UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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Tailene Rabello da Silva

EFEITOS DO EXERCÍCIO ANAERÓBICO GESTACIONAL SOBRE O DESENVOLVIMENTO NO PERÍODO NEONATAL DE FILHOTES DE RATOS WISTAR.

Porto Alegre
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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharel(a) em Biomedicina.

Orientador: Profª Drª Simone Marcuzzo
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BANCA EXAMINADORA

Profª Drª Tatiana Luft

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RESUMO

O exercício físico aeróbico no período gestacional causa diversos efeitos benéficos na prole, como um incremento da cognição, da neurogênese hipocampal e da expressão de fatores de neuroplasticidade. Embora alguns efeitos do exercício gestacional sobre a saúde da prole já sejam conhecidos, poucos estudos têm explorado os efeitos do exercício físico na modalidade anaeróbico. Esse estudo foi desenvolvido para avaliar o desenvolvimento da prole de mães submetidas ao exercício anaeróbico gestacional. O protocolo de treinamento consistiu em subir uma escada com um peso afixado à cauda. Por meio de marcação celular por BrdU, os dados mostram que os filhos de ratas Wistar exercitadas apresentaram aumento da neurogênese observado na zona do hilo do giro denteado no dia pós-natal (P) 8. Também foi possível observar diferenças no desenvolvimento corporal dos filhotes. Ao mesmo tempo em que os filhotes de mães exercitadas apresentaram menor peso corporal, em comparação aos filhotes de mães sedentárias, nos dias pós-natais 7 e 14, eles apresentaram o crânio com dimensões maiores. O protocolo de exercício não gerou alterações no desenvolvimento motor. Não foram observadas diferenças significativas no peso das glândulas adrenais dos filhotes ou das mães após a realização do protocolo de exercício anaeróbico, o que indica que o protocolo utilizado nesse estudo não causou aumento do estresse materno. A partir desses resultados, pode-se concluir que o exercício anaeróbico não causou efeitos adversos no desenvolvimento dos filhotes, bem como foi associado a um aumento da neurogênese hipocampal. Esses dados são promissores e reforçam a importância de se realizar análises adicionais que consolidem essa prática em mães.

Palavras-chave: Exercício Anaeróbico, Gravidez, Neurogênese, Hipocampo, Prole.

ABSTRACT

Gestational aerobic physical exercise produces many beneficial effects in the offspring, such as an increment in cognition, in hipocampal neurogenesis and in the expression of neuroplasticity factors. Although some gestational exercise effects in the offspring's health are well researched, few studies have explored the effects of anaerobic physical exercise in the offspring. This study was constructed to evaluate the development of the offspring from rats that were submitted to gestational anaerobic physical exercise. The exercise protocol consisted of climbing a ladder with a weight fixed to the dam's tail. By BrdU stained cell counting, our data showed that pups from exercised mothers had an increased neurogenesis observed in the hilus of the dentate Gyrus on postnatal day (P) 8. We also observed differences in the offspring's body development. While the offspring from exercised dams presented a lower body weight at P7 and 14, when compared to pups from dams that remained sedentary during pregnancy, they had skulls with larger dimensions. The exercise protocol did not generate relevant alterations in their motor development. No significant differences were observed in the pup's adrenal glands index weights nor an increase in the mother's adrenal glands index weights after performing the gestational anaerobic exercise protocol, which indicates that the protocol used in this study did not elevate the mother's stress levels. Our data suggests that the anaerobic exercise is related to an increase in the postnatal offspring's hippocampal neurogenesis and does not provoke adverse effects in the offspring, like gestational aerobic exercise.

Keywords: Anaerobic exercise, Pregnancy, Neurogenesis, Hippocampus, Offspring.

LISTA DE FIGURAS

LISTA DE ABREVIAÇÕES

ATP: Adenosina trifosfato

ATP-PC: Adenosina trifosfato- Fosfocreatina

BrdU: Bromodeoxiuridina

BDNF: Fator neurotrófico derivado do cérebro

CA: *Cornu ammonis*

DG: Giro denteado

EXE: Grupo exercitado

G: Dia gestacional

GCL: Camada celular granular

IGF: Fator de crescimento semelhante à insulina

MLC: Capacidade de carga máxima

P: Dia pós-natal

PBS: Tampão fostato-salino

SED: Grupo sedentário

SGZ: Camada subgranular

SUMÁRIO

1 INTRODUÇÃO

1.1 EXERCÍCIO FÍSICO

Na maior parte do tempo em que os seres humanos existem, o estilo de vida nômade, em que a comida é obtida por caça e colheita, foi predominante. Nesse tipo de comportamento, a atividade física e o consumo de alimentos são completamente diferentes do que é possível observar hoje em dia na quase totalidade da população mundial. A sobrevivência atual de raras comunidades nômades tornou possível a observação das diferenças entre indivíduos pertencentes a estes grupos e os indivíduos de países desenvolvidos: aqueles são relativamente livres de doenças crônicas, mais magros, têm um nível de condicionamento físico maior, comem uma dieta estritamente natural e têm necessariamente elevados padrões de atividade física, o que não ocorre com a população em geral. Com a invenção de novas tecnologias e a migração para as cidades, houve uma enorme redução da atividade física essencial à sobrevivência dos indivíduos. Para compensar a redução do gasto calórico nas tarefas diárias, os indivíduos “civilizados” começaram a praticar esportes e outras atividades com o objetivo de melhorar sua condição física; o que, de certa forma, é a humanidade tentando retornar ao estilo de vida ativo que seus ancestrais viviam. (COOPER; BLAIR, 2015)

Esse tipo de atividade, que tem como objetivo manter ou melhorar um ou mais componentes do condicionamento físico, é classificado pela Organização Mundial da Saúde (OMS) como “exercícios físicos” e, dentro dessa classificação, as atividades podem ser divididas em aeróbicas e anaeróbicas, conforme a via energética utilizada. Os exercícios aeróbicos são as atividades que consomem oxigênio para produção de adenosina trifosfato (ATP), são geralmente atividades como as corridas ou o ciclismo de média ou longa distâncias. Já os exercícios anaeróbicos, utilizam principalmente duas vias para obtenção de energia na forma de adenosina trifosfato: a via fosfagênio ATP-PC, que consome a fosfocreatina presente nos músculos e é dispendida principalmente em atividades de força e explosão de curta duração; e a via glicolítica que utiliza o glicogênio presente nos músculos e fígado como fonte energética, e é a principal fornecedora de energia nos exercícios em que se utilizam o suporte com peso. O tipo de metabolismo que é utilizado em cada exercício depende da sua intensidade e duração. Enquanto o sistema aeróbico tem como fator limitante a disponibilidade calórica, as vias anaeróbicas somente têm capacidade para fornecer energia para o músculo durante poucos segundos (8 a 10 segundos pela via do fosfagênio e 1,3 a 1,6 minutos pela via glicolítica) (NELSON; COX, 2005).

A prática frequente de atividades físicas é um fator de proteção para muitas doenças, já que, se realizada de forma correta, é capaz de melhorar a saúde cardiorrespiratória, musculoesquelética e mental, o metabolismo, de facilitar o controle do peso corporal e de reduzir os riscos para alguns tipos de câncer (ESTADOS UNIDOS DA AMÉRICA, 2008). Além de prevenir inúmeras doenças, o exercício físico também é utilizado como forma de reabilitar ou melhorar paliativamente pacientes que sofrem com certas condições. Recentemente foi observado que um protocolo de exercício foi capaz de melhorar os sintomas de pacientes com demência grave (FLEINER et al., 2017). Outro estudo mostrou que crianças que passaram por cirurgias para retirada de tumores cerebrais tiveram uma melhora nas suas condições físicas e motoras após realização de um protocolo de exercício físico (RIGGS et al., 2016). Em outra pesquisa, a realização de treinamento de resistência foi capaz de reduzir os níveis de glicose em indivíduos com diabetes mellitus tipo II, deixando-os mais próximos da normalidade (RUSSELL et al., 2017).

1.2 EXERCÍCIO E GRAVIDEZ

Em 2002 a AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS, publicou um documento no qual indicava que as mulheres devem ser incentivadas a iniciar ou manter uma vida saudável com o começo de sua gravidez recorrendo à melhora na dieta, à redução dos níveis de estresse e à realização regular de exercícios. Na opinião desse comitê, a atividade física durante a gravidez pode trazer diversos benefícios, como a redução do risco de diabetes gestacional e de obesidade.

Ao contrário das opiniões mais recentes, as publicações da AMERICAN COLLEGE OF OBSTETRITIANS AND GYNECOLOGYSTS anteriores à 2002 contraindicavam a prática de atividades físicas durante a gestação (THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS, 1994). Essas opiniões conservadoras ainda persistem na população e geram um receio por parte dos médicos, pacientes e outros profissionais da saúde, de que alguns tipos de exercícios estejam relacionados a um prejuízo no crescimento fetal e aumento do risco de aborto espontâneo ou nascimento prematuro. Devido a essa preocupação, e a fim de garantir a saúde das gestantes e seus filhos, ainda se aconselha que atividades físicas mais vigorosas, como corridas e levantamento de peso, sejam realizadas com acompanhamento médico. O comitê classifica, ainda, como contraindicações absolutas às gestantes, a realização de esportes de contato, mergulho e atividades com grandes chances de queda. Além das contraindicações quanto ao tipo de exercício, também estão presentes nessa publicação restrições de atividades físicas para gestantes com anemia grave, doença pulmonar crônica, pré-eclâmpsia ou hipertensão causada pela gravidez, limitações ortopédicas graves e outras condições que possam ser agravadas pela prática de exercício físico (ACOG, 2002).

Outros estudos corroboram a ideia de que exercícios físicos durante a gravidez são seguros e não geram efeitos adversos. PETROV FIERIL et al., 2014, mostraram que mulheres que se exercitaram durante a gestação apresentaram redução de dores nas costas, melhoras no sono e aumento da autoconfiança. Em 2014, WHITE; PIVARNIK; PFEIFFER observaram que exercícios de resistência durante a gestação também estavam relacionados a menores níveis de hipertensão e diabetes gestacional.

Como o processo de desenvolvimento do sistema nervoso é altamente complexo, cada passo de sua formação está vulnerável a alterações causadas por fatores externos ou internos. Diversas doenças ou condições ocorrem por problemas na formação desse sistema. Sendo assim, a gestação é um período de grande vulnerabilidade para a prole, uma vez que o comportamento e a saúde materna durante a gravidez influenciam diretamente no desenvolvimento dos filhos. Em algumas situações, como o consumo de álcool ou infecções virais durante a gravidez, os efeitos negativos sobre a neurotransmissão, neurodesenvolvimento e comportamento dos filhos, são amplamente conhecidos pela população (ADAMS WALDORF; MCADAMS, 2013; KORPI et al., 2015). Outros fatores, entretanto, não tão conhecidos popularmente, podem influenciar o desenvolvimento da prole. Como exemplo, no estudo de PICKETT et al., 2008, foi encontrado que o tabagismo materno durante a gravidez está relacionado com o humor dos filhos, sendo que os filhos de mães fumantes apresentaram um temperamento mais difícil que filhos de mãe não fumantes. Já ARYA et al., 2012, observaram que a exposição materna à música durante a gravidez tem um efeito positivo nas capacidades de habituação e orientação dos recém-nascidos.

Pelo contrário, tem sido demonstrado que os filhos de mães que praticaram exercício aeróbico na gestação têm sua função cognitiva aprimorada em avaliações neonatais, de inteligência e comunicação oral (CLAPP, 1996; CLAPP; LOPEZ; HARCAR-SEVCIK, 1999). Quanto ao exercício anaeróbico, os dados clínicos sugerem que a sua prática é segura na gestação, não alterando parâmetros como tempo de gestação, tipo de parto, idade gestacional ao nascimento e risco de prematuridade (PETROV FIERIL et al., 2014; WHITE; PIVARNIK; PFEIFFER, 2014), mas não há dados disponíveis sobre os efeitos cognitivos na prole desse tipo de exercício físico.

Um dos possíveis mecanismos para explicar o incremento na capacidade cognitiva de filhos de mães que realizaram exercícios aeróbicos durante a gravidez apresentaram é o aumento na neurogênese hipocampal. De fato, foi observado que ratas gestantes submetidas à corrida em esteira tiveram filhotes com esse resultado, que foi relacionado a um aumento da vascularização cerebral dos filhotes e aumento da concentração de fator neurotrófico derivado do cérebro (BDNF) na região hipocampal (AKHAVAN et al., 2012). Entretanto, deve-se levar em consideração o tipo de exercício físico realizado, pois no estudo de WASINSKI et al., 2016, ratas gestantes submetidas ao nado forçado tiveram filhotes com redução da neurogênese no giro denteado nos dias pós-natais P8 e P35. Possivelmente, esse prejuízo deve estar relacionado ao estresse que foi observado pelos altos níveis de corticosterona plasmática que as ratas gestantes exercitadas apresentaram, causado pelo protocolo de nado forçado. Níveis elevados de estresse pré-natal podem levar a uma desregulação do eixo hipotálamo-hipófise-adrenal e dos níveis de fatores de crescimento semelhantes à insulina (IGF) nos fetos, o que por vez leva a um comprometimento do desenvolvimento dos indivíduos sujeitos a esse tipo de estresse (WELBERG; SECKL, 2008).

Apesar da musculação (exercício anaeróbico) ser o segundo tipo de atividade física mais praticado no Brasil, ficando atrás apenas do exercício de caminhada, ainda são raros os estudos a respeito dos efeitos nos filhos da prática desse tipo de atividade no período gestacional (DE SA; GARCIA; CLARO, 2014). Portanto, o presente estudo tem como objetivo analisar parâmetros de desenvolvimento na prole de ratas submetidas a um protocolo de exercício anaeróbico.

2 JUSTIFICATIVA

Esse estudo se faz necessário devido ao grande número de adeptos da prática de exercícios anaeróbicos no Brasil e à escassez de informações a respeito da segurança desse tipo de atividade durante o período gestacional, especialmente nos efeitos na saúde e desenvolvimento da prole. O conhecimento dos efeitos desse tipo de exercício é relevante para melhor orientar as gestantes, proporcionando uma orientação mais segura desse tipo de atividade.

3 OBJETIVOS

i. Objetivo Geral

Observar os efeitos do exercício anaeróbico gestacional no desenvolvimento da prole.

ii. Objetivos Específicos

Analisar os efeitos, do exercício anaeróbico gestacional sobre:

1. O desenvolvimento corporal e neurológico dos filhotes, mensurando seu peso, medidas corporais e aparecimento dos marcos do desenvolvimento;
2. O peso das glândulas adrenais dos filhotes, no oitavo dia pós-natal, como uma medida indireta do nível de estresse.
3. O peso das glândulas adrenais das mães, no oitavo dia pós-parto, como uma medida indireta do nível de estresse.
4. A neurogênese hipocampal, avaliando a proliferação celular pela contagem de células marcadas na imuno-histoquímica para BrdU nas zonas granular, subgranular e no hilo do giro denteado dos filhotes com 8 dias de vida.

4 PESQUISA EM FORMA DE ARTIGO CIENTÍFICO

Artigo será submetido à revista Behavioural Brain Research

Anaerobic Physical Exercise During Pregnancy Increases the Hippocampal Neurogenesis and does not Harm the Physical Development in the Neonatal Period.

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ABSTRACT

Gestational aerobic physical exercise produces many beneficial effects in the offspring, such as an increment in cognition, in hipocampal neurogenesis and in the expression of neuroplasticity factors. Although some gestational exercise effects in the offspring's health are well researched, few studies have explored the effects of anaerobic physical exercise in the offspring. This study was constructed to evaluate the development of the offspring from rats that were submitted to gestational anaerobic physical exercise. The exercise protocol consisted of climbing a ladder with a weight fixed to the dam's tail. By BrdU stained cell counting, our data showed that pups from exercised mothers had an increased neurogenesis observed in the hilus of the dentate Gyrus on postnatal day (P) 8. We also observed differences in the offspring's body development. While the offspring from exercised dams presented a lower body weight at P7 and 14, when compared to pups from dams that remained sedentary during pregnancy, they had skulls with larger dimensions. The exercise protocol did not generate relevant alterations in their motor development. No significant differences were observed in the pup's adrenal glands index weights nor an increase in the mother's adrenal glands index weights after performing the gestational anaerobic exercise protocol, which indicates that the protocol used in this study did not elevate the mother's stress levels. Our data suggests that the anaerobic exercise is related to an increase in the postnatal offspring's hippocampal neurogenesis and does not provoke adverse effects in the offspring, like gestational aerobic exercise.

Keywords: Anaerobic exercise, Pregnancy, Neurodevelopment, Hippocampus, Offspring.

1 INTRODUCTION

A sedentary lifestyle is associated with the emergence of a plethora of chronic diseases, such as diabetes mellitus, high blood pressure and obesity. These conditions, especially when present in pregnant women, can lead to major complications (ABALOS et al., 2014; SRICHUMCHIT; LUEWAN; TONGSONG, 2015; SUGIYAMA et al., 2014). Physical exercise, amongst other things, improves the glucose homeostasis and fat oxidation, which are prevention factors for those diseases (FLÜCK, 2006; HOPPELER; FLUCK, 2003; MOKDAD et al., 2004).

Additionally to having an impact in the mother's health, maternal exercise can change biological and behavioral patterns in the offspring. RIBEIRO et al., 2017, for example, showed that a low intensity physical exercise program prevents obesity in rat pups exposed to early overnutrion. It was also found that the aerobic physical exercise in a treadmill increases the hippocampal brain derived neurotrophic factor (BDNF) and improves spatial learning in the offspring (PARNPIANSIL et al., 2003 and GOMES DA SILVA et al., 2016). One of the possible mechanisms to explain the incremente in cognitive capacity found in the offspring from exercised mothers is the increase in neurogenesis. It was observed that pregnant rats submitted to treadmill running had pups with this result (AKHAVAN et al., 2012).

Currently, the practice of aerobic and anaerobic exercises are recommended in the gestational period, and clinical trials have shown that they are mostly safe and do not cause adverse effects (ESTADOS UNIDOS DA AMÉRICA, 2008; PETROV FIERIL et al., 2014; UZENDOSKI et al., 1990; WHITE; PIVARNIK; PFEIFFER, 2014). As previously mentioned, there are several beneficial effects of gestational aerobic exercise in the offspring. However, the outcomes of anaerobic exercise are still underexplored. A few studies demonstrated the safety of its practice in the gestational period and its benefits in maternal health, but the cognitive effects in the offspring were yet to be studied (BARAKAT; LUCIA; RUIZ, 2009; PERALES; ARTAL; LUCIA, 2017).

In our knowledge, the only study that observed the effects of gestational resistance exercise in the offspring was conducted using a forced swimming exercise protocol. Opposite to aerobic maternal exercise, it produced a decrease in neurogenesis on the dentate gyrus in the postnatal days 8 and 35. This impairment was likely related to the elevated levels of stress that the exercise protocol caused in the dams, which was verified using corticosterone plasma levels analysis (WASINSKI et al., 2016).

Therefore, the present study aimed to add to the research about the safety of gestational exercise. Using BrdU staining, the effects of a maternal anaerobic exercise protocol in the cell proliferation patterns in the dentate gyrus during the offspring neonatal period were studied. Also, their body and motor developments were evaluated with daily measurements and testing, as well as an indirect measure of stress.

2 MATERIAL AND METHODS

2.1 Animals and Groups

Wistar rats utilized in this study were obtained from a local breeding colony (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil). All animals were kept in standard living boxes (LxWxH in mm – 300mm x 190mm x 130mm), – three animals per box. After a week of adaptation, female rats, approximately 90 days old, were submitted to daily colpocytological exams in order to detect the estral cycle phase. When in the male receptive phase, the females were put in individual cages with a male rat overnight. The next day, another exam was performed to confirm the mating. If sperm was found in the vaginal swab, that was considered the gestational day (G) 0. In total, 20 females and 10 male rats were used.

The pregnant rats were isolated two days before the due date to set up the nest. The litter size was standardized as 8 pups per dam. All animals stayed in a controlled temperature environment (20 ± 2 ºC), dark/light cycle of 12 hours, with water and food *ad libitum*, according to the Brazilian law nº 11.794/08 de 08/10/08 that regulates animal use for scientific research. A timeline with the key events of the experiment and experimental groups is showed in Figure 3. All the procedures were approved by the Ethical Committee at the Universidade Federal do Rio Grande do Sul (#29840).

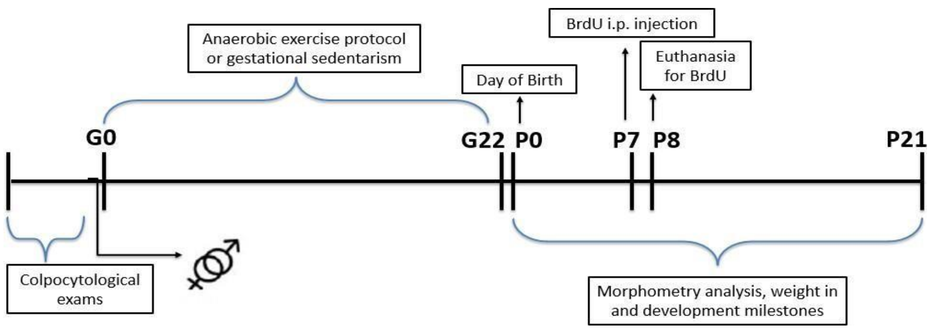


Figure 3 - Timeline of experimental procedures. G: Gestational day, P: Postnatal day.

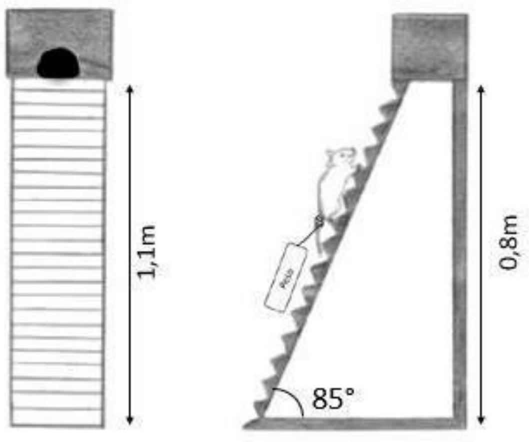
2.2 Exercise Protocol

The following exercise protocol was adapted from HORNBERGER and FARRAR (2004), according to the needs imposed by this study. The training consisted of climbing a slopping ladder (1,1 x 0,8 m) at 85°, each session was composed of 4 to 9 repetitions, with lead weights fixed to the animal's tail (Figure 4). Prior to the start of the protocol, there was an apparatus familiarization and adaptation period, which was done for 3 consecutive days. The adaptation consisted of the rat climbing to a black box located at the end of the apparatus, three times, voluntarily, with a load of 10% its body weight.

The first exercise session, a maximum load capacity test, was performed at G0 and consisted of 4 to 8 climbs with a gradual load increase. The starting load corresponded to 50% of the animal's body weight. Once the top of the stairs was reached the animal could rest for 120 seconds. After successfully completing the repetitions with the starting load, 30g were added. This process was repeated until the animals were no longer able to complete the full climbing. The biggest load that the animal could complete the climb was considered the Maximum Load Capacity (MLC) for that session.

In the following sessions the animals had to climb 4 to 9 times, their loads were 30%, 45%, 55% e 75% of their MLC in the first four sessions, respectively. The next session was another MLC test to define the load for the next four sessions. This exercise regimen was done three times a week, with 48h or 72h intervals between sessions, reaching a total of 9 sessions in the gestational exercise group.

A



B

- 4-9 repetitions;
- 48-72h intervals between sessions;
- Maximum load test;
- 1st 4 sessions: weight=30%, 45%, 55% and 75% of the maximum load capacity.

Figure 4 – A) Anaerobic exercise apparatus. **B)** Gestational anaerobic exercise protocol.

2.3 Indirect stress evaluation in the mothers

In the postnatal day 8 of progeny, the mothers (n =8) were euthanized by decapitation and their adrenal glands were removed, dissected and weighted in a analytical scale to calculate their relative weight (MARCUZZO et al., 2007).

2.4 Physical evaluation of the offspring

The male pups were weighted in the postnatal days (P) 2, 7, 14 and 21. Their growth was also analyzed with respect to their antero-posterior skull axis, latero-lateral skull axis, longitudinal axis and length of tail measurements that were taken at P2 and P21.

The male pups sensorimotor development was analyzed from postnatal day 1 to 8 using the following motor development milestones tests: 1) Surface righting: the pups were placed in supine position, and the result was considered positive when the animal returned to the initial position, placing the four paws on the surface; 2) Cliff aversion: the pups were placed with the forepaws and the snout at the edge of a shelf, the response was considered positive when the animal changed its Direction and moved in the opposite Direction of the Cliff; 3) Negative Geotaxis: the pups were placed head down in a 45° sloped surface, the test was considered positive when the pup turned 180° and moved towards the higher end of the surface; 4) Proprioceptive posterior limb placement: the pups had their heads and body supported while their posterior limbs hung near a platform, the dorsal surfaces of the posterior paws were lightly touched in the edge of the platform in order to generate a tactile stimulus, the test was considered positive when this stimulus was followed by a hip and knee extension and an ankle plantar flexion. The response for each test was considered positive in the first day in which it appeared, in a time limit of 30 seconds, with the exception of the proprioceptive posterior limb placement test, which should be instant. (MARCUZZO et al., 2010)

2.5 Immunohistochemistry for BrdU

At P7, a section of the pups from both groups were injected intraperiotonally with a solution of BrdU 100mg/kg diluted in NH4OH 0,1M, 20 mg/mL. At P8, the animals were euthanized by decapitation and the whole brain was dissected and transferred to a 15% and 30% sucrose solution where they stayed for 24h in each solution. Then, the samples were post-fixed in the 4% paraformaldehyde solution for 24 h. They were then frozen at -20◦C. Serial coronal sections were obtained with a cryostat and collected on pre-gelatinized slides. Five slices of coronal sections per animal were obtained, with 40μm between slices. The sections corresponded to a distance of approximately 2.8 to 4.3mm posterior to the bregma (PAXINOS; WATSON, 1982).

The slices were washed in xylol for 5min (2 times) and in decreasing concentrations of ethanol. Antigenic retrieval was obtained at 92 ◦C with citrate buffer for 40 min. Then, the tissues were washed in distilled water and the endogen peroxidase was inactivated (H2O2 5% dissolved in methanol). The sections were washed in Triton-X dissolved in PBS (PBS-tx), incubated with anti-5'-2'-deoxyuridine monoclonal antibody 1:500 (General Health) for 2h at room temperature and overnight at 4 ◦C. After that, the slices were washed again in PBS-tx and incubated with the secondary antibody IgG Peroxidase anti-mouse rabbit (1:100; sigma-aldrich) for 1h at room temperature. The immunohistochemical reaction was revealed by 0.06% 3,3-diaminobenzidine (DAB) in PBS. After being rinsed in distilled water, sections were counterstained with hematoxylin, dehydrated in ethanol and mounted on slides using Entellan®.

2.6 Quantification of BrdU labeled cells

All immunoreactive cells in the medial end of the dorsal section of the DG were captured with a 100× objective in an Olympus BX40 microscope, and were carefully counted. Cell counts were restricted to the granular cell layer (GCL),the subgranular zone (SGZ) and hilus of the DG. BrdU-immunoreactive cells in the DG were counted with respect to the different layers with the Image Pro Plus 7.0 software

2.7 Statistical Analysis

Differences between sedentary and exercised groups were determined using the Student’s independent T-test and Mann-Whitney Test. Statistical analysis was performed using the IBM SPSS Statistics 24 and statistical significance was established at $p < 0.05$.

3 RESULTS

3.1 Offspring Body Weight and Length

The offspring from exercised mothers presented a lower body weight at postnatal days 7 (t (18.2) = 2.58, p = 0.01) and 14 (t (21.7) = 3.52, p = 0.01), (Figure 5). But the difference did not persist at postnatal day 21.

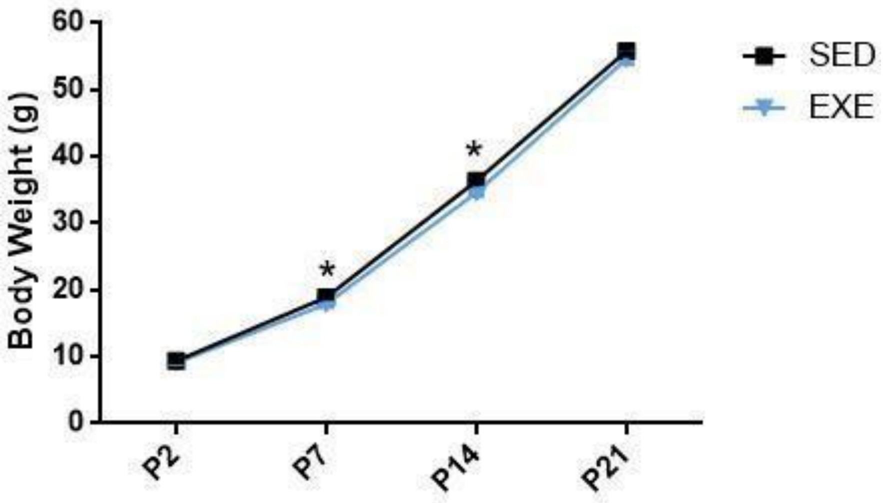


Figure 5 - Body weight progression in pups whose mothers were sedentary (SED) or exercised (EXE) during pregnancy. (SED – n = 16; EXE – n = 16), P: Postnatal day. * = p<0.05.

Our data also revealed a significant difference in the size of the pups' skull (Table 1) the pups whose mothers went through the anaerobic exercise protocol had a larger anterior-posterior (*t* (20) = -2.73, p = 0.01) and latero-lateral skull axis (*t* (20) = 2.09, p = 0.04) at P2 when compared to the control group. A significant antero-posterior skull axis size difference persisted at P21 (*t* (20) = - 2.92, p = 0.001).

Table 1. Growth measurements in offspring of sedentary and exercise mothers.

	SED		EXE		
	n	Mean ± SE	n	Mean ± SE	p
P2	12		10		
Antero-posterior axis of skull		17,16 ± 0,21 mm		17,97 ± 0,19 mm	0.013*
Latero-lateral axis of skull		13,48 ± 0,15 mm		13,01 ± 0,16 mm	0.050*
Longitudinal axis		56,42 ± 0,56 mm		56,10 ± 0,07 mm	0.726
Length of tail		17,89 ± 0,23 mm		18,72 ± 0,46 mm	0.136
P21	12		10		
Antero-posterior axis of skull		34,23 ± 0,60 mm		36,59 ± 0,51 mm	0.008*
Latero-lateral axis of skull		24,16 ± 0,52 mm		25,22 ± 0,41 mm	0.143
Longitudinal axis		119,29 ± 0,79 mm		120,46 ± 0,75 mm	0.304
Length of tail		76,19 ± 0,36 mm		75,98 ± 0,58 mm	0.931

Values are means ± SEM. Abbreviations: SED, sedentary; EXE, exercise; P, postnatal day.*T-Student test utilized ($p < 0.05$).

3.2 Offspring Development Milestones

The motor development of the pups was evaluated using the motor development milestones series of tests in the first 8 days of life (Table 2). No difference was found between the groups.

Table 2. Neurological Reflexes evaluated at postnatal day 1 to postnatal day 8.

	SED		EXE		
	n	Mean ± SE	n	Mean ± SE	p
NEUROLOGICAL REFLEXS	15		7		
Surface Righting		1.06 ± 0.06 d		1.00 ± 0.00 d	0.495[a]
Posterior Limb Placement		5.80 ± 0.31 d		6.28 ± 0.42 d	0.164[b]
Negative Geotaxis		5.80 ± 0.41 d		4.7 ± 0.64 d	0.379[b]
Cliff Aversion		1.80 ± 0.22 d		1.28 ± 0.28 d	0.099[a]

Values are means ± SEM. Abbreviations: SED, sedentary; EXE, exercise; d, day of appearance. [a]Mann-Whitney and [b]T-Student test utilized. ($p < 0.05$).

3.3 BrdU Assay

The number of proliferating cells were assayed at P8, 24h after one injection of BrdU. Our data showed that there were more BrdU-labeled cells in the hilus of pups from exercised mothers (*t*(15)= -2,25, p = 0,04). No difference was found in the subgranular and granular cell layer, or in the total cell count (Figure 6C).

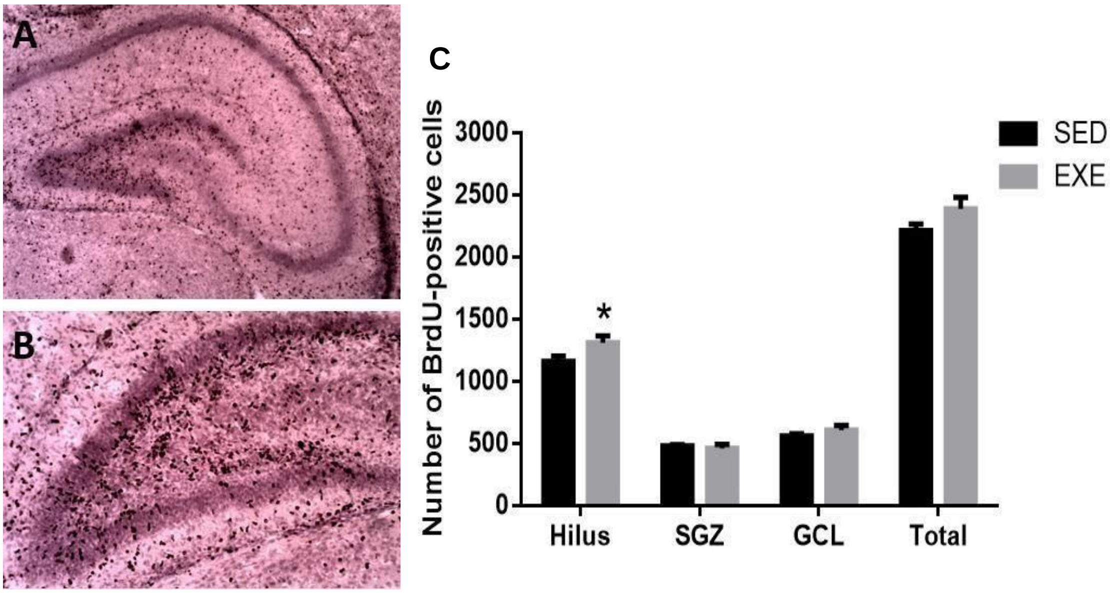


Figure 6 - A) Hippocampus at postnatal day (P) 8 stained for BrdU. with HE 100x. B) Dentate gyrus at P8 stained for BrdU with HE 400x. **C)** BrdU stained cell proliferation in wistar pups at P8. SED group (1167,11 ± 40,83), EXE group (1316,75 ± 53,50), SGZ: Subgranular cell layer, GCL: Granular cell layer. Values are mean ± SEM. *p=0.04.*;* n = 9-8.

3.4 Mothers and Offspring's Adrenal Glands

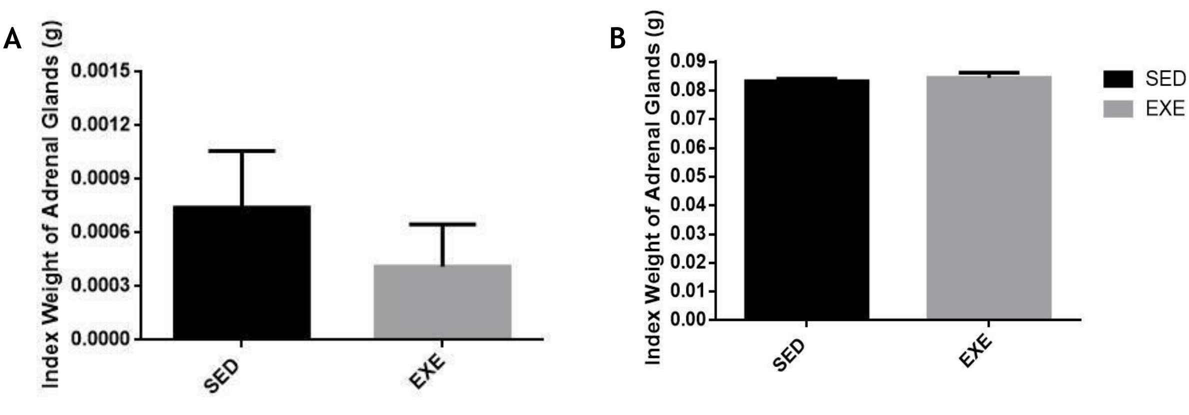


Figure 7 – A. Comparison of the of the index weight of the adrenal glands between sedentary and exercised groups. **B.** Adrenal glands index weight of pups whose mothers were sedentary (SED) or exercised (EXE) during pregnancy. Values are mean ± SEM, mothers - n = 4; pups - n = 10-11; p < 0.05.

In order to observe an indirect measure of stress, the adrenal glands of the mothers and offspring were removed, dissected and weighted at P8. No significant differences were observed in both analysis (Figure 7A and 7B).

4 DISCUSSION

The gestational anaerobic exercise protocol used in this study lead to development alterations in the offspring. The pups from exercise mothers had an overall lower body weight at P7 and P14, larger skull sizes, and an increased neurogenesis in their hippocampus' hilus. It did not, however, produce changes the motor development milestones nor in the adrenal glands index weights of mothers and pups.

These differences found in body weight corroborate with previous studies, as it has been shown that children of mothers who exercise present a lower weight in their initial stages of life, both in humans and animals (BARBALHO et al., 2011; CLAPP, 1996; CLAPP; LOPEZ; HARCAR-SEVCIK, 1999). This may be due to a decrease in their adiposity percentage, but epigenetic factors could also play a role in this balance (BISSON et al., 2017).

While the skull sizes in pups from exercised mothers were larger antero-posteriorly at P2 and P21, and latero-laterally at P2, there were no differences in the other body dimensions evaluated. At the moment, we do not have a direct explanation for this result. This result does not match with the body weight that the pups presented. Therefore, the head size did not contribute to the decreased body weight. This reinforces the hypothesis that the body weight difference that gestational exercise produces is due to a decrease in adipose tissue, as it has been found in previous studies (GUTH et al, 2013). Further evaluation of the offspring body composition should be conducted to fully elucidate the effects of anaerobic maternal exercise in the pups' body development.

In our study, we chose to analyze the neurogenesis in the eight postnatal day due to the peak in cell proliferation at this period, and we found that the heightened neurogenesis was observed only in the hilus of the dentate gyrus, differently from other studies about gestational exercise that observed increases in the total labeled cells and the others hippocampal layers (DAYI et al., 2012; GOMES DA SILVA et al., 2016; LEE et al., 2006). In those studies, high levels of hippocampal BDNF were found, and this could be the biological mechanism involved in this hippocampal neurogenesis alteration (GOMES DA SILVA et al., 2016; KIM et al., 2007; LEE et al., 2006b). Since the cell migration during early-life neurogenesis in the dentate gyrus happens in a radial pattern – from the hilus to the outer layers - it could mean that, when analyzed in other periods, the neurogenesis would have a different pattern (KHALAF-NAZZAL; FRANCIS, 2013; SUGIYAMA; OSUMI; KATSUYAMA, 2013).

As it was found in the indirect stress analysis, our exercise protocol did not alter the levels of stress in the mothers nor in the pups. Opposite to our result, WASINSKI et al. (2016) found that a forced swimming gestational exercise protocol caused a decrease in hippocampal dentate gyrus cell proliferation in the offspring, which, in this case, was attributed to the high levels of stress that this type of activity caused in the mothers – that were evaluated using serum corticosterone levels. This contrast between our data is likely due to the type of exercise performed: voluntary exercise generally does not increase the levels of stress; on the other hand, forced exercise, such as swimming and treadmill running is known to increase the stress and corticosterone levels; (CARLBERG; ALVIN; GWOSDOW, 1996; NOTOMI et al., 2001; OSORIO et al., 2003; ROSA et al., 2011; VINICIUS et al., 2007)(NOTOMI et al., 2001; ROSA et al., 2011).

No significant differences were found in the motor development analysis either. A few studies with humans showed that gestational exercise also did not modify the neurodevelopment of the progeny (CLAPP, 1996; CLAPP; LOPEZ; HARCAR-SEVCIK, 1999).This result is also an indicator that the exercise protocol did not generate stress, as it has been found, in animal and human studies, that children from prenatally stressed mothers presented a retardation in their motor development (FRIDE; SOREQ; WEINSTOCK, 1986; GRACE et al., 2016).

Our results showed that the moderate anaerobic exercise performed in this study did not harm normal physical development of the pups and it increased the cell proliferation in the hilus of the denteate gyrus. As an exploratory investigation, this is the first study, in our knowledge, to verify the effects of maternal anaerobic exercise in developmental aspects of the prole in the neonatal period. Our data joins to other clinical studies to help understand the effects of the gestational anaerobic exercise in the offspring. Further research is necessary to elucidate the full effects of maternal anaerobic exercise.

REFERENCES

ABALOS, E. et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. **BJOG: An International Journal of Obstetrics & Gynaecology**, v. 121, p. 14–24, mar. 2014.

AKHAVAN, M. M. et al. Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring. **Pak. J. Pharm. Sci**, v. 25, n. 1, p. 233–238, 2012.

BARAKAT, R.; LUCIA, A; RUIZ, J. R. Resistance exercise training during pregnancy and newborn's birth size: a randomised controlled trial. **International journal of obesity (2005)**, v. 33, n. 9, p. 1048–57, 2009.

BARBALHO, S. M. et al. Efeito do exercício físico contínuo e intervalado no peso e perfil bioquímico de ratas Wistar prenhes e consequências no peso da prole. **Revista Brasileira de Medicina do Esporte**, v. 17, n. 6, p. 413–415, 2011.

BISSON, M. et al. Physical activity during pregnancy and infant's birth weight: results from the 3D Birth Cohort. **BMJ open sport & exercise medicine**, v. 3, n. 1, p. e000242, 2017.

CARLBERG, K. A; ALVIN, B. L.; GWOSDOW, A R. Exercise during pregnancy and maternal and fetal plasma corticosterone and androstenedione in rats. **The American journal of physiology**, v. 271, n. 5 Pt 1, p. E896-902, 1996.

CLAPP, J. F. Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy. **The Journal of pediatrics**, v. 129, p. 856–863, 1996.

CLAPP, J. F.; LOPEZ, B.; HARCAR-SEVCIK, R. Neonatal behavioral profile of the offspring of women who continued to exercise regularly throughout pregnancy. **American Journal of Obstetrics and Gynecology**, v. 180, n. 1 I, p. 91–94, 1999.

DAYI, A. et al. Maternal Aerobic Exercise during Pregnancy Can Increase Spatial Learning by Affecting Leptin Expression on Offspring's Early and Late Period in Life Depending on Gender. **The Scientific World Journal**, v. 2012, p. 1–8, 2012.

ESTADOS UNIDOS DA AMÉRICA. **Physical Activity Guidelines Advisory Committee Report**. [s.l: s.n.].

FLÜCK, M. Functional, structural and molecular plasticity of mammalian skeletal muscle in response to exercise stimuli. **The Journal of experimental biology**, v. 209, n. Pt 12, p. 2239–48, 2006.

FRIDE, E.; SOREQ, H.; WEINSTOCK, M. Are the effects of gestational stress on motor development and cerebellar cholinesterase activity mediated prenatally? **International journal of developmental neuroscience: the official journal of the International Society for Developmental Neuroscience**, v. 4, n. 5, p. 407–13, 1986.

GOMES DA SILVA, S. et al. Maternal exercise during pregnancy increases BDNF levels and cell numbers in the hippocampal formation but not in the cerebral cortex of adult rat offspring. **PLoS ONE**, v. 11, n. 1, 2016.

GRACE, T. et al. The Impact of Maternal Gestational Stress on Motor Development in Late Childhood and Adolescence: A Longitudinal Study. **Child Development**, v. 87, n. 1, p. 211–220, 1 jan. 2016.

GUTH L.M. et al. (2013). Sex-specific Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mouse Offspring. **Exp Physiol.**, 98(10): 1469–1484.

HOPPELER, H.; FLUCK, M. Plasticity of skeletal muscle mitochondria: structure and function. **Med Sci Sports Exerc**, v. 35, n. 1, p. 95–104, 2003.

HORNBERGER, T. A.; FARRAR, R. P. Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. **Canadian journal of applied physiology = Revue canadienne de physiologie appliquee**, v. 29, n. 1, p. 16–31, fev. 2004.

KHALAF-NAZZAL, R.; FRANCIS, F. Hippocampal development - Old and new findings. **Neuroscience**, v. 248, p. 225–242, 17 set. 2013.

KIM, H. et al. The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. **International Journal of Developmental Neuroscience**, v. 25, n. 4, p. 243–249, 2007.

LEE, H.-H. et al. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. **Brain and Development**, v. 28, n. 3, p. 147–154, 2006a.

LEE, H.-H. et al. Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups. **Brain and Development**, v. 28, n. 3, p. 147–154, abr. 2006b.

MARCUZZO, S. et al. Dendritic spines in the posterodorsal medial amygdala after restraint stress and ageing in rats. **Neuroscience Letters**, v. 424, n. 1, p. 16–21, 2007.

MARCUZZO, S. et al. Different effects of anoxia and hind-limb immobilization on sensorimotor development and cell numbers in the somatosensory cortex in rats. **Brain and Development**, v. 32, n. 4, p. 323–331, 2010.

MOKDAD, A. H. et al. Actual causes of death in the United States, 2000. **Journal of the American Medical Association**, v. 291, p. 1238–1245, 2004.

NOTOMI, T. et al. Effects of tower climbing exercise on bone mass, strength, and turnover in growing rats. **Journal of Bone and Mineral Research**, v. 16, n. 1, p. 166–174, 2001.

OSORIO, R. A. et al. Swimming of pregnant rats at different water temperatures. **Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology**, v. 135, n. 4, p. 605–611, 2003.

PARNPIANSIL, P. et al. **Exercise during pregnancy increases hippocampal brain-derived neurotrophic factor mRNA expression and spatial learning in neonatal rat pupNeuroscience Letters**. [s.l: s.n.].

PAXINOS, G.; WATSON, C. The Rat Brain in Stereotaxic Coordinates. **Academic Press, San Diego**, v. 3rd, 1982.

PERALES, M.; ARTAL, R.; LUCIA, A. Exercise During Pregnancy. **JAMA**, v. 317, n. 11, p. 1113, 21 mar. 2017.

PETROV FIERIL, K. et al. Experiences of Exercise During Pregnancy Among Women Who Perform Regular Resistance Training: A Qualitative Study. **Physical Therapy**, v. 94, n. 8, p. 1135–1143, 1 ago. 2014.

RIBEIRO, T. A. et al. Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. **Scientific Reports**, v. 7, n. 1, 2017.

ROSA, B. V. et al. Voluntary exercise in pregnant rats positively influences fetal growth without initiating a maternal physiological stress response. **AJP: Regulatory, Integrative and Comparative Physiology**, v. 300, n. 5, p. R1134–R1141, 2011.

SRICHUMCHIT, S.; LUEWAN, S.; TONGSONG, T. Outcomes of pregnancy with gestational diabetes mellitus. **International Journal of Gynecology & Obstetrics**, v. 131, n. 3, p. 251–254, dez. 2015.

SUGIYAMA, T. et al. Pregnancy outcomes of gestational diabetes mellitus according to pre-gestational BMI in a retrospective multi-institutional study in Japan. **Endocrine journal**, v. 61, n. 4, p. 373–80, 2014.

SUGIYAMA, T.; OSUMI, N.; KATSUYAMA, Y. The germinal matrices in the developing dentate gyrus are composed of neuronal progenitors at distinct differentiation stages. **Developmental Dynamics**, v. 242, n. 12, p. 1442–1453, 1 dez. 2013.

UZENDOSKI, A. M. et al. Physiological responses to aerobic exercise during pregnancy and post-partum. **The Journal of sports medicine and physical fitness**, v. 30, n. 1, p. 77–82, mar. 1990.

VINICIUS, R. et al. Biomarkers of stress in rats exercised in swimming at intensities equal and superior to the maximal estable lactate phase. **Rev Bras Med Esporte**, v. 13, p. 150–154, 2007.

WASINSKI, F. et al. Maternal Forced Swimming Reduces Cell Proliferation in the Postnatal Dentate Gyrus of Mouse Offspring. **Frontiers in neuroscience**, v. 10, p. 402, 2016.

WHITE, E.; PIVARNIK, J.; PFEIFFER, K. Resistance Training during Pregnancy and Perinatal Outcomes. **Journal of Physical Activity and Health**, v. 11, n. 6, p. 1141–1148, ago. 2014.

5 CONCLUSÂO E PERSPECTIVAS

Este estudo demonstrou que o exercício físico anaeróbico gestacional causa diferenças significativas no desenvolvimento dos filhotes, como as diferenças em suas medidas de crânio, de peso e a proliferação celular aumentada na região do hilo do giro denteado no período pós-natal. Nesse estudo não foram encontrados efeitos adversos desse tipo de exercício nos aspectos analizados, fato este que está diretamente relacionado com o caráter não-estressante do protocolo de exercício utilizado. Como foram observadas diferenças significativas entre os grupos, pesquisas mais aprofundadas devem ser realizadas para compreender a totalidade dos efeitos causados por esse tipo de exercício e garantir a segurança de sua prática. Como perspectivas, temos o aumento do número de animais estudados, a análise dos níveis de corticosterona plasmático das mães após a realização do protocolo de exercício e a observação do comportamento pós-natal materno a fim de verificar possíveis alterações que o exercício possa ter causado.

6 REFERÊNCIAS ADICIONAIS

ACOG. ACOG Committee opinion. Number 267, January 2002: exercise during pregnancy and the postpartum period. **Obstetrics & Gynecology**, v. 99, n. 1, p. 171–3, 2002.

ADAMS WALDORF, K. M.; MCADAMS, R. M. Influence of infection during pregnancy on fetal development. **Reproduction (Cambridge, England)**, v. 146, n. 5, p. R151-62, 1 nov. 2013.

ARYA, R. et al. Maternal Music Exposure during Pregnancy Influences Neonatal Behaviour: An Open-Label Randomized Controlled Trial. **International journal of pediatrics**, v. 2012, p. 901812, 2012.

COOPER, K. H.; BLAIR, S. N. **Exercise** Encyclopædia Britannica, inc., , [s.d.]. (Nota técnica).

DE SA, T. H.; GARCIA, L. M. T.; CLARO, R. M. Frequency, distribution and time trends of types of leisure-time physical activity in Brazil, 2006–2012. **International Journal of Public Health**, v. 59, n. 6, p. 975–982, 22 dez. 2014.

ESTADOS UNIDOS DA AMÉRICA. **Physical Activity Guidelines Advisory Committee Report**. [s.l: s.n.].

FLEINER, T. et al. Structured physical exercise improves neuropsychiatric symptoms in acute dementia care: a hospital-based RCT. **Alzheimer's Research & Therapy**, v. 9, n. 1, p. 68, 29 dez. 2017.

KORPI, E. R. et al. Mechanisms of Action and Persistent Neuroplasticity by Drugs of Abuse. **Pharmacological reviews**, v. 67, n. 4, p. 872–1004, 1 out. 2015.

NELSON, D. L.; COX, M. M. **Lehninger Principles of Biochemistry**. [s.l: s.n.]. v. 4th

PICKETT, K. E. et al. Meaningful differences in maternal smoking behaviour during pregnancy: implications for infant behavioural vulnerability. **Journal of Epidemiology & Community Health**, 2008.

RIGGS, L. et al. Exercise training for neural recovery in a restricted sample of pediatric brain tumor survivors: a controlled clinical trial with crossover of training versus no training. **Neuro-Oncology**, v. 19, n. 3, p. now177, 23 ago. 2016.

RUSSELL, R. D. et al. Skeletal Muscle Microvascular-Linked Improvements in Glycemic Control From Resistance Training in Individuals With Type 2 Diabetes. **Diabetes care**, v. 40, n. 9, p. 1256–1263, 1 set. 2017.

THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS. Exercise during pregnancy and the postpartum period: ACOG technical bulletin number 189 — February 1994. **International Journal of Gynecology & Obstetrics**, v. 45, n. 1, p. 65–70, 1994.

WELBERG, L. A. M.; SECKL, J. R. Prenatal Stress, Glucocorticoids and the Programming of the Brain. **Journal of Neuroendocrinology**, v. 13, n. 2, p. 113–128, 18 jul. 2008.

ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA BEHAVIOURAL BRAIN RESEARCH

BEHAVIOURAL BRAIN RESEARCH

AUTHOR INFORMATION PACK

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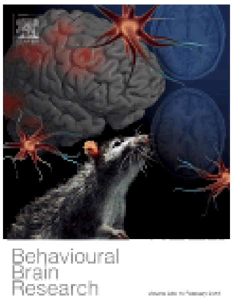

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