrites of near granule cells and dendrites of Golgi cells.

The histological changes resulting from diabetes are present in various tissues since this is a systemic disorder. Much of the histological changes may be caused by the accumulation of AGEs (advanced glycation end productions) in the tissue. The interaction between AGEs and endothelial cells results in an increase in the thickness of the blood vessel wall, impairing the supply of oxygen and nutrients. These vascular changes are directly related to the incidence and progression of other diabetic complications such as neuropathy, nephropathy and retinopathy[22].

Experimental studies have demonstrated that neurons of the dorsal root ganglion (DRG) of diabetic rats show reduced volume[23,24], they have plenty of lipofuscin, an indicative of premature aging, and there is a reduction in blood flow DRG 40%, which causes hypoxia, contributing to injury of DRG neurons[25]. According to Hellweg et al[26], the higher the blood glucose, the lower the axonal transport of NGF (nerve growth factor).

In the four groups (Figures 3-6) it is possible to observe the presence of Purkinje cells. Purkinje cells of phylogenetically belong to the cerebellar neocortex, the evolutionarily the newest region and with a cytoarchitecture characterized by three layers: molecular, Purkinje and granular (1-3). The Purkinje cell is the dominant element of the cerebellar information process and can show degenerative changes in senescence that can be noticed by simple staining methods such as H & E[27].

There is still a range of studies involving exercise, diabetes and cerebellum, these vary greatly from model and exercise configuration to the variables and methodologies used, as well as studies examining the histology of the brain, specifically the cerebellum of diabetic rats trained, which decrease the capacity of comparing the results.

Therefore, the aim of this study was mainly analyze qualitatively differences of trained and untrained diabetic rats' cerebellar tissue, thus enabling further studies with more specific techniques able to evaluate the development of neurons, if the necrosis only occurs by the disease and if the aerobic physical exercise training is able to regenerate some neurons. Thus, there is certainly a need to better understand the effects and mechanisms of physical exercise actions in the central nervous system.

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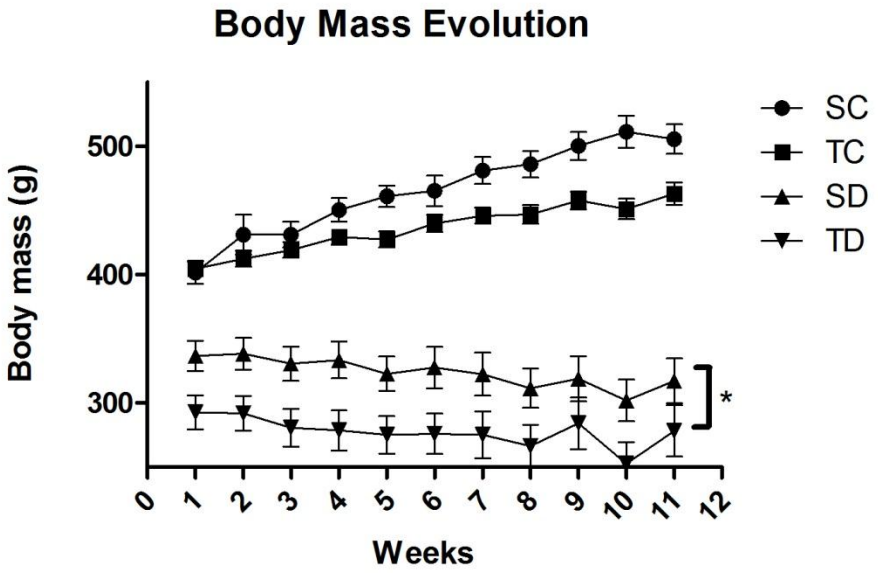

Figure 1. Difference between the initial values (first week) and final (last week)

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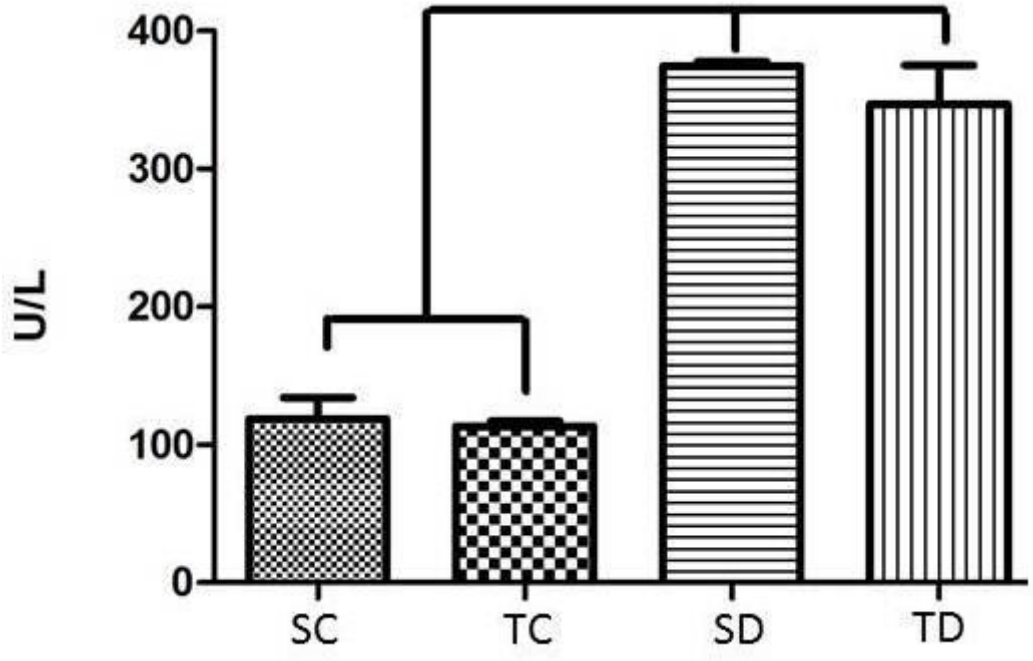

Figure 2. Glycemia after 8 weeks of aerobic training. [The values are the mean ± SD

of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control, TC – trained control,

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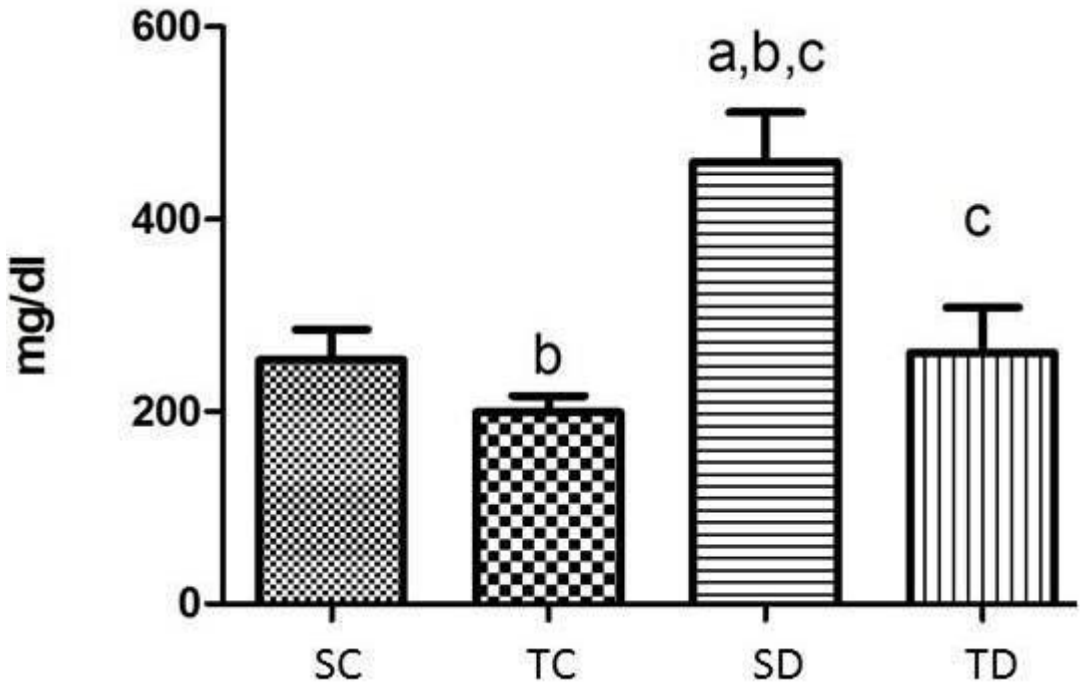
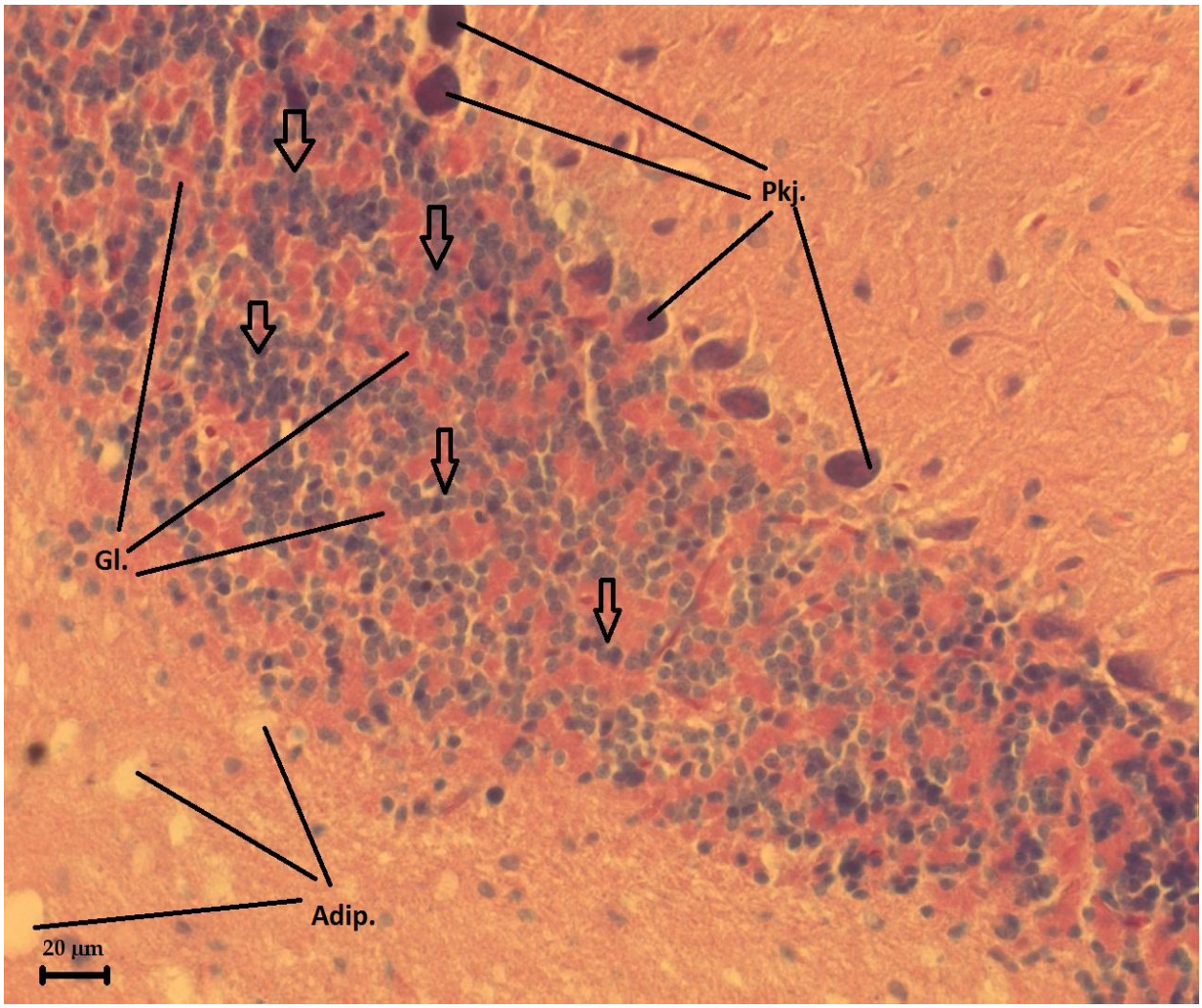


Figure 3. Serum triglycerides (mg/dl) after 8 weeks of aerobic training.

[The values are the mean ± SD of 10 rats per group. ANOVA $p<0.05$]. SC – sedentary control,

TC – trained control, SD – sedentary diabetic, TD – trained diabetic.



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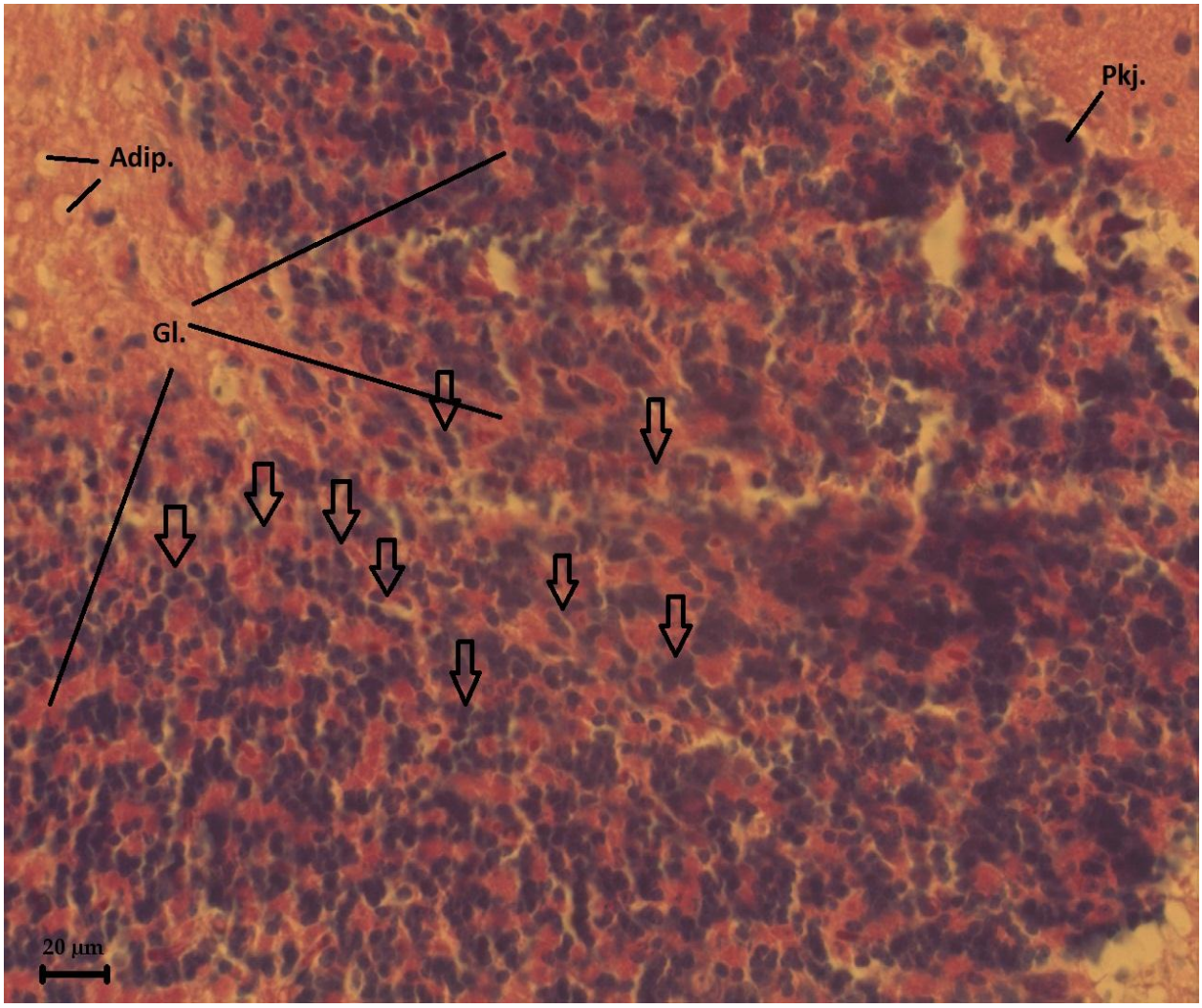

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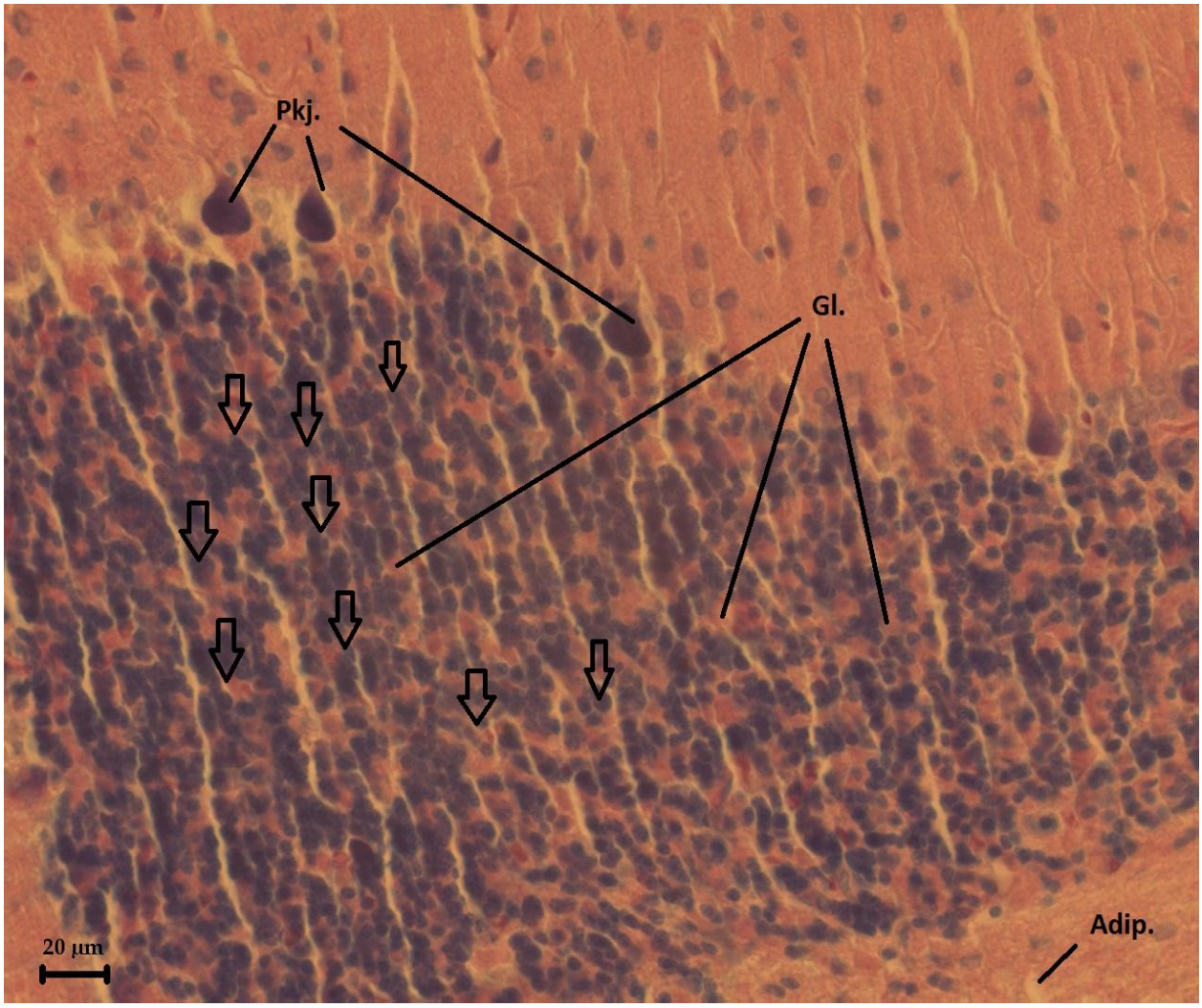

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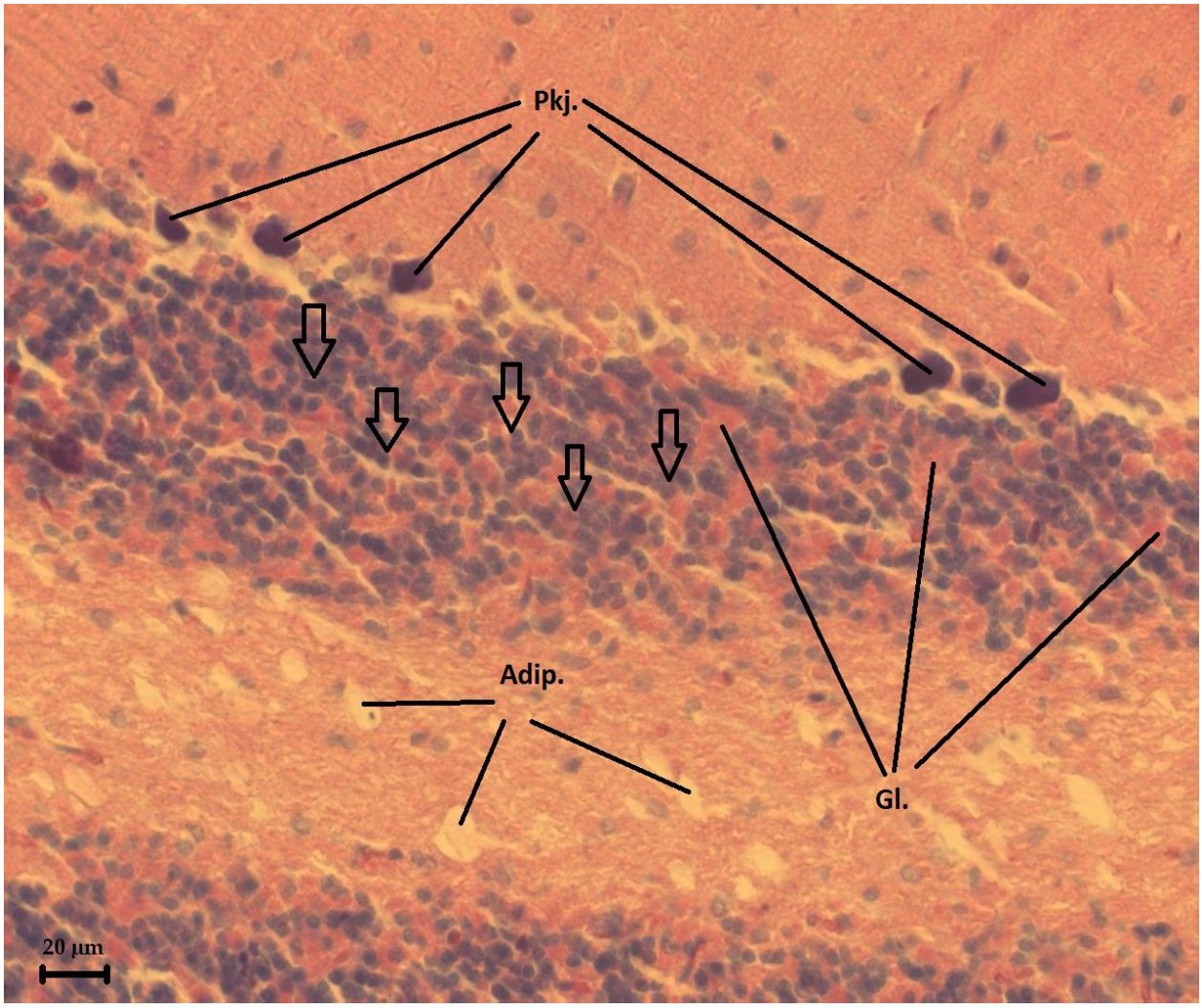

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7. CONCLUSÕES

Este trabalho teve como objetivo geral analisar os efeitos do treinamento físico aeróbio no perfil endócrino-metabólico de ratos diabéticos, com maior ênfase no tecido cerebelar. Os resultados apresentados em forma de artigos científicos nos permitem concluir que:

- Os valores de glicemia e trigliceridemia foram aumentados nos animais diabéticos (DS e DT) e ambos foram reduzidos no grupo diabético treinado quando comparados aos valores do grupo diabético sedentário.

- A indução do diabetes reduziu os valores de insulinemia nos animais de ambos os grupos (DS e DT) e o treinamento físico não alterou os valores deste parâmetro.

- Nenhuma alteração no hematócrito foi observada entre os grupos em estudo, isto sugere que as alterações observadas na glicose sanguínea, provavelmente, induziram a desidratação.

- O treinamento aeróbio não modifica as concentrações de IGF-1 e Insulina no cerebelo.

- Foi observado diferenças no equilíbrio corporal de ratos diabéticos e controles. O grupo diabético apresentou menor desempenho que o grupo controle, mostrando maior dificuldade nas atividades que requerem equilíbrio.

- Com relação à histologia, observamos diferenças qualitativas nos diferentes grupos estudados.

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ANEXO 1. PARECER DO COMITÊ DE ÉTICA E PESQUISA