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EFEITOS DO EXERCÍCIO FÍSICO RESISTIDO MATERNO NA NEUROPLASTICIDADE HIPOCAMPAL DA PROLE DE RATOS WISTAR

PORTO ALEGRE
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Tese apresentada ao Programa de Pós-Graduação em Neurociências do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de doutor em Neurociências.

Orientadora: Profª. Drª. Simone Marcuzzo

PORTO ALEGRE

2020

"...Ilha do Norte onde não sei se por sorte ou por castigo dei de parar
Por algum tempo que afinal passou depressa, como tudo tem de passar
Hoje eu me sinto como se ter ido fosse necessário para voltar
Tanto mais vivo de vida mais vivida, dividida pra lá e pra cá."

Back in Bahia – Gilberto Gil, (1972)

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SUMÁRIO

RESUMO

A prática do exercício aeróbico materno é capaz de gerar benefícios na função cognitiva da prole, melhorando aspectos relacionados à memória e aprendizado, aumentando a expressão de neurotrofinas e gerando neurogênese hipocampal. Entretanto, os efeitos de outras modalidades de exercício físico praticados pelas mães sobre a função cognitiva da prole permanecem desconhecidos. Essa tese teve como objetivo estudar os efeitos do exercício físico materno do tipo resistido em aspectos comportamentais, neuroplásticos e epigenéticos dos descendentes, explorando esses efeitos em diferentes períodos na vida da prole. Para tal, ratos Wistar foram utilizados em dois experimentos. O primeiro teve como objetivo avaliar os efeitos do exercício resistido (ER) materno no período neonatal, e o segundo na fase adulta da prole. O modelo de ER adotado em ambos os experimentos foi a escalada em escada vertical. Neste, após realização de teste de capacidade máxima, a rata teve que escalar a escada com peso fixado ao corpo. Esse protocolo foi realizado três vezes por semana, durante os períodos descritos. No primeiro experimento, ratas grávidas foram divididas em dois grupos: sedentário durante a gestação e o exercitado durante a gestação. Após o nascimento, no dia pós-natal (P) 8, os filhotes machos foram avaliados quanto ao nível de corticosterona (CORT) plasmática, neurogênese hipocampal e metilação global do DNA hipocampal; também foi avaliado a CORT materna e os marcos do desenvolvimento, peso corporal, tamanho do corpo e cabeça do P2 ao P21 da prole. Não foram observadas diferenças estatísticas entre as mães exercitadas e seus filhos em relação aos níveis de CORT quando comparados ao controle, sugerindo que essa modalidade de exercício não gerou níveis elevados de estresse. Não foram observadas diferenças nos marcos do desenvolvimento entre os grupos. Diferenças transitórias, diminuição do peso corporal e aumento do eixo ântero-posterior do crânio, foram observadas nos filhos de mães exercitadas. Os filhos de mães exercitadas apresentaram proliferação celular aumentada no hipocampo, bem como uma diminuição nos níveis de metilação global de DNA hipocampal. O conjunto desses resultados mostrou que o ER gestacional não gerou malefícios ou alterou de forma importante aspectos físicos dos descendentes, e foi capaz de modificar positivamente fatores associados à neuroplasticidade no hipocampo no período neonatal. No segundo experimento, os efeitos do ER maternal praticado em diferentes janelas temporais foram explorados. Quatro grupos compuseram esse experimento: o grupo de filhos de mães que não se exercitaram, filhos de mães que se exercitaram apenas no período gestacional, filhos de mães que se exercitaram apenas no período pré-gestacional e os filhos de mães que se exercitaram no período pré-gestacional e gestacional. Os descendentes machos foram avaliados em parâmetros comportamentais de memória e aprendizado (Labirinto Aquático de Morris), fatores de neuroplasticidade hipocampais (IGF-1 e BrdU+) e mecanismos epigenéticos hipocampais (Metilação global de DNA, Acetilação Global das Histonas H3 e H4, e a atividade da HDAC2). O ER praticado durante a gestação influenciou de forma discreta, mas positivamente a memória e aprendizado dos descendentes, diminuindo a atividade hipocampal da HDAC2 mas sem causar modificações nos marcadores de neuroplasticidade. Os filhos de mães exercitadas no período pré-gestacional, por sua vez, apresentaram aumento na expressão dos fatores neuroplásticos hipocampais (BrdU+ e IGF-1), acompanhado de diminuição da metilação global de DNA e aumento da acetilação da histona H4. Surpreendentemente, os filhos de mães exercitadas nos dois momentos não apresentaram modificações em nenhuma das análises feitas. O conjunto dos resultados obtidos nessa tese demonstra que o ER materno é capaz de gerar modificações hipocampais importantes relacionadas com a função cognitiva. Essa influência positiva parece estar mais relacionada à prática dessa modalidade de exercício realizada de forma isolada nos períodos pré-gestacional e gestacional.

Palavras-chave: metilação; cérebro; hipocampo; neuroplasticidade; anaeróbico.

ABSTRACT

Maternal aerobic exercise practice can cause benefits on offspring cognitive function, improving memory and learning, increasing neurotrophins expression and causing hippocampal neurogenesis. However, the effects of other modalities of exercise practiced by mothers on cognitive function of offspring, remain unknown. This Ph.D. thesis aimed to study the effects of maternal resistance physical exercise on behavior, neuroplastic and epigenetic aspects of the descendants, exploring these effects in different periods of mother's life and in distinct periods of progeny's life. Thus, Wistar rats were used in two groups of experiments, the first aimed to study the effects of maternal resistance exercise (RE) on the neonatal period, and the second on adult phase. In the first experiment, pregnant rats were divided into two groups: sedentary during pregnancy and exercise during pregnancy. The RE model used in both experiments was the vertical ladder climbing. After the maximum capacity test, the rat had to climb the ladder with weight in their tails. This protocol was performed three times per week, during the described periods. After the birthday, on postnatal day (P) 8, the male pups were evaluated for: plasmatic corticosterone (CORT) levels, hippocampal neurogenesis and global DNA hippocampal methylation; maternal CORT and motor milestones, body weight, body length and head length on P2 and P21, also were evaluated. No differences were observed in CORT levels of mothers and offspring, indicating that the modality of exercise did not cause high levels of stress. No differences were reported in motor milestones between groups; transitory differences were observed in some body weight and length measures. Offspring from exercise mothers presented an increase in hippocampal cellular proliferation, and a decrease of global DNA hippocampal methylation was also reported. These results demonstrated that maternal RE did not cause harm or alter of an important way the physical development of offspring and was able to cause positive modifications in the hippocampus at the neonatal period. In the second experiment of this thesis, the effects of maternal RE practiced in different temporal windows were explored. This experiment was composed of four groups: offspring of sedentary mothers before and during gestation, offspring of mothers exercised just at pregestational period, offspring of mothers exercised during pregnancy, and offspring of mothers exercised before and during the gestational period. The adult male offspring were evaluated to: behavior analysis (Morris water maze), expression of neuroplastic factors (BrdU and IGF-1), and hippocampal epigenetic mechanisms (Global Hippocampal DNA methylation, Global hippocampal H3 and H4 acetylation, and HDAC2 activity). The RE practiced during pregnancy discretely influenced the memory and learning of descendants, mitigating the hippocampal HDAC2 activity, but without altering neuroplastic factors. Offspring from exercised mothers before pregnancy showed overexpression of hippocampal neuroplastic factors (BrdU and IGF-1), a decrease of global hippocampal DNA methylation, and an increase of H4 acetylation. Surprisingly, the offspring of exercised mothers in the two moments did not show any modifications in assays performed in this group of experiments. The group of data reported in this thesis demonstrated that maternal RE can cause important hippocampal modifications related to cognitive function. This positive

influence seems to be linked to the maternal practice of this modality at gestational and pregestational periods.

Keywords: methylation; brain; hippocampus; neuroplasticity; anaerobic.

LISTA DE ABREVIATURAS

5-mc: 5- metilcitosina

ACOG: Colégio Americano de Geriatria e Obstetrícia

ANOVA: Análise de Variância

ATP: Adenosina Trifosfato

ATP-CP: Sistema Anaeróbico Aláctico – Creatina fostato

BDNF: Fator Neurotrófico Derivado do Encéfalo

BrdU: Bromodesoxiuridina

BSA: Albumina Sérica Bovina

CAPES: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico

CORT: Corticosterona

DAB: Diaminobenzidina

DAPI: 4',6'-diamino-2-fenil-indol

DG: Giro Denteado

DNA: Ácido Desoxiribonucleico

DNMT: DNA Metiltransferase

EE: Grupo experimental descendentes de fêmeas exercitadas em ambos os períodos, pré-gestacional e gestacional

ER: Exercício Resistido

ES: Grupo experimental descendentes de fêmeas exercitadas antes da gestação e mantidas sedentárias no período gestacional

FAPERGS: Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul

G: Dia gestacional

GCL: Camada celular granular

GLM: *General Linear Models*

H3: Histona H3

H4: Histona H4

H3K4me3: Tri-metilação da lisina 4 na histona H3

H19: Gene H19

HAT: Histona Acetiltransferase

HDAC: Histona desacetilase

IBRO: *International Brain Research Organization*

I.P.: Intraperitoneal

IGF-1: Fator de Crescimento Semelhante à Insulina Tipo 1

Igf2: Gene do Fator de Crescimento Semelhante à Insulina Tipo 2

Igf2r: Gene do Receptor do Fator de Crescimento Semelhante à Insulina Tipo 2

IgG: Imunoglobulina G

LTP: Potenciação de Longa Duração

M: Molar

Meg3: *Gene Maternally Expressed 3*

Min: Minutos

ml: Mililitro

MLC: *Maximum load capacity test*

mm: Milímetros

ng: Nanograma

NGF: Fator de Crescimento Nervoso

NH4OH: Hidróxido de Amônio

P: Dia pós-natal

PBS: Tampão Fosfato-Salino

pg: Picograma

pH: Potencial de Hidrogênio

QI: Quociente de Inteligência

RE: *Resistance exercise*

RNA: Ácido Ribonucleico

S: Segundos

SE: Grupo experimental descendentes de fêmeas que foram mantidas sedentárias antes da gestação e exercitadas durante o período gestacional

SEM: *Standard error of the mean*

SGZ: Zona Subgranular

SPSS: *Statistical Package for the Social Sciences*

SS: Grupo experimental descendentes de fêmeas que foram mantidas sedentárias antes e durante a gestação

TRKB: Receptor Tirosina Quinase B

UFRGS: Universidade Federal do Rio Grande do Sul

VEGF: Factor de Crescimento Endotelial Vascular

µm: Micrômetro

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INTRODUÇÃO

1.1 Impacto positivo do exercício físico na saúde e cognição dos praticantes

Já está bem estabelecido que o exercício físico é benéfico para diversos sistemas corporais, sendo sua prática recomendada por diversas entidades e associações médicas (HASKELL et al., 2007; WARBURTON; NICOL; BREDIN, 2006). Dentre os benefícios da prática do exercício físico, podemos destacar a prevenção de doenças cardiovasculares, doenças metabólicas, prevenção de osteoporose, melhora de força muscular, prevenção de alguns tipos de cânceres, melhora da saúde mental, melhora da função cognitiva e prevenção de algumas doenças neurológicas (CHEKROUD et al., 2018; FEBBRAIO, 2017; KOKKINOS, 2012; WARBURTON; NICOL; BREDIN, 2006).

Mais precisamente sobre o sistema nervoso central, diversas evidências suportam que a prática de exercício físico melhora a função cognitiva e aspectos relacionados à memória e aprendizado (MANDOLESI et al., 2018), previne doenças como Alzheimer (REGE et al., 2016) e Acidente vascular encefálico (PESCATELLO, et al., 2004), e auxilia no processo de reabilitação de doenças neurológicas (VAN DEN BERG et al., 2016). O impacto do exercício físico em relação ao sistema nervoso central pode ser observado em diferentes idades. Colcombe et al., (2006), em um ensaio clínico controlado-randomizado, observou que 6 meses de exercício aeróbico em pacientes idosos saudáveis foi capaz de aumentar a substância cinzenta em córtex frontal e em algumas regiões do hipocampo. Outros estudos com populações semelhantes relataram que a prática de exercício físico previne declínio cognitivo e diminui o risco do desenvolvimento de demência (COLBERG; SOMMA; SECHRIST, 2008; HOLLAMBY; DAVELAAR; CADAR, 2017; YAFFE et al., 2009). Em crianças e adultos a prática do exercício físico também pode gerar melhor desempenho acadêmico (principalmente em crianças), otimiza habilidades cognitivas, melhora aspectos relacionados à memória e aprendizado, e processos executivos e atencionais (DONNELLY et al., 2016; GREGO et al., 2005; VOSS et al., 2011; WINTER et al., 2007).

A maioria dos estudos explorando os efeitos do exercício físico sobre aspectos cognitivos utilizaram o exercício aeróbico para obtenção dos seus resultados. Essa modalidade de exercício físico é caracterizada por utilizar o oxigênio como principal fonte de energia, sob a forma de adenosina trifosfato – ATP, para a geração de trabalho

muscular. Esse tipo de exercício é geralmente composto de atividades de longa duração e que exigem a utilização de diversos grupos musculares, como a corrida. Em contrapartida, o exercício do tipo resistido ou anaeróbico, que utiliza duas vias principais como fonte de energia, a fosfagênio ATP-CP, presente nos músculos e caracterizada por atividades de força e explosão de muito pouca duração; e a via glicolítica, que utiliza o glicogênio presente nos músculos e fígado como fonte energética, e caracterizada por exercícios com resistência à contração muscular (UNESCO, 2013).

Embora menos estudado que o exercício aeróbico, evidências sugerem efeitos semelhantes da prática do exercício resistido aos observados no exercício físico aeróbico sobre a cognição de seus praticantes. Liu-Ambrose et al., (2010), demostraram que um protocolo de 52 semanas de exercício resistido, executado duas vezes por semana por idosas, foi capaz de gerar melhores resultados na função executiva e resolução de conflitos, quando comparado ao grupo de idosas controle. Resultados semelhantes também foram reportados por Cassilhas et al., (2007); nesse estudo os autores relatam que um protocolo de vinte e quatro semanas de exercício físico moderado e intenso em idosos foi capaz de gerar benefícios na função cognitiva.

Estudos pré-clínicos também demonstram o impacto positivo do exercício físico sobre o sistema nervoso, explorando com um pouco mais de profundidade alguns mecanismos biológicos e moleculares relacionados a esses benefícios. Huang et al., (2018), por exemplo, utilizando um protocolo de 12 semanas de corrida em esteira – modelo de exercício forçado – com ratos adultos, relataram melhor desempenho no labirinto aquático de Morris, indicando benefícios na memória e aprendizado de ratos exercitados. Em um protocolo de exercício aeróbico mais curto, Ding et al., (2006) observaram que ratos adultos expostos apenas 5 dias à roda de corrida de livre acesso – aparato de exercício voluntário – apresentaram maiores níveis hipocampais de Fator Neurotrófico Derivado do Encéfalo (BDNF) e Fator de Crescimento Semelhante à Insulina tipo 1 (IGF-1). Em outro estudo, Cassilhas et al., (2012), relataram que um protocolo de oito semanas de corrida em esteira ergométrica foi capaz de melhorar o desempenho em teste de memória e aprendizado, associado a maiores níveis de BDNF, IGF-1, sinaptofisina, receptor tirosina quinase (Trkb) e sinapsina hipocampal de ratos adultos.

De fato, o exercício físico aeróbico é capaz de melhorar a memória e aprendizado de seus praticantes e essa melhora está relacionada ao aumento de fatores neurotróficos hipocampais expressos pela prática do exercício físico, essencialmente o BDNF

(MANDOLESI et al., 2018). Essa expressão se dá tanto a nível encefálico, pela repetida ativação de neurônios necessária durante a prática de atividade física, quanto a nível periférico pela indução da síntese da D-β-hidroxibutirato pelo fígado, o qual é transportado pela circulação sistêmica ao hipocampo e modula a expressão de BDNF via inibição da histona deacetilase 2 (HDAC2) (LOPRINZI; FRITH, 2019; SLEIMAN et al., 2016). O aumento de BDNF hipocampal está diretamente relacionado com o aumento da neurogênese e potencialização de longa duração (LTP) no hipocampo, que são fatores essenciais para a consolidação de processos cognitivos (CASSILHAS; TUFIK; DE MELLO, 2016; SLEIMAN et al., 2016; VAN PRAAG et al., 1999).

Assim como com humanos, estudos pré-clínicos também suportam que a prática de exercício físico do tipo resistido, especificamente, gera um impacto positivo na função cognitiva de seus praticantes. Cassilhas et al., (2012), explorando diferentes modalidades de exercícios físicos sobre a função cognitiva e possíveis mecanismos relacionados a mesma, observaram que um protocolo de oito semanas de exercício físico resistido (escalada em escada vertical) foi capaz de gerar benefícios no aprendizado e memória, associados com um aumento de IGF-1, sinaptofisina e sinapsina hipocampal, esses achados foram associados a níveis de corticosterona (CORT) semelhantes ao grupo de ratos sedentários. Novaes Gomes et al., (2014) relataram que um protocolo de exercício físico resistido, escalada em escada vertical, realizada por 4 semanas em ratos adultos, foi capaz de gerar aumento da proliferação celular hipocampal. Utilizando outro modelo de exercício resistido, roda de corrida de livre acesso com resistência, por 4 semanas, Lee et al., (2013) mostraram que ratos adultos após serem submetidos a esse protocolo de treino apresentaram um aumento da proliferação celular hipocampal, sem mudanças em níveis plasmáticos de CORT.

Os mecanismos envolvidos na melhora cognitiva gerada pela prática do exercício resistido ainda não estão muito claros. Expressão hipocampal de IGF-1, BDNF e homocisteína são os mais relatados na literatura, sendo o IGF-1 o provável principal modulador desse efeito. De fato, alguns estudos investigando o exercício resistido sobre a função cognitiva e expressão de neurotrofinas no hipocampo, não observaram aumento da expressão hipocampal de BDNF, enquanto a expressão de IGF-1 é bem vista em estudos com animais e humanos (CASSILHAS et al., 2012; CHANG et al., 2012; HEROLD et al., 2019). O IGF-1 é produzido principalmente pelo fígado (70%), músculo e encéfalo, e, pela sua capacidade de atravessar a barreira hematoencefálica, ele pode ser encontrado em grandes concentrações no encéfalo. No encéfalo, o IGF-1 é capaz de

desencadear vários mecanismos que contribuem para a função cognitiva como: potencialização de longa duração, proliferação celular hipocampal e inibição de apoptose de células neurais (BASSIL et al., 2014; DORÉ; SATYABRATA; QUIRION, 1997; POPKEN et al., 2004).

1.2 Influência do exercício físico materno na função cognitiva da prole

De forma semelhante ao observado com o exercício físico *per se* em seus praticantes, diversos estudos clínicos e pré-clínicos mostram que a prática do exercício físico realizado pelos progenitores pode influenciar o processo de saúde e doença dos descendentes em diferentes idades, inclusive influenciando a função cognitiva, memória e aprendizado (GOMES DA SILVA; ARIDA, 2015; HOPKINS; CUTFIELD, 2011).

De forma geral, há evidências que a prática de exercício físico pode influenciar a cognição dos filhos, refletido em um melhor escore linguístico, quociente de inteligência (QI) e de vocabulário, menores níveis de ansiedade, melhor aprendizado e memória, maior expressão de fatores ligados a neuroplasticidade e aprendizado no hipocampo, e diversos outros benefícios em outros sistemas (ALVAREZ-BUENO et al., 2017; CLAPP, 1996; DOMINGUES et al., 2015; GOMES DA SILVA; ARIDA, 2015).

Em estudos com humanos, por exemplo, Esteban-Cornejo et al., (2015) em ensaio longitudinal, observaram que crianças entre 6 e 18 anos de idade, filhos de mães ativas antes e durante a gestação, apresentaram maiores escores linguísticos. Resultados semelhantes foram observados por Clapp, (1996); Clapp relatou que um programa de exercício físico realizado durante a gestação, por 20 mulheres, aumentou o escore de dois testes de vocabulário (*Wechsler Scale and Oral language skills*) em seus filhos, quando comparados aos filhos de mães sedentárias aos 5 anos de idade. Nesse mesmo estudo, as crianças descendentes de mães exercitadas durante a gestação apresentaram um melhor desempenho em um teste de inteligência (*Primary Scale of Intelligence – Revised*). Resultados semelhantes relacionados à inteligência de filhos de mães exercitadas durante a gestação também foram observados por Domingues et al., (2015), em um estudo de coorte com filhos de mães exercitadas durante a gestação.

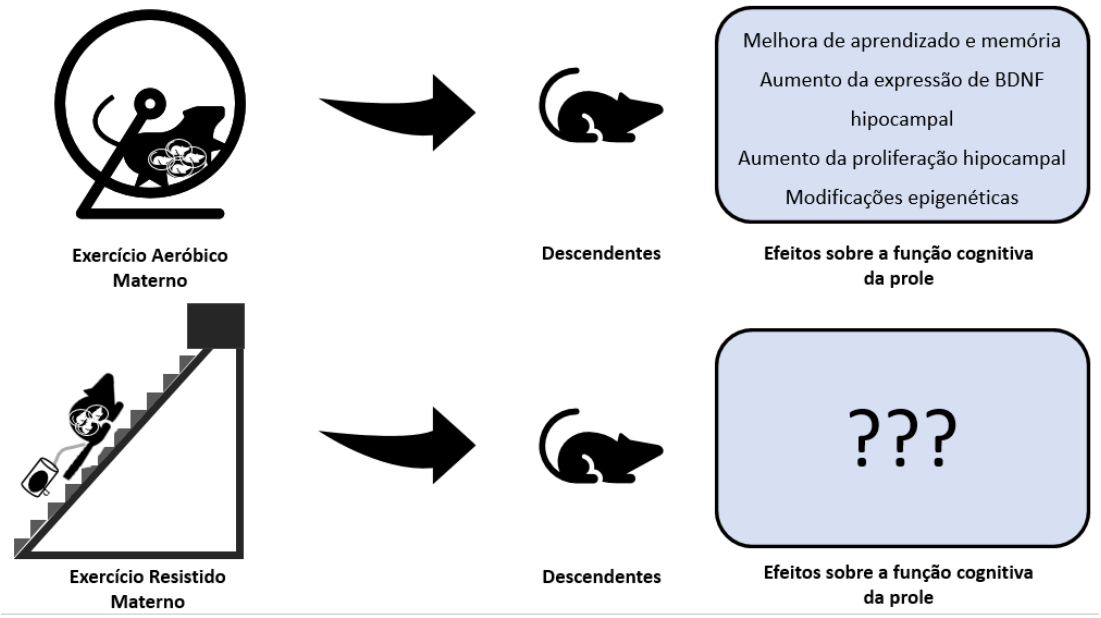
Até o momento não há estudos clínicos investigando possíveis vias e mecanismos relacionados à melhora da função cognitiva em filhos de mães exercitadas. Entretanto, algumas hipóteses sugerem que fatores neurotróficos providos pela prática de exercício físico materno, como o BDNF, seriam transferidos por via placentária para o feto

(FERNANDES; ARIDA; GOMEZ-PINILLA, 2017a; GOMES DA SILVA; ARIDA, 2015; ROJAS VEGA et al., 2010). Outra hipótese sugere que mudanças no ambiente materno tais como dieta, exposição a doenças e estresse durante a gestação, podem mudar a expressão de genes que serão expressos pelos descendentes no período neonatal e em alguns casos também na vida adulta, influenciando o processo de saúde e doença do ser (HANSON et al., 2011).

A pesquisa básica vem explorando os efeitos do exercício físico materno sobre a função cognitiva da prole, investigando mecanismos biológicos e moleculares (Quadro 1). Já está bem estabelecido que o exercício físico materno do tipo aeróbico praticado durante a gestação promove benefícios na função cognitiva da prole em diferentes idades por alguns mecanismos biológicos já bem descritos (GOMES DA SILVA; ARIDA, 2015). Bick-Sander et al., (2006), por exemplo, observaram que um protocolo de exercício aeróbico voluntário, com o modelo de roda de corrida de livre acesso durante a gestação e lactação, foi capaz de aumentar a proliferação celular hipocampal em filhos de mães exercitadas no dia P (dia pós-natal) 8 e P36. Utilizando outro modelo de exercício físico aeróbico – corrida em esteira ergométrica – e observando os efeitos do exercício físico gestacional nos descendentes de ambos os sexos, Dayi et al., (2012) também relataram aumento da proliferação celular hipocampal em diferentes idades (P21 e P120), em ambas as idades e sexos a proliferação celular foi acompanhada do aumento da expressão de leptina no hipocampo.

Além do aumento da proliferação celular hipocampal ser bastante observada em filhos de mães exercitadas no período gestacional, outro achado comumente reportado em estudos com esse cenário é o aumento da expressão de BDNF hipocampal, geralmente associado também com melhora em testes de memória e aprendizado. Kim et al., (2007) e Lee et al., (2006), relataram que corrida em esteira ergométrica e natação, respectivamente, praticados por ratas grávidas, do décimo quinto dia de gestação até o seu fim, foram capazes de melhorar a memória dos descendentes (avaliado em P29) acompanhado de aumento da proliferação hipocampal e aumento da expressão de mRNA BDNF. De maneira similar, Gomes da Silva et al., (2016) relataram que um protocolo de corrida em esteira ergométrica durante a gestação foi capaz de melhorar de forma discreta a curva de aprendizado no aparato labirinto aquático de Morris, essa melhora foi acompanhada de aumento do número de células hipocampais e aumento da expressão mRNA de BDNF hipocampal.

Figura 1. Efeitos do exercício aeróbio e resistido materno sobre a função cognitiva dos descendentes.



(Parnpiansil et al., 2003; Bick-Sander et al., 2006; Lee, et al., 2006; Kim, et al., 2007; Askua et al., 2012; Dayi, et al., 2012; Park, et al., 2013; Akhavan, et al., 2013; Robinson e Bucci, et al., 2014; Da Silva, et al., 2016; Wasinski, et al., 2016; Xu et al., 2017; Jang, et al 2017; Segabinazi et al., 2019)

Fonte: Elaborado pelo autor.

Em contraste do que observado nos efeitos do exercício materno sobre a função cognitiva da prole, outras modalidades de exercício físico, como o exercício resistido, são pobremente investigados nesse contexto. Uma das prováveis razões para essa modalidade de exercício físico não ser tão explorada em ensaios clínicos e estudos de forma geral que envolvam mães e filhos, é a crença de que a prática dessa modalidade possa gerar malefícios para saúde das mães e de seus descendentes (FIERIL et al., 2014; HAMMER; PERKINS; PARR, 2000). Apesar disso, essa modalidade de exercício físico é indicada e sua prática aconselhada pela ACOG (*American College of Obstetricians and Gynecologists*) (JAFFE, 2016) para gestantes sem risco na gravidez. O aconselhamento da ACOG se baseia em alguns ensaios clínicos que reportaram a segurança da prática dessa modalidade tanto para saúde da gestante quanto para saúde de seus filhos (BARAKAT; LUCIA; RUIZ, 2009; FIERIL et al., 2014; WHITE; PIVARNIK; PFEIFFER, 2014).

Até o momento não há estudos clínicos ou pré-clínicos que investiguem os efeitos do exercício físico materno resistido *per se* sobre a função cognitiva ou sistema nervoso central dos descendentes. Todavia, assim como observado na prática de exercício físico aeróbico, a prática do exercício físico resistido também é capaz de gerar benefícios à função cognitiva de seus praticantes. Essa melhora cognitiva, como supracitado, está relacionada a maior expressão de fatores de neuroplasticidade hipocampais como IGF-1, sinaptofisina e sinapsina, geralmente acompanhados por aumento da proliferação celular hipocampal.

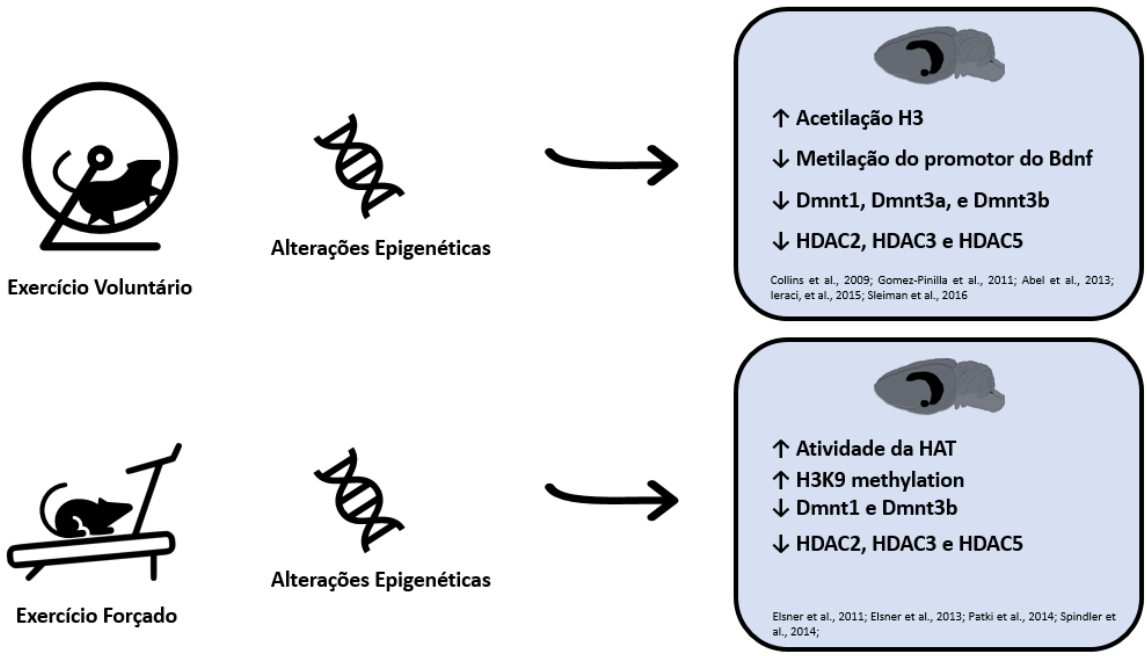
1.3 Mecanismos epigenéticos envolvidos na melhora cognitiva relacionada à prática do exercício físico e sua herança intergeracional

Já está claro que hábitos adotados durante a vida e/ou exposição a fatores negativos como estresse e toxinas são capazes de influenciar os processos de saúde e doença (BALE, 2015). Um dos mecanismos envolvidos nesse processo de mudança frente a fatores que influenciarão positivamente ou negativamente o processo de saúde e doença é a epigenética. A epigenética pode ser definida como mudanças na expressão de genes que acontecem independente de modificações na sequência do DNA e que são mitoticamente estáveis e, em alguns casos, podem ser herdadas (HANSON; SKINNER; HOSPITAL, 2016; SANDMAN et al., 2011). Alguns mecanismos epigenéticos estão diretamente relacionados com a função cognitiva, dentre eles a metilação do DNA. A metilação é uma modificação que gera uma conversão da citosina para 5-metilcitosina, com consequente redução da acessibilidade do DNA para fatores de transcrição. A inibição da DNA metiltransferase (DNMT), por exemplo, prejudica a LTP no hipocampo e altera a metilação de regiões promotoras dos genes *BDNF* e *Relina*, dois genes que estão diretamente relacionados com a plasticidade sináptica hipocampal (DENHAM, 2018; LEVENSON et al., 2006).

Alguns estudos demonstram que o exercício físico gera diminuição dos níveis de metilação de DNA hipocampal e/ou são capazes de inibir a ação da DNA metiltransferase. Gomez-Pinilla et al., (2011), por exemplo, demonstraram que um protocolo de exercício em roda de livre acesso em ratos adultos foi capaz de diminuir a metilação de DNA no gene *BDNF* na região promotora IV. Utilizando outro modelo de exercício (corrida em esteira ergométrica), Elsner et al., (2013) relataram diminuição das enzimas DMNT1 e DMNT3b no hipocampo de ratos adultos.

Outro mecanismo epigenético bastante estudado é a acetilação de histonas, que consiste na adição de um grupo de lisinas presente na cauda N-terminal da superfície do nucleossoma, e está associada com a transcrição ativa. Utilizando protocolo de exercício em roda de livre acesso com camundongos, Abel; Rissman, (2013) demonstraram um aumento da acetilação da histona H3 e aumento da atividade da HDAC2 no cerebelo e hipocampo dos animais treinados. Já com animais velhos, Lovatel et al., (2013), demonstraram que um protocolo de corrida em esteira ergométrica foi capaz de aumentar a acetilação da histona H4 em ratos. Os níveis de acetilação das histonas são controlados pelo balanço de proteínas com a histona acetiltransferase (HAT) e a atividade da histona deacetilase (HDAC) (PEIXOTO; ABEL, 2013). O aumento da expressão da HDAC2 (mas não da HDAC1), por exemplo, diminui a plasticidade sináptica e a formação de memórias; enquanto um modelo *knockout* da HDAC2 apresenta melhoras na memória (GUAN et al., 2009). Uma relação das principais modificações em mecanismos epigenéticos hipocampais causados pelo exercício físico voluntário e forçado encontram-se no quadro 2.

Quadro 1. Principais alterações epigenéticas geradas pela prática do exercício físico no hipocampo em diferentes tipos de aparatos.



Legenda: H3, Histona 3; Bdnf, Fator Neurotrófico Derivado do Encéfalo; Dnmt1, DNA metiltransferase 1; Dmnt3a, DNA metiltransferase 3a; Dmnt3b, DNA metiltransferase 3b; HDAC2, Histona deacetilase 2; HDAC3, Histona deacetilase 3; HDAC5, Histona

deacetilase 5; HAT, Histona acetil-transferase; H3K9, metilação da lisina 9 da histona H3. Fonte: Elaborada pelo próprio autor.

Além do exercício físico gerar benefícios para função dos seus praticantes, evidências vêm apontando que a sua prática pode gerar benefícios para os descendentes dos seus praticantes, impactando positivamente em seus processos cognitivos. Estudos utilizando um protocolo de corrida em esteira ergométrica em ratos, observaram que os filhos de pais exercitados tinham uma discreta melhora do aprendizado espacial, acompanhado de diminuição da metilação global do DNA hipocampal (MEGA et al., 2018; SPINDLER et al., 2019). Xu et al., (2017), reportaram que um protocolo de exercício físico – corrida em esteira ergométrica – executado em camundongos no período pré-gestacional, ajudou no desenvolvimento de embriões produzidos em vitro; de forma adicional, os autores também observaram que os embriões derivados de mães exercitadas apresentaram menores níveis de ATP mitocondrial, e níveis normalizados de metilação das ilhas CpGs nas regiões de *impriting* dos genes (*Igf2, Igf2R, Meg3* e *H19*) quando comparados a embriões derivados de mães inativas no período pré-gestacional. Em outro estudo, também explorando protocolos de exercício aeróbico com corrida em esteira ergométrica, Segabinazi et al., (2019) observaram os efeitos do exercício materno em diferentes períodos – antes da gestação, durante a gestação, e a associação dos dois períodos – sobre a função cognitiva da prole; foi observado que filhos de mães exercitadas no período pré-gestacional apresentaram maiores níveis de expressão de fatores de neuroplasticidade, neurogênese hipocampal e menor metilação global do DNA hipocampal.

Apesar de vários estudos demonstrarem os benefícios do exercício aeróbico materno sobre a função cognitiva da prole, e explorando possíveis fatores intergeracionais de herança, ainda não há estudos investigando os efeitos do exercício físico materno do tipo resistido sobre a função cognitiva da prole. Sendo esse ponto o principal objetivo desse estudo.

2. JUSTIFICATIVA E HIPÓTESE

A prática de exercício físico aeróbico durante a gestação promove benefícios à saúde da mãe, e um corpo crescente de evidências sugere que esses benefícios são transmitidos para seus descendentes. Entre os benefícios observados em filhos de mães exercitadas podemos destacar a melhora da função cognitiva associada ao aumento de expressão de fatores de neuroplasticidade hipocampais.

Até o presente momento não há evidências que suportem ou não a afirmativa de que a prática de exercício físico resistido pelas mães possa gerar benefícios na função cognitiva da prole, e nem se há um período ideal para sua prática que potencialize ou iniba esses possíveis efeitos. Dessa forma, nos propomos a investigar os efeitos do exercício resistido materno em diferentes janelas temporais sobre a função cognitiva dos descendentes em diferentes fases da vida. A hipótese central dessa tese é que a prática de exercício resistido materno gera benefícios para a função cognitiva da prole em diferentes fases da vida. Outras hipóteses também serão verificadas: (1) o exercício materno gestacional do tipo resistido não gera malefícios para saúde da mãe e da prole; (2) o impacto positivo maior na função cognitiva de filhos de mães exercitadas continuamente, pela provável sinergia da prática de exercício nos períodos pré-gestacional e gestacional; (3) o exercício materno resistido promove influências em mecanismos epigenéticos hipocampais relacionados a melhora da função cognitiva.

3. OBJETIVOS

3.1 Objetivo Geral

Investigar os efeitos do exercício físico materno do tipo resistido sobre o comportamento, neuroplasticidade e fatores epigenéticos da prole em diferentes fases da vida dos descendentes.

3.2 Objetivos Específicos

Experimento 1

Relatar o impacto do exercício físico resistido gestacional sobre os níveis de corticosterona materna e neonatal;

Investigar a influência do exercício físico resistido gestacional sobre parâmetros físicos da prole no período neonatal (peso, tamanho corporal, tamanho de cabeça e marcos do desenvolvimento).

Reportar os efeitos do exercício físico resistido gestacional sobre a neurogênese hipocampal e níveis de metilação global do DNA hipocampal no período neonatal.

Experimento 2

Estudar o impacto do exercício resistido materno realizado em diferentes janelas temporais (antes da gestação, durante a gestação, e nos dois momentos de forma conjunta) sobre os descendentes durante a vida adulta, observando:

Memória e aprendizado espacial;

Modificação de marcadores neurobiológicos de plasticidade hipocampal: IGF-1 e BrdU;

Modificações epigenéticas hipocampais – níveis globais de metilação do DNA, acetilação das histonas H3 e H4, e atividade da HDAC2.

4. COLETÂNEA DE ARTIGOS

4.1CAPÍTULO I

"Strength training during pregnancy influences hippocampal plasticity but not body development in neonatal rats"

(Artigo aceito na revista *Journal of Musculoskeletal and Neuronal Interactions* – ISSN:1108-7161).

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Original Article

Strength training during pregnancy influences hippocampal plasticity but not body development in neonatal rats

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Abstract

Objective: To describe the effects of strength exercise practice during pregnancy on the offspring's development parameters: growth and motor performance, hippocampal neuroplasticity, and stress levels. **Methods:** Pregnant Wistar rats were divided into two groups: sedentary and exercised rats. Exercised pregnant rats were subjected to a strength training protocol (vertical ladder climbing) throughout the gestational period. Male offspring's body weight, length, and head size were evaluated during the neonatal period (postnatal days [P]2–P21), as well as motor milestones during PO–P8. At P8, a set of male pups were subjected to global hippocampal DNA methylation, hippocampal cell proliferation, and plasma corticosterone concentration. **Results:** Offspring from trained mothers presented a transient change in body morphometric evaluations, no differences in milestone assessments, enhancement of cell proliferation in the dentate gyrus of the hippocampus, and decreased global hippocampal DNA methylation compared with the offspring from sedentary mothers. Furthermore, strength training during pregnancy did not change the corticosterone concentration of exercised mothers and their offspring. **Conclusions:** These data indicate that strength training can protect offspring's development and could impact positively on parameters linked to cognitive function. This study provides a greater understanding of the effects of strength exercise practiced during pregnancy on the offspring's health.

Keywords: Epigenetics, Neural Plasticity, Neurogenesis, Physical Activity

Introduction

The gestational period is considered a critical development window, and environmental influences, such as physical exercise practice and diet, in this period can be important for offspring's health outcomes[1]. However, the influence of progenitor's health habits on descendants is more studied in diseases scenarios[2].

The healthy lifestyle of parents could influence offspring by programming somatic cells in utero, influencing the development of tissues and organs, such as the brain[3]. One of the mechanisms involved in the programming of somatic cells is epigenetic, which can be defined as changes to gene expression that are independent of sequence modifications and that are mitotically stable and, in some cases, heritable[3,4]. One of the epigenetic marks is DNA methylation. This modification leads to a conversion of cytosine to 5-methylcytosine, with reduced accessibility of the DNA to transcription factors. Inhibition of DNA methyltransferases

The authors have no conflict of interest.

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Table 1. Maternal resistance exercise variables in experimental group.

Group	Set	Repetitions	Intensity / Maximum load loaded	Frequency	Rest
SS	-	-	-	-	-
SE	4 – 9 climbs per day of training	8 – 12 repetitions per climb	35 – 75% MLC/ 439.81 ± 43.81	3 times per week / total of 8 - 9 days of training	120 sec

MLC, Maximum load capacity test. n=8 - 9 per group. Data are expressed as mean ± standard error of the mean.

(enzymes that catalyze the transfer of a methyl group to DNA), for example, prejudices long-term potentiation in the hippocampus and alters the methylation of the promoter regions of *Reelin* and *Bdnf*, two hippocampal genes that are directly involved in synaptic plasticity[5,6].

Basic and clinical research has studied the effects of maternal aerobic exercise on progeny health. Animal studies demonstrate that maternal aerobic exercise during pregnancy improves offspring's spatial learning by increasing neuroplasticity factors[7,8]; in turn, clinical studies revealed positives influences of pregnancy aerobic exercise on language and intelligence scores of the descendants[9,10]. However, other modalities such as strength exercise, acrobatic exercise, and functional exercise are not widely explored.

Strength training during the gestation period has been avoided by pregnant women, due to concerns that the practice of this modality could cause harm to mother and child[11,12]. However, clinical trials[13,14] concluded that this modality of exercise practiced during pregnancy does not cause any harmful effects to mothers and children. Studies investigating the effects of strength exercise, specifically in adult rats, demonstrated improvement in cognitive performance and enhancement in hippocampal neurogenesis[15,16]. Cassilhas et al.[16], for example, showed that a strength training protocol yields positive results on learning and spatial memory and increases the concentration of some neuroplasticity markers (synapsin, synaptophysin, and IGF-1) in the hippocampus. Until now, there are no studies exploring the effects of strength exercise during pregnancy on offspring brain.

Thus, the present study describes the effects of strength training during the gestational period on physical and motor development, as well as on the offspring's hippocampal neuroplasticity and corticosterone (CORT) concentration in the neonatal period.

Material and Methods

Animals and Groups

Twenty-four females and eight males Wistar rats (80 days old) from a local breeding colony (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil) were used for obtaining male progeny. To avoid the effects of hormonal variation in females – that could influence the results of the behavioral and molecular outcomes studied here – only male offspring were used for the study. The estrous cycle of the primiparous females was monitored daily and in the proestrus phase, the rats were housed overnight with a sedentary male to mate. If the spermatozoa were detected in the vaginal smear in the next morning to the mate, that day was considered as the fertilization day or day 0 of gestation (G0); after the mating period, male progenitor was discarded from the present study. Pregnant rats were divided into sedentary and exercised. After birth, two experimental groups were composed of male offspring (n=72) from sixteen different litters, [1] offspring from sedentary mothers and [2] offspring from exercised mothers. Animals were kept in standard living boxes (300 mm x 190 mm x 130 mm), – one animal per box, with your litter (n=7–8), in a controlled temperature environment (20±2°C), dark/light cycle of 12 hours (lights on at 7:00 a.m.), with water and food *ad libitum*. Procedures performed in this study were approved by the Ethical Committee at the Universidade Federal do Rio Grande do Sul (#29840).

Physical Exercise Protocol

The physical exercise protocol consisted of pregnant rats climbing a vertical ladder (1,1 x 0,8 m), with an inclination of 85°, with lead weights fixed to animal tail with adhesive tape.

Two weeks before the pregnancy, female rats were familiarized with the apparatus by climbing it three consecutive days. On the first day, 3 climbs without weight fixed in their bodies. On the second and third days, 3 climbs with 30 g of weight fixed in their bodies.

One week before the pregnancy, a maximum load capacity test (MLC) was performed individually in the future exercised mothers, which consisted of 4 to 8 climbs while carrying progressively heavier loads. The starting load in the MLC test corresponded to 50% of the animal's body weight. Once the rat reached the top of the stairs, the animal could rest for 120 seconds. After successfully completing climbing with the starting load, 30g of lead was added. This process was repeated until the animals were no longer able to complete the full climb. The highest load successfully carried in the complete length of the vertical ladder was considered the MLC of the animal and was used for the first four training days.

In the firsts 4 training days (after pregnancy confirmation) animals had to climb a vertical ladder 4 to 9 times, with 30%, 45%, 55% and 75% of their MLC load. Before the next training day (training day five) another MLC test

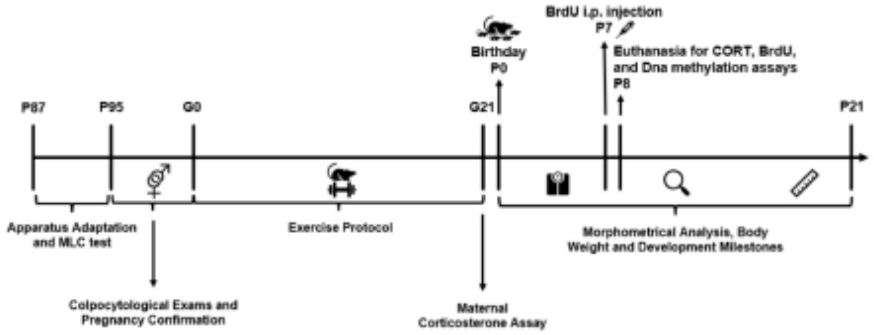

Figure 1. Timeline of key experimental procedures. MLC (maximum load capacity test), CORT (corticosterone), G (Gestational day), P (postnatal day).

was performed to define the load for the next four or five sessions. This exercise protocol was done three times a week, beginning at G0 and finishing at G21, with 48 h or 72 h intervals between training days. In all periods of training, rats that declined to climb the ladder voluntarily were encouraged by gently tapping on their backs. If they persisted in refusing to climb the ladder, they were discarded from the study. In this study, one rat refused to perform the exercise protocol.

A total of 8–9 days of training were performed by exercised mothers. A summary of information about the training variables per group is shown in Table 1. The present exercise protocol was adapted[17], according to the needs imposed by our study.

A timeline with the key events of the experiment and exercise protocol is shown in Figure 1.

Stress Evaluation

After the period of exercise protocol, a set of mothers of both groups and their litters with 8 days old, were euthanized. Trunk blood was collected between 9:00 a.m. and 11:00 a.m. in heparinized tubes, centrifuged at 10.000 rpm for 15 min. to attain the plasma and then stored at -80°C until the assay. Total CORT concentration was measured with a commercial ELISA kit (Enzo ADI-900-097 Enzo Life Sciences), according to the manufacturer's specifications.

Physical Evaluation and Neonatal Development

Body weight and length, and cephalic measurements were evaluated at P2, P7, P14 and P21, using an analytic balance and a digital pachymeter. Additionally, at P1 to P8, the following development milestones were examined: surface righting, cliff aversion, negative geotaxis, and proprioceptive posterior limb placement. The day of milestone emergence was when the pup performed the task in less than thirty seconds[18]. All neonatal physical evaluations were performed in the light cycle of animals.

Immunohistochemistry for BrdU

At P7, a set of the pups received one BrdU injection (5-Bromo-2'-deoxyuridine; Sigma, 100 mg/kg, intraperitoneally, dissolved in 0.1 M NH_4OH, 20 mg/mL). At P8, male offspring were euthanized by decapitation and the entire brain was dissected and transferred to cryoprotected by immersion in 15% and 30% sucrose solution (Synth, Brazil) at 4°C. Then, brains were post-fixed in the 4% paraformaldehyde solution for 24 h. After, they were frozen in isopentane, cooled in liquid nitrogen and stored at -80°C until processing.

Serial coronal sections from the dorsal hippocampus of 20 µm thickness were obtained utilizing a cryostat (CM1850, Leica, Germany) at -20°C. Five slices of coronal sections per animal were obtained, with a range of 30 µm between slices. The sections corresponded to approximately 2.8 to 4.3 mm posterior to the bregma[19]. Slices were washed in xylol for 5 min. (2 times) and in decreasing concentrations of ethanol. Antigenic retrieval was obtained at 92°C with citrate buffer for 20 min. Then, tissues were washed with distilled water and the endogen peroxidase was inactivated (H_2O_2 5% dissolved in methanol). Next, sections were washed in Triton-X dissolved in PBS (PBS-tx), incubated with anti-5'-2'-deoxyuridine monoclonal antibody 1:500 (General Health) for 2 hours at ambient room temperature and overnight at 4°C. Slices were then washed again in PBS-tx and incubated with the secondary antibody IgG Peroxidase anti-mouse rabbit (1:100; Sigma-Aldrich) for 1 hour at room temperature. The immunohistochemical reaction was revealed by

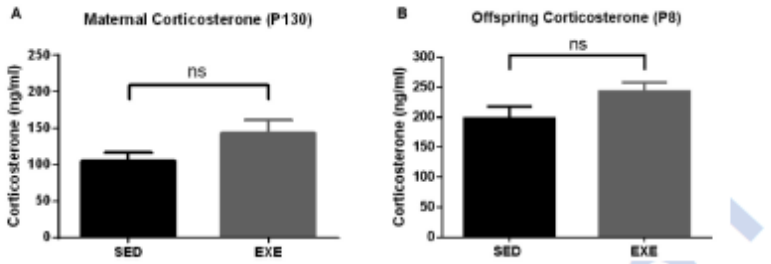

Figure 2. Plasma concentration of corticosterone in mothers exercised or sedentary (A) and in their offspring (B). SED (sedentary); EXE (exercised); ns (non-significant). n=4 - 5. Values are expressed in mean ± SEM.

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®. The negative control was performed by switching the primary antibody with PBS[20].

Quantification of BrdU labeled cells

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

Epigenetic assay

DNA isolation

Another set of animals was destined for epigenetic assay at P8. Pups of both groups were euthanized by decapitation and the hippocampi were quickly dissected. On the day of assay, the male offspring's hippocampus DNA was isolated using a commercial Tissue Section DNA isolation Kit (TRIzol™ Reagent catalog #15596026, Thermo Fisher Scientific) according to the manufacturer's instructions. Hippocampus DNA yield was quantified by absorbance using NanoDrop™ 2000 Spectrophotometer.

Global DNA methylation

The pup's hippocampal global DNA methylation was assessed utilizing a commercial kit MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Epigentek™) (Base catalog # P-1030). Briefly, 100 ng of DNA per well was added to the plates and incubated with 28 µL of DNA ligation buffer for 60 min. at 37°C.

Thereafter, 150 µL of blocking buffer was added and incubated for 50 min. at room temperature followed by washed five times with washing buffer. Developer solution (100 µL/well) was then added and incubated with 1–5 min. in a dark place. Absorbance at 450 nm was measured using an automatic microplate reader (Anthos Zenyth® – 200rt).

Statistical Analysis

Initially, data were analyzed by Shapiro-Wilk normality test, and homogeneity of variance was calculated with the Levene's test. Differences between sedentary and exercised groups were determined using the Student's unpaired t-test and Mann-Whitney test. Statistical analysis was performed using IBM SPSS Statistics 24.00, and statistical significance was considered when $p<0.05$. Data are expressed as mean ± standard error of the mean (SEM).

Results

Corticosterone concentration levels

Plasma concentration of CORT was observed in the mothers and their litters. No significant difference in plasma concentration of CORT was observed between exercised and sedentary mothers ($t(8)= -1.788$, $p=0.112$), nor between the different groups of pups ($t(6)= -1.822$, $p=0.118$) (Figures. 2A and 2B).

Physical evaluation – weight body and length, and motor milestones

Offspring body weights of each group at P2, P7, P14, and P21 are summarized in Table 2. Student's unpaired t-test indicated that the male offspring from exercised mothers had lower body weight at P7 ($t(18,2)=2.58$, $p=0.015$) and P14 ($t(21,7)=3.52$, $p=0.002$). No differences were observed for the other days (Table 2).

At P2 ($t(20)= -2.73$, $p=0.013$) and P21 ($t(20)= -2.92$, $p=0.008$), male offspring from mothers that exercised had an anteroposterior skull size that was larger than that of the descendants from sedentary mothers. Lengths of skull, body,

Table 2. Body weight, pachymetry and motor milestones in male offspring of sedentary and exercise mothers.

		SED		EXE	
	N	Mean ± SEM	n	Mean ± SEM	p
WEIGHT					
P2	16	9.35 ± 0.11	16	9.15 ± 0.11	0.207
P7	16	18.94 ± 0.12	16	17.93 ± 0.36	0.019*
P14	16	36.35 ± 0.21	16	34.64 ± 0.43	0.002*
P21	16	55.84 ± 0.57	16	54.68 ± 0.71	0.217
PACHYMETRY (P2)	12		10		
Anteroposterior 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		
Anteroposterior 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
MOTOR MILESTONES	15		7		
Surface Righting		1.06 ± 0.06 d		1.00 ± 0.00 d	0.495#
Posterior Limb Placement		5.80 ± 0.31 d		6.28 ± 0.42 d	0.164
Negative Geotaxis		5.80 ± 0.41 d		4.7 ± 0.64 d	0.379
Cliff Aversion		1.80 ± 0.22 d		1.28 ± 0.28 d	0.099#

Values are means ± SEM. Abbreviations: SED, sedentary; EXE, exercise; P, postnatal day; d, day of appearance. Unpaired Student T-test and # Mann-Whitney test, and utilized (p<0.05).*

and tail at days P7 and P14 were not different and are not presented in Table 2.

No differences were observed between the groups in motor milestone evaluations (Table 2).

Hippocampal cells proliferation

Male offspring from exercised mothers presented a higher number of cells in the hilus of the dentate gyrus of the hippocampus compared with the offspring from sedentary mothers (t (15)= -2.25, p=0.040) (Figure 3A). Representative images of sedentary and exercise groups are exposed in Figures 3C and 3D, respectively.

Hippocampal global DNA methylation

Male offspring from exercised mothers presented a lower global DNA methylation in the hippocampus when compared with the offspring from sedentary mothers (t (6)=2.77, p=0.032) (Figure 3B).

Discussion

This study showed that a strength exercise protocol during pregnancy did not influence the CORT levels in the mothers or the descendants, or cause modifications in the male offspring's physical development. However, the development of the hippocampus was influenced by the mothers' training. An increase in the number of cells in the hilus of the DG within the hippocampus and a decrease in global hippocampal DNA methylation were observed.

High-intensity exercise protocols and the use of involuntary apparatuses in the gestational period may abruptly raise CORT levels, causing deleterious effects to offspring. Wasinski et al.[21] exploring the effects of forced swimming during pregnancy, for example, observed an increase in maternal CORT and a decrease in neuroplasticity markers in the progeny at neonatal life. Similar results were also observed by Jang et al.[22]. Investigating a protocol of involuntary swimming exercise during pregnancy, the authors reported that exercised mothers presented a severe reduction in the number of embryos implanted and amniotic fluid with a high concentration of cortisol and a low concentration of growth factors (NGF and VEGF). Due to possible deleterious effects caused by high-intensity protocols during pregnancy, here we adopted a low to moderate intensity exercise protocol. In fact, this training protocol did not increase the CORT concentration levels in mothers or offspring. A similar result to that of our study was observed by Rosa

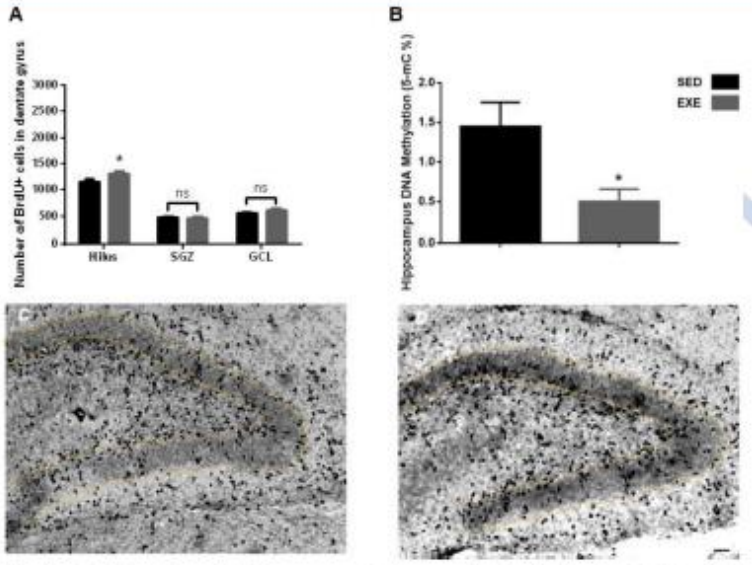

Figure 3. (A) Number of BrdU positive cells in hippocampus dentate gyrus of male offspring (n=8 - 9); (p=0.040). (B) Global hippocampal DNA methylation of the male offspring (n=4); (p=0.032). (C-D) Digitalized image of the DG hematoxylin counterstaining of SED and EXE groups (captured at 40x). SED, sedentary group; EXE, exercised group; DG, dentate gyrus; SGZ, Subgranular zone; GCL, Granule cell layer; ns (non-significant). Unpaired Student's t-test. Values are expressed in mean ± SEM and percentage of mean, respectively. Scale bar = 80µm.

et al.[23], using two different strength exercise protocols, tower climbing and squat exercise, during the gestational period. The authors reported that exercised mothers had a high concentration of fecal CORT, but this increment was not significant. Another similarity between our data and those of Rosa et al.[23] is the model of exercise adopted–voluntary exercise. Protocols of involuntary exercises, such as forced swimming, are related to high levels of CORT concentration, while voluntary exercise, such as vertical ladder climbing and wheel running, is considered less stressful for the animals[21,24].

High maternal levels of stress, restriction diet, and high-intensity exercise could cause a delay in the acquisition of neurodevelopmental milestones and important negative changes in body morphometric measures in the offspring[21,25–27]. Here, descendants' physical evaluation revealed that the strength exercise protocol did not alter the physical development of male offspring but just transiently changed some morphometric parameters studied. This is in accordance with clinical and basic research, which shows that physical exercise during pregnancy could cause changes in the weight of some organs, fat, and various structures, but these changes are transitory and do not cause any functional change or have clinical relevance[2,28]. Thus, strength exercise does not seem to significantly alter the body's morphometric development, and this outcome could be considered a positive result, because it indicates that this modality of exercise practiced by mothers is safe for the development of male offspring's body.

Two potential parameters involved in the influences of maternal strength exercise on the cognitive processes of the descendants were studied: cell proliferation and global DNA methylation in the hippocampus. Both were found to be modified in the offspring from mothers submitted to strength training. Several studies conclusively demonstrated that physical exercise can affect the expression of genes related to cognitive processes, memory behavior tests, and synaptic plasticity in the brain by epigenetic mechanisms[6]. One of these epigenetic mechanisms is the methylation of DNA, which is associated with the silencing of genes, by an increase in chromatin compaction; it is also related to the enhancement in the expression of plasticity-promoting genes, such as the *Bdnf* and *reelin* genes. Furthermore, the actions of DNMT1 and DNMT3A are directly involved in

neurogenesis[29,30]. Although it is not possible to make a direct comparison of our results with those of others, in one of the few studies reporting modifications in epigenetic marks in the offspring from exercised mothers, Xu et al.[31] observed a lower level of global DNA methylation in the embryos of exercised mothers. With methods more similar to ours, Segabinazi et al.[32] showed that the offspring from exercised mothers during the pregestational period presents a decrease in global hippocampal DNA methylation associated with a high expression of hippocampal neuroplasticity factors in adult life.

The hippocampal hilus is considered an important neurogenic niche, and it is the area from which granule cells are derived in late development[33]. The enhancement in hippocampal cells in the male offspring from exercised mothers, besides demonstrating a possible positive influence of strength training. Exploring the effects of aerobic training during pregnancy, Gomes da Silva et al.[7] also observed high hippocampal cell proliferation, associated with an overexpression of the hippocampal BDNF level. This is the most described biological mechanism for the increase in cellular proliferation caused by aerobic exercise in this and other studies. Nevertheless, other neuroplasticity markers could also explain the enhancement in hippocampal cell proliferation in the descendants. Studies using strength exercise protocols in adult rats reported that hippocampal neurogenesis is associated with an enhancement in IGF-1 and synapsin levels in the hippocampus, together with better performance in memory and learning tests[15,16]. Thus, as observed in maternal aerobic exercise, strength training during pregnancy also seems to have a positive influence on the offspring's hippocampal neuroplasticity factors.

This study had as objective to investigate the influence of maternal strength training on male offspring's development and brain plasticity in order to advance knowledge in this area. Some issues that were not explored here, and were limitations of this study, should be investigated in future researches: [1] the expression of other neuroplasticity markers and epigenetic mechanisms, [2] the effects of maternal strength training in different periods of offspring's life, and [3] the influence of maternal strength training in female offspring.

The present data agree with current clinical research evidence on the safety of this modality of exercise for mothers and offspring. Furthermore, as with maternal aerobic exercise, strength training during pregnancy had a positive impact on markers linked to descendants' cognitive function. These initial results open a range of possibilities for further investigations about the influence of this physical exercise modality on the offspring's cognitive function and other aspects related to their development.

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4.2CAPÍTULO II

"Maternal Resistance Exercise Promotes Changes in Neuroplastic and Epigenetic Marks of Offspring's Hippocampus During Adult Life"

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Title*:*

Maternal Resistance Exercise Promotes Changes in Neuroplastic and Epigenetic Marks of Offspring's Hippocampus During Adult Life

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Abstract

Studies indicate that gestational exercise practice positively impacts the offspring's cognition. Nevertheless, the influence of maternal resistance exercise, different periods of exercise practice, and the inter- and transgenerational effects involved in these responses are not known. This study sought to report the influence of the maternal practice of resistance exercise on offspring's cognitive function, exploring behavior, and neuroplastic and epigenetic marks in the hippocampus. Female Wistar rats were divided into four groups: sedentary (SS), exercised during pregnancy (SE), exercised before pregnancy (ES), and exercised before and during pregnancy (EE). Exercised rats were submitted to a resistance exercise protocol (vertical ladder climbing). Between postnatal days P81 and P85, male offspring were submitted to the Morris water maze test. At P85, the following analyses were performed in offspring's' hippocampus: expression of IGF-1 and BrdU+ cells, global DNA methylation, H3/H4 acetylation, and HDAC2 activity. Only the offspring of SE mothers presented subtly better performance on learning and memory tasks, associated with lower HDAC2 activity. Offspring from ES mothers presented an overexpression of hippocampal neuroplastic marks (BrdU+ and IGF-1), as well as a decrease of DNA methylation and an increase in H4 acetylation. Offspring from EE mothers (continuously exercised) did not present modifications in plasticity or epigenetic parameters. This is the first study to observe the influence of maternal resistance exercise on offspring's brains. The findings provide evidence that offspring's hippocampus plasticity is influenced by exercise performed in isolated periods (pre- or gestationally) more than that performed continually.

Keywords: histone acetylation, physical activity, brain, intergenerational, memory, methylation.

1. Introduction

It is widely recognized that progenitors' habits can affect their offspring's health. Parents' exposure to toxins, diet, and stress are commonly known factors that can interfere in their offspring's development and promote changes in phenotypes during adulthood [1,2]. The progenitor's physical exercise practice has been studied as a possible positive influence factor on offspring's health, and some current reviews attempt to elucidate this topic by exploring how progenitors' physical exercise can influence the epigenetic machinery of their descendants [3,4].

The epigenetic machinery has been noted to exert a pivotal role in inter- and transgenerational effects in response to the rodent parent's lifestyle [5]. Some epigenetic modifications are related to better performance on learning and memory tests, directly influencing cognitive function [6]. Histone acetylation, the addition of an acetyl group to lysine residues in the N-terminal tails on the surface of the nucleosome, for example, is directly associated with active transcription and with the establishment of long-term memory [7]. The histone level is regulated by the balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) protein content. HATs catalyze the transfer of acetyl groups to histone proteins, whereas histone HDACs cause the removal of acetyl groups [8]. Precisely, overexpression of HDAC2 in the hippocampus impairs memory and blunts long-term potentiation (LTP), whereas *Hdac2*-knockout mice display memory enhancements [9]. Another widely studied epigenetic mark is DNA methylation, which involves the transfer of a methyl group into the 5c position of the cytosine ring of DNA. Some evidence indicates that, as histone modifications, DNA methylation changes could play an important role in the molecular formation and maintenance of long-term memories [8,10]. One of the main epigenetic marks involved in the relation between methylation and modification of memory is the action of DNMTs (DNA methyltransferase). The DNMT inhibitors can impair the induction of LTP at hippocampal synapses [11,12]. Feng et al. [13] demonstrated that DNMT neuronally impaired animals have deficits in different types of memory, including spatial memory, and in hippocampal LTP.

Physical exercise practice, in either voluntary or involuntary modalities, could be a potent intervention able to modify histone acetylation status and DNA methylation levels in the rat brain at different stages of development [14–17]. Although some studies have demonstrated the benefits of maternal aerobic physical exercise practice to memory

and learning tasks in offspring, which are associated, at least in part, with high levels of brain-derived neurotrophic factor (BDNF) and hippocampal neurogenesis [18–20], the influence of the maternal practice of exercise on offspring's hippocampus epigenetic marks is not so abundant. Xu et al. [21], for example, observed potentially enhanced histone methylation modifications and better development in embryos from mother mice that exercised during the pregestational period. In another study, Segabinazi et al. [22] showed that offspring of mothers that exercised before the gestational period presented a decrease in global hippocampal DNA methylation during their adult life, which was associated with cell proliferation in the hippocampus.

Indeed, all reports involving the impact of maternal exercise on the central nervous system of descendants, in a health context, explore aerobic exercise, and mostly during pregnancy. The influence of resistance exercise practice by progenitors on their offspring's brains is unknown. Evidence supports that the practice of resistance exercise by adult rodents has a similar positive impact to that observed in aerobic exercise [23]. Better performance on memory and learning tests, overexpression of neuroplastic factors (IGF-1 and synaptophysin), and enhanced hippocampal cell proliferation have been reported in adult rats submitted to this modality of exercise [23,24].

This study was designed to investigate the influence of the maternal practice of resistance exercise in different periods (before pregnancy, during pregnancy, and performed continually – before and during pregnancy) on offspring's spatial memory and learning, as well as on their epigenetic and plasticity markers related to cognitive function. The main hypothesis of this study is that the maternal practice of resistance exercise can promote positive impacts on the cognitive function of descendants, as observed in the maternal practice of aerobic exercise.

2. Material and methods

2.1 Ethical considerations and animals

All experiments were approved by The Ethical Committee at the Universidade Federal do Rio Grande do Sul (#29840) following the Brazilian Society for Neuroscience, Committee of the School of Veterinary Surgery, University of Buenos Aires, International Brain Research Organization (IBRO) and the National Institute of Health's

Guidelines for Care and Use of Laboratory Rats (publication no 85–23, revised 1985). All efforts were made to diminish the number of animals used and their discomfort as much as possible. Furthermore, the present study is following the animal ethics checklist as outlined in Grundy, [25].

Thirty-eight females and fifteen males Wistar rats (65 days old) from a local breeding colony (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil) were used for obtaining male progeny. Animals were kept in standard living boxes (300 mm x 190 mm x 130 mm), – one animal per box, with their litter (n = 7 – 8), in a controlled temperature environment (20 ± 2ºC), dark/light cycle of 12 hours (lights on at 7:00 a.m.), with water and food *ad libitum*.

2.2 Maternal exercise protocols, experimental procedures, and experimental groups

Nulliparous Wistar rats were distributed into four groups: (1) sedentary before and during the pregnancy (SS), (2) exercised before the pregnancy (ES), (3) exercised during the pregnancy (SE), and (4) exercised before and during the pregnancy (EE). Females from the SS group were manipulated with the same frequency as the other groups and were exposed to the apparatus of exercise in horizontal disposition for 5 min/day and 3 times per week, without any stimulus to climbing or walking, for the total duration of the exercise protocols (Fig. 1).

The protocol used here was adapted from [26], according to the needs imposed by our study. The physical resistance exercise protocol consisted of climbing voluntarily a vertical ladder (1,1 x 0,8 m, 85° incline), with lead weights fixed to animal tail with adhesive tape. Before the maximum load capacity test (MLC) and beginning of exercise protocol, the rats were familiarized with the apparatus by climbing it three consecutive days. On the first day, 3 climbs without weight fixed in their bodies. On the second and third days, 3 climbs with 30g of weight fixed in their bodies.

The SE, ES and EE groups, were submitted to the MLC test before the beginning of exercise protocol, which was repeated after each 4 training days. The MLC test of the SE group was performed before the mate and confirmation of pregnancy. More details see below.

MLC test was performed individually in all exercised females, the test consisted of climbing the vertical ladder while carrying progressively heavier loads. The starting load in the MLC test corresponded to 50% of the animal's body weight on the day of the test (the rats of all groups were weighed on this day). Once the rat reached the top of the

stairs, the animal could rest for 120 seconds in a black box at the top of the stairs. After completing the climbing with the starting load, 30g of lead was added. This process was repeated until the animals were no longer able to complete the full climb (unable complete full climb happened when the animals did not reach the last rung of the ladder). The heavier load successfully carried by the animal in the complete length of the vertical ladder was considered the MLC and was used for the first four training days.

On the first four training days of ES and EE groups, the animals had to climb the ladder 4 to 9 times, carrying 50%, 75%, 90% and 100% of their MLC load. Before the next training day (training day five) another MLC test was performed to determine the load for the next four training days and so forth. A total of 18 training days were performed by ES mothers, and 26 – 27 training days by EE mothers. All groups performed the training 3 times per week, with 48 h or 72 h intervals between training days. In all periods of training, rats that declined to climb the ladder voluntarily were encouraged by gently tapping on their backs. If they persisted in refusing to climb the ladder, they were discarded from the study.

After the exercise protocol performed previously the gestational period in groups ES and EE, and before the beginning of exercise protocol in SE rats, the estrous cycle of the female rats of all groups was monitored daily, for one week and four days. In the proestrus phase, the rats were housed overnight with a sedentary male to mate. If the spermatozoa were detected in the vaginal smear in the next morning to the mate, that day was considered as day 0 of gestation (G0); after the mating period, male progenitor was discarded from the present study. The same protocol of exercise performed before the gestational period was adopted in the SE group and the EE group in the gestational period, just changed the load percentages to 35%, 45%, 55% and 75% of the MLC load, and the number of training days, 8 – 9 days of training spread within the gestational period. Dams were not submitted to exercise protocol in the lactation period. A summary of information about the training variables per group is shown in table 1.

The birthday of pups was considered the postnatal day (P)0. The four experimental groups were composed of 40 – 45 male offspring from 38 litters that were used for distinct analyses. Each litter was composed of 6 to 8 offspring, including males and females, to prevent litters of discrepant sizes. The weaning happened at P21, the female offspring and the mothers were discarded from the study. At this experimental time point, the mother's body was weighed, and their tibialis anterior and soleus muscles were dissected out and weighed. Offspring male rats of each group were designated for the Morris water maze

task (n = 15 – 11 / group), histological analyses (n = 4 / group), global hippocampal DNA methylation, global hippocampal DNA H3/H4 acetylation, and global hippocampal DNA HDAC2 activity (n = 8 – 6 / group). There was no overlapping of using animals in the experiments reported above. Therefore, the male progeny was divided into four groups, according to the period in which their mothers were exercised:

(A) Offspring from mothers sedentary during the pregestational and gestational period (SS);

(B) Offspring from mothers exercised only during the pregnancy (SE);

(C) Offspring from mothers exercised only during the pregestational period (ES);

(D) Offspring from mothers exercised during the pregestational and gestational periods (EE).

2.3 Offspring body weight

Offspring total body weight was measured using an analytical balance on postnatal days 2, 56 and 85.

2.4 BrdU administration

From P81 to P84, a set of offspring of all groups received one BrdU injection per day (5-Bromo-2′-deoxyuridine; Sigma, 100 mg/kg, intraperitoneally, dissolved in 0.1 M NH4OH, 20 mg/mL). At P85, offspring were euthanized to analyze the cellular proliferation phase of hippocampal neurogenesis [27].

2.5 Spatial and learning analyses

At P80 to P85 the Morris water maze task was performed to evaluate the spatial learning and memory of male pups (adapted from Pereira et al. [28]). A circular plastic pool, 120 cm in diameter and 40 cm deep, was filled with water (23 ± 2°C) and divided into four equal virtual quadrants. One visual cue was fixed on the walls of the experimental room in each quadrant of apparatus, and a plexiglass platform (10 cm in diameter) was 2 cm underwater in the target quadrant. Rats were subjected to four trials during five training days, with a 15 minutes intertrial interval and one training per day. In each trial, the rats were put at a starting point with their head positioned to the pool wall and the escape latency to find the platform was recorded. The animals that did not find the platform within 60 s were gently conducted to it, where stay for 30 s. In each trial, the

quadrant starting position changed. The time that the animals took to reach and move up to the platform was recorded as the escape latency. On the probe day, the sixth day of the task, the platform was removed. The rats were put on the opposite quadrant from the platform location and during 60 s swam freely in the apparatus. Latency to find the platform quadrant and time spent in target, and opposite quadrants were recorded by a video acquisition system for the measure of retention of information.

2.6 Histological procedures

At P85, a set of rats of each group were euthanized by the administration of sodium thiopental (50 mg/kg, i.p.; Cristália, Brazil) combined with lidocaine (10 mg/mL) and, posteriorly, 1000 IU heparin (Cristália, Brazil) via the left ventricle, for immunohistochemistry of (Insulin-like Growth Factor 1) IGF-1 and BrdU. Then, rats underwent transcardiac perfusion with 200 mL of saline solution, followed by 200 mL of a solution containing 4% paraformaldehyde diluted in 0.1 M phosphate buffer (PB; pH 7.4, Synth, Brazil) at room temperature, using a peristaltic pump (Milan, Brazil, 30 mL/min). The brains were dissected, collected, post-fixed in the same fixative solution at room temperature for 4 h and cryoprotected by immersion in 15% and 30% sucrose solution (Synth, Brazil) in PB at 4°C until they sank. After, brains were congealed in isopentane, cooled in liquid nitrogen and kept at -80°C until processing. Coronal sections from the dorsal hippocampus of 40 μm thickness were acquired using a cryostat (CM1850, Leica, Germany) at -20°C. The sections were successively collected on gelatin treated slides with an interval of 200 μm between them [27]. The coordinates used was around 2.30 to 4.52 mm posterior to the bregma [29].

2.7 Immunohistochemistry for IGF-1 and BrdU

Each immunohistochemical assay was executed independently. The slices were washed in phosphate buffer saline (PBS, pH 7.4) and then heated for 20 min in 0.01 M sodium citrate buffer (pH 6.0) in a thermostatic bath at 92°C. The sections were washed in PBS and the endogenous peroxidase was inactivated with 3% hydrogen peroxide (Synth, Brazil) diluted in PBS for 30 min and washed in PBS, later they were washed in PBS with 0.4% Triton X-100 (PBS-Tx) for 15 min and preincubated with 3% bovine

serum albumin (BSA; Sigma Aldrich, USA) in PBS-Tx for 30 min. After this procedure, the slices were incubated with mouse anti-IGF-1 antibody (diluted 1:100 in 3% BSA; Millipore, USA) for 48 h at 4ºC or with a monoclonal mouse anti-BrdU antibody (diluted 1:100 in nuclease, GE Healthcare, Amersham Biosciences, USA) at room temperature for 2 h and 4°C overnight. Then, the slices were washed in PBS-Tx and incubated with the secondary antibody Alexa Fluor™ 555 anti-mouse IgG (1:500, Molecular Probes™, Invitrogen™, USA) in PBS-Tx at room temperature for 2 h. Finally, the slices were washed in PBS and the slides were covered with Fluoroshield™ with DAPI in BrdU slides, and Fluoromont™ in IGF-1 slides (both aqueous mounting medium), and coverslips.

2.8 Quantification of IGF-1 and BrdU labeled cells

All immunoreactive cells in the medial end of the dorsal section of the DG were captured with a 20× and 40× objectives in an EVOS FL Auto and Olympus Fluoview FV1000 microscopes and were carefully counted with two independent observers. In BrdU immunohistochemistry, cell counts were restricted to the granular cell layer (GCL), and the subgranular zone (SGZ) of the DG. In IGF-1 immunohistochemistry, cell counts were restricted to the hilus of the DG. Both cell counts were performed using the Image Pro Plus 7.0 software.

2.9 Epigenetic assay

2.9.1 Brain tissue extraction

Another set of animals was used to epigenetic assay at P85. Male offspring of the four groups were euthanized by decapitation, the hippocampi were quickly dissected on ice, immediately snap-frozen in liquid nitrogen, and finally stored at -80°C. In the H3 and H4 levels, and HDAC2 activity assays, the samples were prepared as previously described by [14]. The samples used in the global DNA methylation assay were processed using a commercial Tissue Section DNA isolation Kit (TRIzol™ Reagent catalog # 15596026, Thermo Fisher Scientific) according to the manufacturer's instructions. Hippocampus DNA yield was quantified by absorbance using NanoDrop™ 2000 Spectrophotometer.

2.9.2 Epigenetic measurements

The global DNA methylation, global histone H3/H4 acetylation levels, and global HDAC2 activity of the hippocampus were determined using the Colorimetric Detection, catalog number P1030, MethylFlash™ USA; Colorimetric Detection, catalog number P4008; EpiQuik™ USA; Colorimetric Detection, catalog number P4009, EpiQuik™ USA; and Colorimetric Detection, catalog number P4006, EpiQuik™ USA, respectively according to the manufacturer's instructions.

In the global histone H4/H3 acetylation levels and HDAC2 protein content, the protein concentration of each sample was measured by the Coomassie Blue method using bovine serum albumin as standard, and the samples were run in unicate according to the manufacturer's protocol [30]. The samples were incubated with the capture antibody followed by incubation with the detection antibody. Thereon, were incubated with developing solution followed by the addition of the Stop Solution. H4/H3 acetylation levels and HDAC2 enzyme protein content were measured on a microplate reader (excitation = 360 nm, emission = 450 nm). The results were calculated using a standard curve and expressed as ng/mg of protein.

Global hippocampal DNA methylation was expressed as 5-mc%. The protein concentration of each sample was measured by the Coomassie Blue method using bovine serum albumin as the standard [31]. A DNA input of 100 ng per sample was used and the samples were run in duplicates according to the manufacturer's protocol. The absorbance at 450 nm was measured using an automatic microplate reader. The results were calculated using a standard curve and expressed as a percentage of 5-mC. More details about this assay could be founded in Mega et al. [32].

2.10 Statistical analysis

Initially, samples were submitted to the Shapiro-Wilk normality test to verify if the data follow a normal distribution. Latency to find the platform of Morris water maze task in training days were evaluated by General Linear Models (GLM) for repeated measures (time *vs*. group). Other differences among the groups were analyzed by two-way ANOVA – pregestational and gestational exercise were considered as independent variables. The Bonferroni's *post hoc* test was used for pairwise comparison in GLM and

two-way ANOVA statistical techniques. Data without normal distribution were evaluated by Kruskal-Wallis test followed by Dunn's *post hoc* test and Bonferroni's adjustment. Data were expressed as mean ± standard error of the mean (S.E.M.) or percentage. Statistical analysis was performed using the IBM SPSS Statistics 24 and statistical significance was established at $p < 0.05$.

3. Results

3.1 Variables of maternal resistance exercise training, maternal body weight and maternal muscle weight gain

Expectedly, due to the time of exposure to the exercise protocol, the SE group of mothers carried less weight than the ES ($p = 0.004$) and EE ($p = 0.001$) groups. Table 1 summarizes the variables of volume in experimental groups submitted to the resistance exercise protocol.

The total body weight of future mothers did not differ between the groups before the exercise protocol. However, at euthanasia day sedentary mothers had a higher body weight compared to SE ($p = 0.001$), EE ($p = 0.001$), ES ($p = 0.178$) groups of mothers (Table 2).

The relative weight of the tibialis anterior and soleus muscles, sedentary mothers presented less relative weight of Soleus muscle than experimental groups SE ($p = 0.001$), ES ($p = 0.001$) and EE ($p = 0.001$) (Table 2). Mothers exercised just during pregnancy presented a lighter relative weight of soleus muscle compared to the EE group of mothers ($p = 0.022$). No differences were observed in the relative weight of the tibialis anterior muscle (Table 2).

3.2 Maternal resistance exercise did not change the total body weight of descendants

Two-way ANOVA revealed no difference in total body weight of descendants between the groups on the evaluated days (data not presented).

3.3 Resistance exercise during pregnancy improved offspring's memory and learning

Just one statistical difference was observed among the groups comparing the latency to find the platform in the Morris water maze task on any of the 5 days of training. The offspring from the mothers exercised just during the pregnancy (SE), spent less time to find the platform on the fourth day of training ($p = 0.001$) compared to the offspring of sedentary mothers (SS) (Fig. 2A). When analyzing the learning curve from each group individually, a statistical difference between the first test day and the second day was observed in all groups SS ($p = 0.022$), SE ($p = 0.041$), ES ($p = 0.005$) and EE ($p = 0.007$) (Fig. 2A).

Although no statistical differences were observed in latency time to find the platform or time spent in the target quadrant at probe day (Fig. 2B and 2C), SE offspring spent more time in the target quadrant and less time to find the platform quadrant in probe day compare to the other groups; and the statistical difference was observed in the time spent in the opposite quadrant (lower time) in probe day ($p = 0.024$) when compared to SS offspring (Fig. 2D), indicating a possible memory enhancement caused by gestational exercise. More information about statistical differences in the WM task could be found in legends of figure 2.

3.4 Resistance exercise before the pregnancy enhances cell proliferation and IGF-1 expression in the dentate gyrus of the hippocampus

Just the offspring from mothers exercised before the pregnancy period (ES) had a significant hippocampal cell proliferation compared to the offspring from sedentary mothers (SS) ($p=0.007$) (Fig. 3A). No differences were observed in comparison between SE ($p = 0.526$) and EE ($p = 0.380$) groups with SS group.

ES offspring also had an enhanced in IGF-1 expression in the dentate gyrus of the hippocampus when compared to the SS offspring ($p=0.005$) (Fig 4A). No differences were observed in the comparison between SE ($p = 1.000$) and EE ($p = 0.321$) groups with the SS group.

3.5 Resistance exercise practiced before the pregnancy decrease global hippocampal DNA methylation

Offspring from mothers exercised before the pregnancy (ES) presented a lower global level of hippocampal DNA methylation compared to offspring whose mothers were sedentary (SS) (p=0.017) (Fig. 5A). Offspring from mothers exercised during pregnancy (SE) (p =0.869) or before and during pregnancy (EE) (p = 1.000) did not differ from the offspring of the SS group (Fig 5A).

3.6 Resistance exercise during pregnancy decreases the hippocampal HDAC2 activity without change H3/H4 acetylation status.

Offspring whose mothers were exposed to resistance exercise during the pregnancy (SE) presented a lower global concentration of HDAC2 activity in the hippocampus compared to the offspring of sedentary mothers (p=0.016) (Fig. 6C). Offspring whose mothers were exposed to resistance exercise during the pregestational period (ES) (p = 0.473) or continuously in pregestational and gestational periods (EE) (p = 0.264) did not differ to offspring of sedentary mothers (SS) (Fig. 6C).

The data about HDAC2, surprisingly, was not accompanied by an increase of H3 or H4 acetylation. Just ES offspring showed an increase of global hippocampal DNA H4 acetylation compared to SS offspring (p=0.012) (Fig. 6B). Other statistical differences were not observed when compared SS group with SE (p = 1.000) and EE groups (p = 0.195) in global hippocampal H4 acetylation (Fig. 6B).

Moreover, two-way ANOVA (pregestational x gestational exercise) did not reveal any effect or interaction between the periods of exercise. No differences were observed in global hippocampal DNA H3 acetylation between the groups (Fig 6A).

4. Discussion

The purpose of this study was to highlight the effects of maternal resistance exercise on the offspring's hippocampus. The different periods of training were chosen in order to mimic the situations that generally happen in the clinic: mothers who exercise before pregnancy but stop exercising during pregnancy, mothers who do not exercise before pregnancy but begin exercising during pregnancy, and mothers who keep exercising. The data pointed out that, in different ways, hippocampal plasticity was more influenced by maternal exercise performed in isolated periods. Specifically, offspring of

mothers exposed to resistance exercise just during pregnancy presented subtly better performance on memory and learning tasks and a decrease in hippocampal HDAC2 activity. The offspring of mothers that exercised before the gestational period presented high cell proliferation and high expression of IGF-1 in the dentate gyrus of the hippocampus and modifications in global levels of DNA methylation and H4 acetylation. The offspring of mothers that exercised continuously did not present modifications in the behavioral or plasticity parameters evaluated.

Exercised mothers presented a lighter total body weight, no difference in the relative weight of the tibialis anterior muscle, and a heavier relative weight of the soleus than sedentary mothers. These results are an indirect indication of hypertrophy caused by the exercise protocol. These data are in accordance with previous studies exploring the effects of resistance exercise on body morphology and muscular hypertrophy. Secchi et al. [33], analyzing the effects of stretching and resistive exercise on skeletal muscles in rats, reported that an 8-week exercise protocol increased the relative weight and length of the soleus when compared to the control group. Other studies using different periods of resistance exercise also observed soleus hypertrophy with muscle weight gain and histomorphology assays [33–35]. Indeed, the soleus and plantaris muscles are very well recruited by the vertical ladder climb apparatus, unlike the tibialis anterior (which showed no change here) and the extensor digitorum longus, which are not primary movers during this type of exercise [36].

Offspring of mothers exposed to a resistance exercise protocol just during pregnancy presented discreetly better performance on the Morris water maze task, reflected as a better learning curve on training days and less time spent in the opposite quadrant on the probe day. As this is a pioneering study, the only possible discussion of these results is in comparison with the results of the effect of aerobic training during pregnancy on offspring's cognitive function. Dayi et al. [18], for example, observed that adult male offspring whose mothers were exposed to a treadmill protocol during pregnancy presented a better learning curve and spent more time in the target quadrant and less time in the opposite quadrant in the Morris water maze task. Similarly, Dayi et al. [18] and Parnpiansil et al. [37] showed that offspring of mothers exercised during pregnancy took less time to find the target and made a smaller number of errors in the first 4 days of the T-maze test. In contrast to these two studies, Gomes Da Silva et al. [20] just observed subtly better performance on the Morris water maze task by offspring of

exercised mothers. The authors reported that the offspring of exercised mothers were faster learners than control animals, but in the probe test, no difference in retention of spatial memory was observed. In a related study, Segabinazi et al. [22] also showed that a protocol of aerobic exercise in different phases of the mother's life did not alter important learning and spatial memory of offspring in their adult life. Apparently, data about learning and spatial memory in the offspring of exercised mothers are not yet robust. Our results, as observed by Gomes da Silva et al. [20] and Segabinazi et al. [22], reported just a slight improvement in the memory and spatial memory of offspring of mothers exercised during pregnancy. Until now, as observed in aerobic exercise, it is not possible to state with precision that maternal resistance exercise can strongly influence the learning and memory of male offspring, at least with this test and evaluated at that age.

However, the offspring of these mothers (SE) presented a decrease in hippocampal HDAC2 activity. HDAC2 is an important enzyme linked to the learning and memory process, and its overactivity prejudices memory formation and synaptic plasticity [38]. Some studies have observed that rats submitted to different protocols of physical exercise present a decrease in the hippocampal activity of HDAC2, HDAC3, and HDAC5 [16,30,39]. Therefore, the lower hippocampal HDAC2 activity presented by the offspring of mothers exercised during pregnancy is one of the evaluated parameters that could be linked to this group's performance on the Morris water maze task. Selective knockout of HDAC2 in mice, for example, causes an acceleration of the extinction rate of conditioned fear responses and could lead to reduced dendritic spine density, synapse number, synaptic plasticity, and memory formation [9,38]. Normally, the down-regulation of HDAC2 may be related to an increase of acetylation by removing acetyl groups from histones, thereby forming a compacted chromatin configuration closed to transcription factors [38,40]. Some studies investigating the influence of exercise on hippocampal epigenetic modifications have shown that a down-regulation of HDACs is mainly linked to an increase in H3 acetylation [16,41]. Surprisingly, an expected modification in H3/H4 acetylation status in the offspring from mothers exercised just in the pregnancy period was not observed. This result may have occurred due to the reason that the down-regulation of HDAC2 does not necessarily result in a global increase in acetylation. Vecsey et al. [42], for example, reported that HDAC inhibition increases the acetylation with just a subset of genes. Besides that, HAT and DNMT'S activity – were not studied

here – can also regulate the hippocampal histone acetylation [43]. A possible imbalance between HAT and HDACs or an inhibition of DNMTs could explain the association of low HDAC2 activity with the lack of observation of histone acetylation modifications in this group.

No differences in IGF-1 and hippocampal cell proliferation of offspring of mothers exercised during pregnancy were observed when compared to control offspring, diverging from most studies investigating the effects of aerobic exercise during pregnancy on the hippocampus of descendants [18,44,45]. The lack of observation of the neuroplastic factors studied here may have been related to the short time of exposure and low intensity of exercise imposed on this group of mothers. The authors that observed enhancement of cell proliferation and IGF-1 overexpression in the hippocampus of adult rats submitted to resistance exercise used long-term (8 weeks) and moderate- to high-intensity (50% to 100% of MLC test) exercise protocols [23,24]. In contrast, the protocol of training used in the pregnancy group was short (3 weeks) and had a low to moderate intensity (35% to 75% of MLC test). It is worth noting that strenuous and longer periods of exercise could be stressful to pregnant rats and could cause negative effects on them and their offspring [46,47]. Thus, investigate how these maternal exercise variables could influence the expression of neuroplastic factors in descendants over time, it is an important question for future research.

Contrary to SE descendants, offspring of mothers exercised in the pregestational period presented an overexpression of the neuroplastic marks studied, but without significant changes in spatial memory evaluation. The discussion of these results will focus on the effects of exercise during gestation, as there are few equivalent studies during the pregestational period. Investigating a maternal voluntary exercise protocol during pregnancy and lactation, Bick-Sander et al. [45], for example, reported an increase in offspring's hippocampal cells in adult life. Similarly, Kim et al. [48] also reported an increase in the hippocampal cells in the offspring of mothers submitted to a treadmill running protocol during pregnancy. However, just one study explored the effects of aerobic maternal exercise in the pregestational period on offspring's cognitive function. Segabinazi et al. [22] reported that offspring whose mothers were submitted to a treadmill protocol just in the pregestational period presented subtly better performance on the learning curve without changes in memory assays, but associated with an overexpression in hippocampal neuroplastic marks – Reelin and BrdU.

IGF-1 is mainly produced in the liver, muscles, and brain. Due to its ability to cross the blood–brain barrier, it is found in large concentrations in the brain, directly influencing mechanisms involved in cognitive function [49–51]. Long-term and moderate- to high-intensity resistance training protocols are associated with high levels of hippocampal IGF-1, synaptophysin, and synapsin [23,24]; probably due to the fact that mothers exercised in the pregestational period had a longer period of higher-intensity exercise, their offspring showed greater expression of the studied neuroplastic factors than the offspring of mothers exercised just during pregnancy.

In addition to the increased expression of hippocampal neuroplastic factors, the group of offspring of mothers exercised in the pregestational period also showed changes in H4 acetylation levels and hippocampal DNA methylation. Studies investigating the epigenetic modifications caused by aerobic physical exercise have demonstrated that lower levels of hippocampal methylation, a decrease in DNMTs, and higher levels of histone H4 acetylation are involved in better cognitive function and other biological mechanisms linked to cognitive processes [41,52]. In one of the unique studies to explore the epigenetic modifications to the brain made by resistance exercise, De Meireles et al. [53] reported that exercise attenuated age-induced effects on hippocampal *Bdnf* promoter *H3K4me3* in aged rats. Still, pregestational anaerobic exercise seems to cause a similar effect to pregestational aerobic exercise on the hippocampal epigenetics of offspring. One of the few studies to explore the relationship between maternal exercise and epigenetic marks presented in offspring demonstrated that embryos of mothers exercised before gestation presented histone modifications and normalized methylation levels of CpG sites in imprinting control regions of the genes *Igf2*, *Igf2r*, *Meg3*, and *H19* [21]. Segabinazi et al. [22] observed that offspring of mothers submitted to aerobic exercise in the pregestational period presented lower levels of global hippocampal methylation than the offspring of sedentary mothers; in addition, the authors found no difference in hippocampal methylation between the offspring of sedentary mothers and mothers exercised just during pregnancy or mothers exercised continuously during the pregestational and gestational periods. Although the expression of specific genes and other epigenetic mechanisms such as HAT and DNMT activity were not explored here, maternal exercise was able to influence hippocampal epigenetic mechanisms related to memory and learning improvements in offspring.

Surprisingly, a possible synergism of the isolated exercise's effects was not observed in the offspring of mothers that performed the resistance training continuously (pregestational and gestational periods). That is, the results of this experimental group (EE) were equivalent to those of the unexercised group (SS). There are two main possible explanation for these data. First, mothers exercised continuously were subjected to a possible exercise dose-response and greater manipulation compared to those exercised in isolated periods. This greater manipulation and exposure to a longer and higher-load exercise protocol could have influenced the mothers' behavior toward their offspring, possibly producing moderate variations in the early life environment (including higher levels of plasmatic corticosterone), mitigating a possible positive impact on offspring's hippocampal function in this group. Some evidence exploring involuntary exercise has shown that maternal exercise practice before or during pregnancy could affect maternal corticosterone levels and influence hippocampal cell proliferation and oxidative balance [46,54,55]. A previous study already raised this hypothesis, demonstrating that aerobic exercise during the pregestational and gestational periods also did not promote changes in hippocampal neuroplastic marks or epigenetic modifications compared to isolated exercised groups [22]. Second, there could have been an imbalance in energy intake and expenditure, probably due to the high weight carried and long exposure to exercise. Indeed, imbalance of intake/expenditure before and during pregnancy could affect the metabolism and change the hippocampal function in the offspring [56,57]; however, data about feeding of mothers were not collected in this study, impeding us from being sure about this hypothesis. Finally, as it showed similar results to the control, it is worth mentioning that resistance exercise performed continually is harmless to offspring, because they presented typical neurodevelopment and no signs of stress during life, discarding a harmful effect.

There are some limitations in this study that should be explored in future research: First, the influence of maternal resistance exercise in female progeny was not investigated. Some studies have reported differences between male and female in behavioral and biological assessments. Second, we did not observe the effects of maternal resistance exercise in different periods of offspring's life. Here, we opted to study the offspring just in adult life for more precious data about spatial memory and because rats reach sexual maturity around day 80 of life. However, several studies with similar backgrounds have shown that maternal exercise's influence on cognitive function could

be transient or change during the lifespan of the offspring. Third, there are other biological mechanisms and epigenetic marks that we did not explore. Some neuroplastic marks linked to resistance exercise's influence on rats' cognitive function during adult life should be studied, such as synapsin, synaptophysin and BDNF. Fourth, there are other exercise variables to explore. How are offspring in different phases of life affected by their mothers' having undergone a high-intensity or very-low intensity exercise protocol during pregnancy? The effects observed here could change when the exercise variables such as intensity, frequency, and volume of training are modified. Likewise, more precise epigenetic marks, such as DNMTs, other HDACS, and HATs, could clarify the heritable effects of this modality of exercise, as well the expression of specific genes.

This is one of the few studies to explore the relationship between the effects of the maternal practice of exercise on offspring's cognitive function and epigenetic modifications. Yet, this report is the first to explore the influence of maternal resistance exercise on the offspring's cognitive function during adult life. Our findings indicate that gestational exercise could discretely impact the learning and memory behavior of offspring, as it is associated with lower hippocampal HDAC2 activity. Although pregestational exercise practice did not influence the evaluated behavior of offspring, an overexpression of neuroplastic factors was observed, which was associated with important hippocampal epigenetic modifications. This study highlights biological mechanisms, supporting the hypothesis that maternal resistance exercise is safe for offspring's hippocampal plasticity and could even positively impact the offspring's hippocampal function in adult life.

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Competing Interests:

None.

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5. References

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Figures

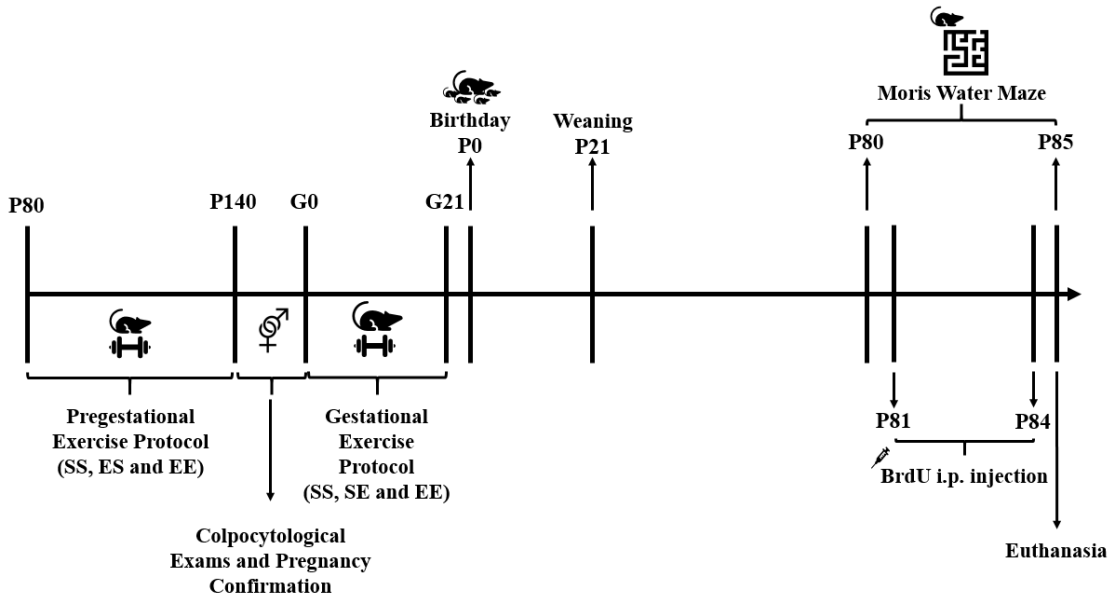

Figure 1. Timeline of key experimental procedures. P, postnatal day; G, gestational day, *SS, group of sedentary mothers in both periods; ES, group of mothers exercised before the gestational period; EE, group of mothers exercised before and during pregnancy; SE, group of mothers exercised just during pregnancy; BrdU, 5-Bromo-2′-deoxyuridine; i.p., intraperitoneal.

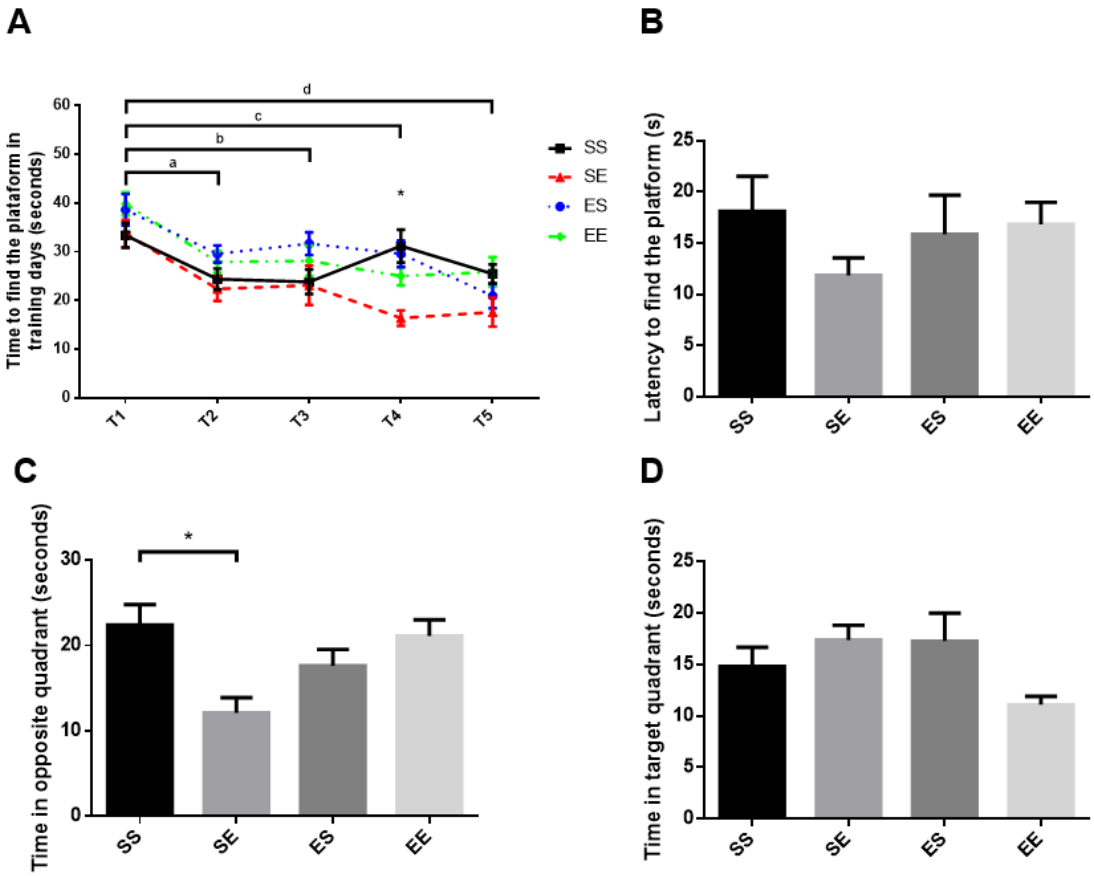

Figure 2. Spatial learning and memory evaluation in the Morris water maze task. (A) Latency to find the platform across the five training days (learning curve). General Linear Model with Repeated Measures (time x group): Time effect ($F_{(4,200)}$= 18.139, p = 0.001); interaction between time and groups ($F_{(12,200)}$= 2.980, p = 0.001). [a]p < .05, indicates decreased latency to find the platform between training day 1 vs. 2 in all four groups. [b]p < .05, indicates decreased latency to find the platform between training day 1 vs. 3 only EE group. [c]p < .001, indicates decreased latency to find platform between training day 1 vs. 4 only in the groups SE and EE. [d]p < .001, indicates decreased latency to find platform between training day 1 vs. 5 only in the groups SE, ES and EE. *p<0.01. (B) Latency to find the platform in the probe day. (C) Time spent in the target quadrant in probe day. (D) Time spent in the opposite quadrant in the probe day; Two-way ANOVA (pregestational x gestational exercise): Gestational exercise effect ($F_{(1,45)}$= 4.958; p = 0.031), indicates that the SE group spent less time in the opposite quadrant in probe day compared to SS offspring (*p = 0.001). Bonferroni *post hoc* test was used in GLM and two-way ANOVA statistical analysis. n = 11–15 per group. Data are expressed as mean ± standard error of the mean.

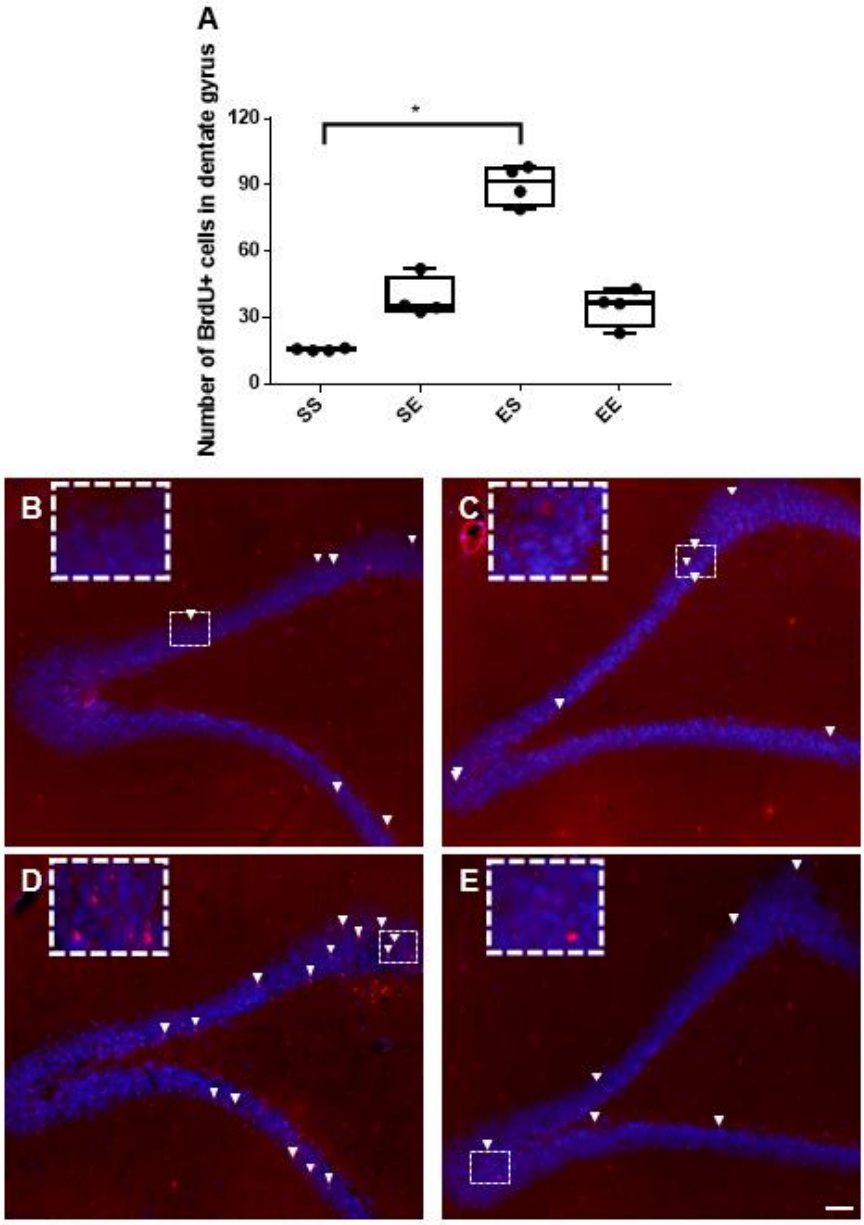

Figure 3. The proliferation of BrdU+ cells in DG of the hippocampus. (A) Quantitative analysis of the immunohistochemistry for BrdU. Kruskal-Wallis test followed by Dunn's *post hoc* test, and Bonferroni adjustment ($X^2(3) = 12.728$, p =0.005), revealed that ES offspring presented high cell proliferation in DG of the hippocampus compared to SS offspring (*p = 0.007). (B-E) SS, SE, ES, and EE, respectively digitalized images of the DG stained for $BrdU^+$ showing cell proliferation (red spots) in the SGZ and GCL (arrows; 40× magnification) and DAPI stained in other cells (blue spots). Data are expressed as mean ± standard error of the mean. n = 4 per group. Scale bar = 50 μm.

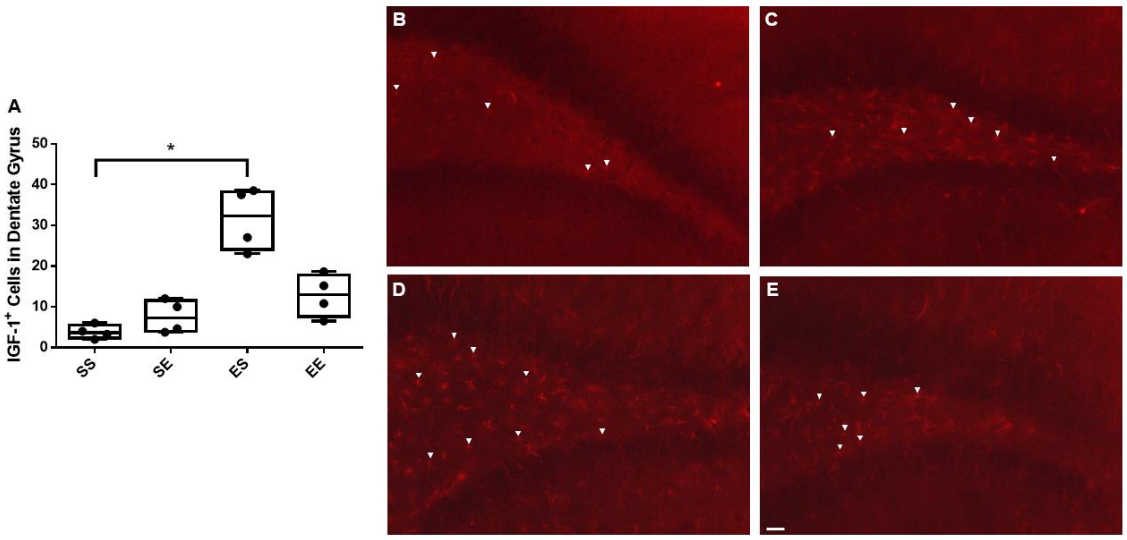

Figure 4. IGF-1 cell labeled in DG of the hippocampus. (A) Quantitative analysis of the immunohistochemistry for IGF-1. Kruskal-Wallis test followed by Dunn's *post hoc* test, and Bonferroni adjustment ($X^2(3) = 12.199$, p = 0.007), indicates that ES offspring presented a high number of IGF-1 cells labeled in the DG of the hippocampus compare do SS offspring (*p = 0.005). (B-E) SS, SE, ES and EE, respectively digitalized images of the DG stained for IGF-1 (arrows; 40× magnification). Data are expressed as mean ± standard error of the mean. n = 4 per group. Scale bar = 50 μm.

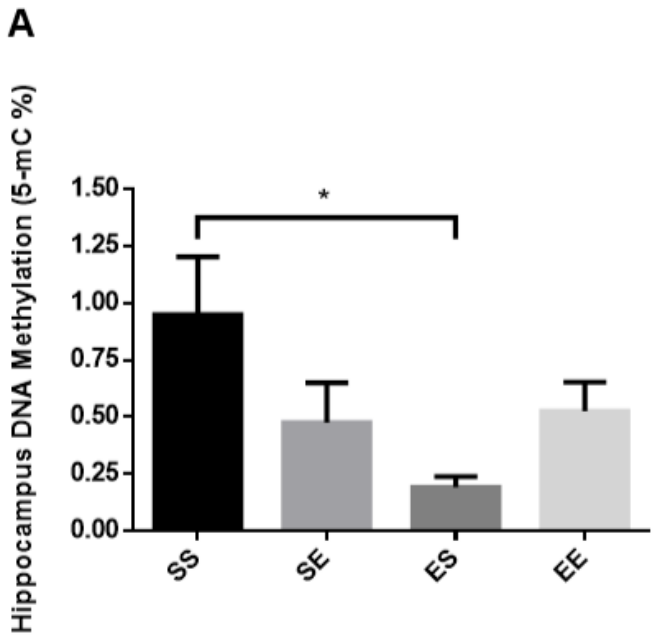

Figure 5. (A) Hippocampal DNA methylation. Kruskal-Wallis test followed by Dunn's *post hoc* test, and Bonferroni adjustment (X^2 $(_3)$ = 9.992, p = 0.019), indicates that ES offspring presented a decrease in hippocampal global DNA methylation compared to SS offspring (*p = 0.017). Data are expressed as mean ± standard error of mean and percentage. n = 6 – 8 per group.

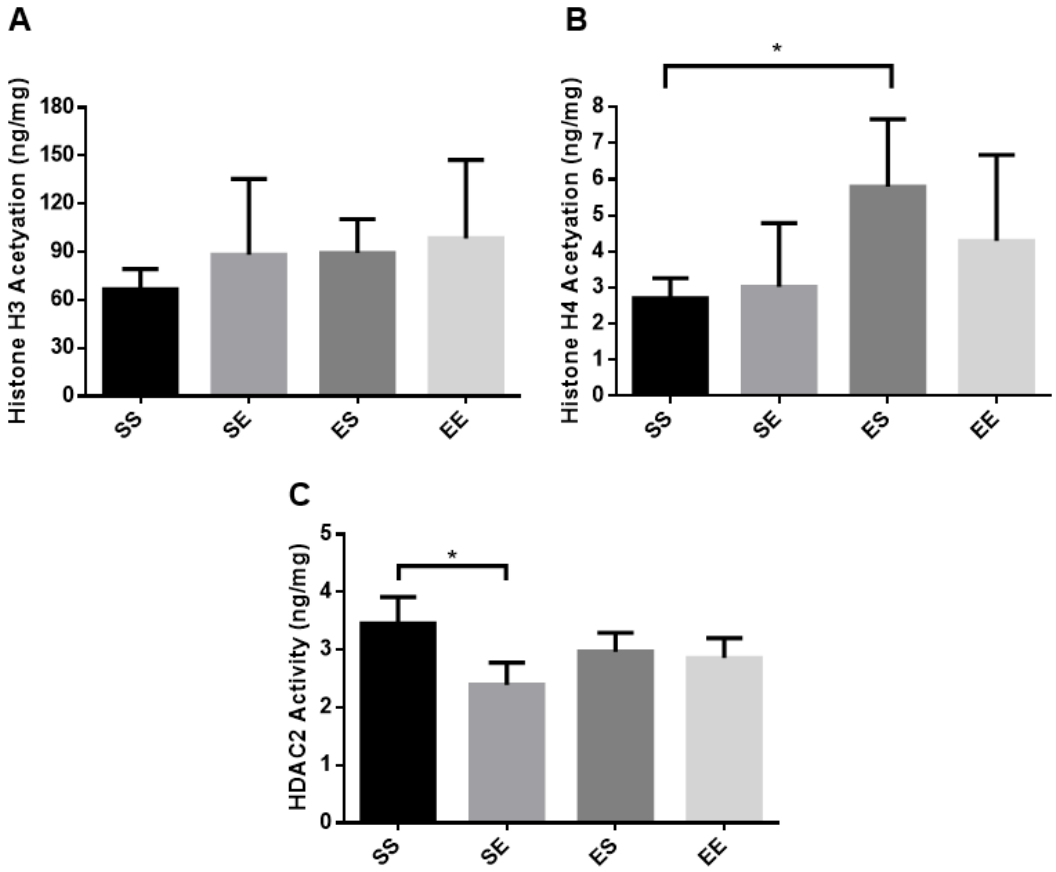

Figure 6. (A) Global hippocampal DNA histone H3 acetylation; Two-way ANOVA. (B) Global hippocampal DNA histone H4 acetylation; Kruskal-Wallis test followed by Dunn's *post hoc* test, and Bonferroni adjustment ($X^2(3) = 10.760$, p = 0.013), indicates that ES offspring presented an increase in hippocampal global DNA H4 acetylation (*p = 0.012). (C) Global hippocampal DNA HDAC2 activity; Two-way ANOVA (pregestational x gestational exercise): gestational exercise effect ($F_{(1,20)} = 13.020$, p = .002), interaction between pregestational and gestational exercise ($F_{(1,20)} = 10.166$, p = .005), followed by Bonferroni's test indicates that SE offspring presented a decrease in global hippocampal DNA HDAC2 activity (*p = 0.001). Data are expressed as mean ± standard error of the mean. n = 6 – 8 per group.

Table 1. Maternal resistance exercise variables in experimental groups.

Group	Set	Repetitions	Intensity / Maximum lead loaded	Frequency	Rest
SS	-	-	-	-	-
SE	4 – 9 climbs per day of training	8 – 12 repetitions per climb	35 – 75% MLC / 486.25 ± 20.03	3 times per week / total of 8 - 9 days of training	120 sec
ES	4 – 9 climbs per day of training	8 – 12 repetitions per climb	50 – 100 % MLC / 653.44 ± 6.60*	3 times per week / total of 18 days of training	120 sec
EE	4 – 9 climbs per day of training	8 – 12 repetitions per climb	50 -100 % and 35 – 75% 701.77 ± 25.85**	3 times per week / total of 26 – 27 days of training	120 sec

Legends: Kruskal-Wallis test followed by Dunn's post hoc test, and Bonferroni adjustment ($X^2(2) = 16.410$; $p = 0.001$), revealed that SE mothers carried less weight than the ES ($p = 0.004$)* and EE ($p = 0.001$)** groups. MLC, Maximum load capacity test. n = 9 – 10 per group. Data are expressed as mean ± standard error of the mean.

Table 2. Total body weight, soleus and tibialis anterior muscle relative weight of mothers.

Group	SS	SE	ES	EE
(A) Weight before the exercise protocol[α] (g)	223.60 ± 2.96	225.90 ± 4.48	223.44 ± 0.69	225.44 ± 2.89
(B) Weight at Euthanasia day (g)	328.30 ± 1.86	311.70 ± 4.90*	318.88 ± 3.22*	301.88 ± 1.93*
(C) Soleus relative weight (g)	0.34 ± 0.01	0.41 ± 0.01*	0.49 ± 0.005*	0.53 ± 0.01*
(D) Tibialis anterior weight (g)	2.28 ± 0.09	2.33 ± 0.13	2.29 ± 0.02	2.30 ± 0.05

Legends: (A) Two-way ANOVA did not reveal any significant effect or interaction between the factors in initial weight. (B) Two-way ANOVA (pregestational x gestational exercise): gestational exercise effect ($F_{(1,34)}$= 32.868; p = 0.001), pregestational exercise effect $F_{(1,34)}$ = 10.757; p = 0.002), followed by Bonferroni's test indicates that SS mothers had a heavier body mass than SE and EE (p = 0.001) mothers. Additionally, EE mothers presented a lighter body weight than SS (p = 0.000) and ES (p = 0.002) groups. (C) Two-way ANOVA (pregestational x gestational exercise): gestational exercise effect ($F_{(1,34)}$ = 28.568; p = 0.001), pregestational exercise effect ($F_{(1,34)}$ = 161.945; p = 0.001), followed by Bonferroni's test indicates that SS offspring presented a lighter soleus relative weight compared to SE (p = 0.001), ES (p = 0.001) and EE (p = 0.001) mothers, and SE mothers had a lighter soleus relative weight compared to ES group of mothers (p = 0.000). Additionally, EE mothers had a heavier soleus relative weight compared to ES group (p = 0.022). (D) Two-way ANOVA did not reveal any significant effect or interaction between the factors in tibialis anterior relative weight. Relative weight (body muscle/ body weight × 100). g, grams. [α]All groups were weight in the same day and hour. *Statically different. n = 9 – 10 per group. Data are expressed as mean ± standard error of the mean.

5. DISCUSSÃO GERAL

O objetivo geral desse estudo foi investigar os efeitos da prática do exercício físico resistido materno sobre a função cognitiva da prole, explorando mecanismos comportamentais, neuroplásticos e epigenéticos em diferentes idades. Para tal, dois grupos de experimentos foram realizados, um explorando os efeitos do exercício resistido gestacional sobre a prole no período neonatal e outro explorando os efeitos dessa modalidade de exercício materno praticada em diferentes janelas temporais na função cognitiva da prole durante a vida adulta.

A nossa hipótese de que o exercício materno resistido influenciava positivamente os descendentes, sem causar malefícios para saúde das mães e prole foi confirmada. O exercício materno gestacional não gerou malefícios para as mães e seus filhos no período neonatal; além de proporcionar maior proliferação celular hipocampal e diminuição da metilação global de DNA também no hipocampo dos descendentes. Também foi observado que esse impacto positivo no período neonatal nos filhos de mães exercitadas na gestação também se refletiu na vida adulta; a prole de filhos de mães exercitadas durante a gestação apresentou benefícios do aprendizado e memória, associada com diminuição da atividade da HDAC2. Apesar de não apresentar melhora no comportamento, os filhos de mães exercitadas no período pré-gestacional apresentaram maior expressão de fatores neuroplásticos hipocampais e modificações epigenéticas importantes na vida adulta. Por fim, uma segunda hipótese desse estudo, a de que a associação do exercício resistido materno nos períodos pré-gestacional e gestacional iriam impactar de forma sinérgica na função cognitiva da prole na vida adulta não foi confirmada. Entretanto, os filhos de mães expostas a esse cenário não apresentaram nenhuma mudança em nenhuma análise feita nesse grupo de experimentos, se igualando ao grupo sedentário em todas as análises.

O exercício resistido gestacional não altera os níveis de CORT materno e de seus filhos, e não modifica parâmetros físicos e corporais dos neonatos

Alguns estudos explorando a exposição materna gestacional à restrição alimentar, dietas hipocalóricas, modelos de estresse condicionado e exposição a toxinas demonstram que a prole dessas mães apresentam alterações corporais importantes como menor peso ao nascimento, menor tamanho corporal e alterações comportamentais que podem

persistir até a sua vida adulta (JAHAN-MIHAN et al., 2015; ROSENFELD, 2017; THORSELL; NÄTT, 2016). Algumas evidências demonstram que o exercício físico do tipo resistido é evitado por algumas gestantes pela crença que sua prática possa ser nociva para a sua saúde e a dos seus filhos (FIERIL et al., 2014; HAMMER; PERKINS; PARR, 2000). Aqui, observamos que tanto as mães submetidas ao protocolo de exercício resistido durante a gestação quanto seus filhos, apresentaram níveis de CORT plasmática iguais a seus controles. Além disso, os filhos de mães exercitadas durante a gestação não tiveram alterações em seus marcos do desenvolvimento motor, e as mudanças de peso e tamanho corporal foram transitórias durante o período neonatal.

Um estudo observando o efeito de diferentes modalidades e aparatos de exercício físico sobre os níveis de CORT materna relatou que dois aparatos de exercício resistido em ratos – escalada de torre e agachamento *squat* – durante o período gestacional aumentaram a concentração de CORT fecal das ratas, mas esse incremento não foi estatisticamente significativo (ROSA et al., 2011). Dois estudos que observaram os efeitos do exercício físico gestacional sobre níveis de CORT observaram resultados divergentes ao observado nessa tese. Wasinski et al., (2016), relataram que um protocolo de nado forçado durante a gestação gerou aumento da concentração de CORT plasmática nas mães, e uma diminuição da proliferação celular hipocampal nos descendentes. Também explorando os efeitos do nado forçado durante a gestação – Dia gestacional(G)5 a G15 – Jang et al., (2018) demonstraram que essa modalidade de exercício causou severa diminuição no número de implantações de embriões no útero de mães exercitadas, aumentou o nível de cortisol no líquido amniótico e diminuiu os níveis de NGF e VEGF.

Provavelmente o protocolo de baixo a moderado, adotado no grupo de experimentos em mães exercitadas no período gestacional nessa tese, não foi capaz de aumentar os níveis de CORT materna e de seus filhos de forma significativa. A provável razão de termos observado resultados semelhantes à Rosa et al., (2011), e diferente de Wasinski et al., (2016) e Jang et al., (2018) seja a escolha do aparato utilizado. Assim como nós, Rosa et al., escolheu utilizar aparatos que tem caraterísticas voluntárias em sua execução; tanto na escalada da torre quanto na subida da escada vertical os animais optam por realizar a subida, tornando esse aparato parcialmente voluntário (CASSILHAS et al., 2012). Enquanto os aparatos utilizados por Jang et al., e Wasinki et al., são considerados modelos de exercício forçado, o que pode gerar aumento do estresse (MORGAN; CORRIGAN; BAUNE, 2015). Faz-se importante destacar que mesmo exercícios

forçados quando realizados de forma crônica podem gerar normalidade nos níveis de corticosterona (DROSTE et al., 2003; GIRARD; GARLAND, 2002). Aksu et al., (2012), em um cenário parecido com o nosso, utilizou um protocolo de corrida em esteira ergométrica em ratas gestantes e observou melhora em parâmetros de neuroplasticidade em córtex cerebral e comportamento dos descendentes, associado com a ausência de modificações nos níveis de CORT materno.

Alguns estudos clínicos demonstram que o exercício gestacional é capaz de gerar uma diminuição transitória do peso corporal dos filhos. Entretanto, essa diminuição é considerada, por parte dos autores, clinicamente irrelevante (CLAPP, 1990; CLAPP et al., 2002; HOPKINS et al., 2010; HOPKINS; CUTFIELD, 2011). De forma semelhante, alguns estudos básicos também observaram flutuações em relação ao peso corporal total e de órgãos, e características morfológicas dos descendentes de mães ou pais exercitados (CARTER et al., 2013; MEGA et al., 2018; MURASHOV et al., 2016; ROSENFELD, 2017). Esses achados estão em concordância aos resultados reportados nessa tese em relação ao peso corporal e alterações morfométricas dos neonatos. Também reportamos que os filhos de mães exercitadas não apresentaram mudanças nos marcos do desenvolvimento motor durante o período neonatal. Os marcos do desenvolvimento motor são comumente utilizados para detecção de atrasos no desenvolvimento neuropsicomotor, geralmente relacionados com insultos pré, peri e/ou pós-natais (DOSMAN et al., 2012). Esse dado indica que o exercício materno resistido não interfere diretamente no desenvolvimento motor, e corrobora com outros autores que utilizaram exercício aeróbico materno e paterno investigando o mesmo desfecho (KLEIN et al., 2018; MEGA et al., 2018; SEGABINAZI et al., 2019).

Esse conjunto de resultados demonstra que o exercício resistido materno praticado no período gestacional foi seguro tanto para saúde das mães quanto para seus filhotes, gerando uma manutenção do fenótipo do desenvolvimento motor semelhante ao observado em filhos de mães sedentárias.

O exercício resistido gestacional aumenta a proliferação celular hipocampal e gera diminuição da metilação global de DNA hipocampal no período neonatal

Tanto o aumento da proliferação celular hipocampal quanto a diminuição dos níveis de metilação hipocampal são indicativos de melhora da função cognitiva dos descendentes de mães exercitadas em outras modalidades de exercício, explorando idades mais avançadas da prole (GOMES DA SILVA et al., 2016; SEGABINAZI et al., 2019). Ambos os parâmetros foram alterados em filhos de mães submetidas ao protocolo de exercício resistido gestacional no período neonatal.

Diversos estudos já demonstraram que o exercício físico é capaz de gerar modificações epigenéticas que irão modificar a expressão de genes relacionados com a plasticidade sináptica, memória e aprendizado (FERNANDES; ARIDA; GOMEZ-PINILLA, 2017b). Dentre as diversas modificações epigenéticas existentes, uma frequentemente observada é a metilação do DNA. O aumento dos níveis de metilação de DNA está associado com o silenciamento de genes pelo aumento da compactação da cromatina, e está relacionada com o aumento da expressão de genes plasticidade-promotores, como o gene *BDNF*. Além disso, a ação das DNMT1 e DNMT3A, enzimas essenciais para catalisação da metilação, estão diretamente relacionadas com o processo de neurogênese hipocampal (GOLDBERG; ALLIS; BERNSTEIN, 2007; GUO et al., 2011; OKANO et al., 1999). Um dos poucos estudos a explorar o impacto do exercício materno *per se* sobre a mudança de mecanismos epigenéticos da prole é o estudo de Xu et al., (2017); os autores relataram que o exercício físico realizado no período pré-gestacional foi capaz de melhorar o desenvolvimento embrionário in vitro, além de diminuir níveis globais da metilação do DNA em embriões de mães exercitadas, ratificando os resultados observados no período neonatal.

Embora a metilação global do DNA hipocampal não nos forneça informações precisas sobre qual ou quais genes foram metilados, esse dado demonstra que o exercício materno resistido pode ser capaz de gerar modificações na maquinaria epigenética hipocampal da prole, assim como é observado com a prática materna do exercício aeróbico, sendo esse achado inédito com esse tipo de modalidade de exercício.

Durante o período neonatal células estarão em abundância na região do hilo do hipocampo, sendo esse um importante nicho neurogênico. As células derivadas do hilo do período neonatal irão migrar para a camada granular durante o desenvolvimento

(BROWN et al., 2003). O aumento da proliferação celular hipocampal hilar nos neonatos de mães submetidas ao protocolo de exercício resistido no período gestacional demonstra que o exercício materno foi capaz de gerar uma influência positiva na neurogênese hipocampal, um dos fatores relacionados com a melhora da função cognitiva; esse dado também poderia indicar um aumento de células na vida adulta na região da camada granular. Entretanto, esse achado só foi confirmado nos filhos de mães exercitadas durante o período pré-gestacional no segundo grupo de experimentos dessa tese.

Alguns estudos demonstram que o exercício aeróbico materno, principalmente no período gestacional, é capaz de impactar positivamente a proliferação celular e expressão de neurotrofinas hipocampais. Gomes da Silva et al., (2016), por exemplo, relataram que um protocolo de corrida em esteira ergométrica em ratas grávidas foi capaz de aumentar a proliferação celular e níveis hipocampais de BDNF nos descendentes. Em outro estudo, Bick-Sander et al., (2006) reportaram que um protocolo de corrida em esteira ergométrica durante a gestação e lactação foi capaz de gerar aumento da proliferação celular hipocampal nos descendentes em P8 e P36. Ao contrário do observado nos dois estudos previamente supracitados, Wasinski et al., (2016) demonstraram que um protocolo de nado forçado materno no período gestacional gerou aumento dos níveis de CORT plasmática nas mães, causou diminuição da proliferação celular hipocampal (P8), e sobrevivência celular hipocampal (P38) em filhos de mães submetidas a esse protocolo de exercício. O achado negativo reportado por Wasinski et al., (2016), provavelmente tem relação com o tipo de exercício escolhido, o nado forçado é um exercício não voluntário, que pode gerar aumento exacerbado dos níveis de corticosterona, como já reportado em outro estudo semelhante (JANG et al., 2018).

O exercício resistido gestacional melhora a função cognitiva da prole na vida adulta, juntamente com a diminuição da atividade na HDAC2 hipocampal

Um achado interessante no segundo conjunto de resultados dessa tese, explorando os efeitos do exercício materno sobre a função cognitiva da prole na vida adulta, foi a discreta melhora na performance do aprendizado e memória dos filhos de mães exercitadas no período gestacional, associado a uma diminuição da atividade da HDAC2. Os achados relativos à memória e ao aprendizado são similares aos encontrados na literatura sobre os efeitos do exercício gestacional aeróbico sobre a prole.

Robinson e Bucci, (2014), por exemplo, demonstraram que um protocolo de corrida em roda de livre acesso praticada por ratas grávidas durante toda a gestação foi capaz de melhorar a memória e o aprendizado da prole no teste de reconhecimento de objetos, essa melhor performance foi acompanhada de um aumento da expressão de C-FOS em células do córtex perirrinal. Corroborando com Robinson e Bucci, (2004), Dayi, et al., (2012) demonstraram que filhos de mães submetidas a um protocolo de corrida em esteira ergométrica durante a gestação apresentaram uma melhor curva de aprendizagem no labirinto aquático de Morris, gastaram mais tempo no quadrante alvo e menos tempo do quadrante oposto no dia teste. Gomes da Silva et al., (2016) relataram que os filhos de mães submetidas à corrida em esteira ergométrica durante a gestação apresentaram uma discreta melhora no aprendizado – labirinto aquático de Morris – associado com maior expressão de BDNF hipocampal e maior número de células neurais no hipocampo. De forma semelhante a Gomes da Silva et al., (2016), Segabinazi et al., (2019) observaram que um protocolo de exercício aeróbico em esteira ergométrica em diferentes fases da vida da mãe (períodos pré-gestacional, gestacional e a associação dos dois períodos) não alterou de forma importante o aprendizado e a memória espacial dos descendentes. Aparentemente, os efeitos do exercício materno sobre a memória espacial dos descendentes ainda não são tão robustos. Nossos resultados, como observado pelos dois estudos citados previamente, demonstram uma discreta melhora no aprendizado e memória dos descendentes machos de mães exercitadas durante a gestação. Não é possível nesse momento atestar com precisão que o exercício resistido influencie fortemente na memória e aprendizado dos descendentes, pelo menos usando esse teste e avaliando nessa idade específica.

A melhora observada na memória e aprendizado no grupo de filhos de mães exercitadas no período gestacional não foi acompanhada de aumento estatisticamente significativo dos fatores de neuroplasticidade hipocampal estudados – BrdU+ e IGF-1 – embora ambos tenham apresentado aumento ao serem comparados com o grupo controle. Entretanto, um dos marcadores epigenéticos hipocampais investigados teve sua atividade diminuída, a HDAC2. Embora não haja artigos explorando os efeitos do exercício físico materno sobre a atividade da HDAC2 no hipocampo de seus descendentes, estudos explorando diferentes tipos de exercício – voluntário e forçado – demonstram que seus praticantes apresentam uma diminuição da atividade dessa enzima, assim como também a de outras como a HDAC3 e HDAC5 (ELSNER et al., 2011; GOMEZ-PINILLA et al.,

2011; PEIXOTO; ABEL, 2013; SLEIMAN et al., 2016; SPINDLER et al., 2014). Como comentado na introdução dessa tese, modelos *knockout* da HDAC2 apresentaram melhoras na memória e aprendizado, estando seu aumento relacionado com à diminuição da plasticidade sináptica e formação de memórias (GUAN et al., 2009). Dessa forma, podemos sugerir que a melhora do aprendizado e memória observados pelo grupo de filhos de mães exercitadas no período pré-gestacional pode estar relacionada com à diminuição dos níveis da HDAC2.

Normalmente a diminuição da atividade da HDAC2 está relacionada com o aumento da acetilação pela remoção de grupos acetil das histonas, tornando a cromatina mais compacta e fechando-a para fatores transcricionais (MORRIS et al., 2013; DELCUVE et al., 2012). Surpreendentemente, a diminuição da atividade da enzima HDAC2 observada nessa tese não foi acompanhada de modificações na acetilação das histonas H3/H4. Esse achado pode ter sido observado pela razão de que a diminuição da atividade da HDAC2 não necessariamente irá resultar em um aumento global da acetilação. Vecsey et al. (2007), por exemplo, demonstraram que a inibição da HDAC aumentou a acetilação de apenas um conjunto de genes. Fora isso, a atividade das DNMTs e HAT – não exploradas nessa tese – podem regular a acetilação de histonas (MILLER et al., 2008). Provavelmente uma alteração no balanço entre a HAT e as HDACs, ou uma inibição das DNMTs pode explicar a associação entre uma diminuição da atividade da HDAC2 e a não alteração no status das histonas H3/H4 observadas nos filhos de mães exercitadas no período pré-gestacional.

A prática de exercício resistido no período pré-gestacional aumenta a expressão de fatores neuroplásticos e modifica marcadores epigenéticos no hipocampo durante a vida adulta da prole

Já está bem estabelecido que a prática do exercício resistido é capaz de gerar aumento da expressão de fatores neuroplásticos ligados a função cognitiva como IGF-1, sinaptofisina e neurogênese hipocampal. Entretanto, ainda não há estudos investigando se os efeitos benéficos observados pela prática do exercício materno na prole, também são vistos com a prática do exercício resistido maternal.

Foi observado que os filhos de mães exercitadas no período pré-gestacional apresentaram maior proliferação celular hipocampal e maior expressão de IGF-1

hipocampal. O aumento da expressão desses marcadores já foi relatado em outros estudos explorando os efeitos do exercício físico resistido sobre o sistema nervoso central no próprio praticante (CASSILHAS et al., 2012; NOVAES GOMES et al., 2014).

Embora não seja possível realizar uma comparação direta entre outros estudos, alguns autores que exploraram os efeitos do exercício materno aeróbico sobre a função cognitiva da prole, observaram resultados que corroboram os nossos. Kim et al., (2007) e Lee et al., (2006), explorando os efeitos de um protocolo de natação e corrida em esteira ergométrica em ratas grávidas, respectivamente, reportaram que os filhos de mães exercitadas apresentaram um aumento da proliferação celular no giro denteado, CA1 e CA2 hipocampais, além de apresentarem um aumento da expressão de mRNA BDNF. Explorando os efeitos do exercício materno em um modelo de exercício voluntário – corrida em roda de livre acesso – Bick-Sander et al., (2006) demonstraram que filhos de mães exercitadas durante a gestação apresentaram maior proliferação celular hipocampal com 8 e 36 dias de vida. O único estudo, de nosso conhecimento, que reportou os efeitos do exercício materno no período pré-gestacional sobre parâmetros neuroplásticos hipocampais foi Segabinazi et al., (2019); os autores reportaram que filhos de mães exercitadas no período pré-gestacional apresentaram maior expressão de reelina no giro denteado hipocampal e aumento da proliferação celular hipocampal.

Observamos que o exercício materno pré-gestacional além de gerar maior expressão de fatores neuroplásticos hipocampais, como mencionado, também foi capaz de diminuir a metilação global de DNA hipocampal e aumentar a acetilação da histona H4 no hipocampo. O silenciamento e repressão da transcrição genica está, geralmente, associado à metilação de DNA, isso pode ser explicado pela interferência na ligação de fatores transcricionais e na montagem da maquinaria de transcrição nos locais reguladores dos genes (CURRADI et al., 2002). Por sua vez, a acetilação de histonas está associada com a ativação transcricional gerada por um relaxamento nas cargas de atração entre a cauda da histona e o DNA, aumentando, assim, o acesso a fatores transcricionais e ao RNA polimerase aos sítios de DNA (DAY; SWEATT, 2011; FERNANDES; ARIDA; GOMEZ-PINILLA, 2017b; PEIXOTO; ABEL, 2013). Estudos investigando os efeitos do exercício aeróbico na vida adulta sobre modificações epigenéticas no hipocampo de seus praticantes reportam que a prática de exercício é capaz de gerar menores níveis de metilação hipocampal, diminuição das DNMT'S, e aumento dos níveis acetilação da histona H4, que estão diretamente envolvidos com a melhora da função cognitiva

proporcionada pelo exercício (GOMEZ-PINILLA et al., 2011; LOVATEL et al., 2013). O único estudo a explorar os efeitos do exercício resistido sobre modificações epigenéticas cerebrais de seus praticantes, De Meireles et al., (2019), mostraram que ratos idosos submetidos a protocolo de exercício resistido apresentaram diminuição da H3K4me3 – marcador repressivo – no promotor de *BDNF* hipocampal.

Como já comentado, ainda não há estudos que explorem modificações epigenéticas geradas nos filhos de mães submetidas a algum protocolo de exercício resistido, limitando, assim, a profundidade e comparações da discussão desses resultados. Entretanto, há alguns estudos que investigaram tais modificações sobre o exercício aeróbico. Em um deles os autores investigaram os efeitos do exercício materno *per se* sobre mecanismos epigenéticos da prole; Xu et al., (2017) reportaram que embriões de mães exercitadas apresentaram um melhor desenvolvimento embrionário, uma menor metilação global, e metilação da H3K9m3 quando comparados ao seu controle. Em outro estudo, realizado em nosso laboratório, Segabinazi et al., (2019) usaram um desenho experimental semelhante ao nosso com a finalidade de investigar os efeitos do exercício materno aeróbico em diferentes momentos, observando aspectos cognitivos da prole na vida adulta. Os autores reportaram que assim como observado em nosso estudo, o período pré-gestacional foi a janela temporal de exercício que mais beneficiou a prole em relação a função cognitiva, tendo os descendentes maior expressão de fatores neuroplásticos hipocampais – BrdU+ e Reelina – assim como menor metilação global do DNA hipocampal.

Embora não tenhamos observado modificações epigenéticas de genes específicos relacionados com a função cognitiva, como os genes *BDNF* e *Igf-1*, as modificações epigenéticas reportadas aqui estão em alinhamento com achados prévios sobre as modificações epigenéticas geradas pelo exercício físico no hipocampo. O aumento da expressão de fatores neurotróficos e as alterações epigenéticas observadas nos filhos de mães exercitadas durante a vida adulta sugere que o exercício materno resistido é um fator influenciador positivo, sendo capaz de gerar influências intergeracionais positivas.

O exercício resistido praticado nos períodos pré-gestacional e gestacional de forma associada não gera impactos sobre a função cognitiva da prole na vida adulta.

Uma das hipóteses desse estudo desde sua fase de projeto era que a associação de exercício físico materno em diferentes períodos – pré-gestacional e gestacional – fosse potencializar um possível efeito benéfico do exercício materno resistido sobre a função cognitiva da prole. Surpreendentemente, o grupo de filhos de mães exercitadas nos dois períodos de forma conjunta apresentaram dados comportamentais, expressão de fatores neuroplásticos e modificações epigenéticas semelhantes ao grupo controle, refutando, assim, nossa hipótese inicial.

Uma possível justificativa para a não observação de um efeito sinérgico da prática de exercício materno nos dois períodos pode ter sido o excesso de manipulação desse grupo frente aos grupos de intervenções em períodos isolados. Apesar de todos os grupos, inclusive o grupo controle, terem sido expostos basicamente as mesmas condições experimentais durante toda a linha de tempo do estudo, o grupo de mães submetido aos dois períodos de exercício obviamente foi exposto mais tempo ao aparato de exercício resistido e a progressão de carga. Além de possíveis efeitos em relação aos níveis de estresse pelo aumento dos níveis de CORT, a manipulação pode influenciar o comportamento materno, que por sua vez poderá gerar variações no ambiente no período neonatal, tendo impacto na função hipocampal do rato em seu desenvolvimento e vida adulta (NEELY et al., 2018; WEAVER et al., 2004). SEGABINAZI et al., (2019) testaram essa mesma hipótese explorando os efeitos do exercício aeróbico materno em diferentes janelas de tempo sobre a função cognitiva da prole. Corroborando os nossos achados, os autores demonstraram que os filhos de mães exercitadas nos períodos pré-gestacional e gestacional de forma conjunta tiveram resultados semelhantes ao grupo controle em relação a comportamento – memória espacial – fatores neuroplásticos – BrdU+ e Reelina hipocampal – e sobre a metilação global de DNA hipocampal. Outro fator que pode ter gerado esses achados é um possível desbalanço entre o gasto energético e consumo calórico nesse grupo de mães. Isso pode ter acontecido devido ao longo período de exposição e altas cargas às quais esse grupo de animais foram expostos, afetando o metabolismo materno e influenciando da função hipocampal dos descendentes (WALKER et al.., 2008; YAFFE et al., 2009). Entretanto, dados sobre a alimentação da mãe não foram coletados nessa tese, nos impedindo de ter certeza sobre essa hipótese.

Apesar de não apresentarem melhoras específicas referentes à função cognitiva, a prole do grupo de mães exercitadas continuamente não apresentou sinais de estresse, modificação em seu desenvolvimento ou mudanças importantes em seus fenótipos. A ausência de malefícios nessas avaliações também foi reportada por Segabinazi et al., (2019).

6. CONCLUSÃO

Essa tese contém os primeiros dados sobre a função cognitiva dos descendentes de mães submetidas a um protocolo de exercício físico resistido em diferentes fases da vida. O exercício resistido materno se mostrou, além de seguro para as mães e seus filhos, capaz de influenciar de forma positiva a função cognitiva da prole, assim como já reportado com o exercício materno do tipo aeróbico.

Os resultados aqui apresentados indicam que o exercício resistido gestacional é capaz de modificar fatores de neuroplasticidade hipocampal da prole no período neonatal, sem causar modificações em níveis de corticosterona materna e dos descendentes, e sem gerar modificações importantes em seu desenvolvimento motor. Reportamos também que a influência benéfica observada no período neonatal perdura para a vida adulta, tendo os filhos de mães exercitadas durante a gestação uma discreta melhora no desempenho de teste de memória e aprendizado espacial, associado a menor atividade hipocampal da enzima HDAC2. Além disso, a prática de exercício materno no período pré-gestacional é capaz de aumentar a expressão de fatores neuroplásticos dos descendentes, juntamente com modificações epigenéticas hipocampais. Por fim, os resultados apontam não haver efeito sinérgico da prática de exercício físico materno resistido nos períodos pré-gestacional e gestacional, tendo os filhos de mães exercitadas nesse cenário resultados semelhantes ao grupo de filhos de mães sedentárias.

Essa tese investigou marcadores neurobiológicos e moleculares relativos à função cognitiva da prole de mães exercitadas, os quais suportam a hipótese de que o exercício físico materno resistido também pode gerar impacto positivo na função hipocampal durante o período neonatal e vida adulta dos descendentes, provavelmente pela prática dessa modalidade de exercício físico nos períodos gestacional e pré-gestacional.

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