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IMPACTO DO EXERCÍCIO FÍSICO GESTACIONAL SOBRE MODELOS DE OBESIDADE EM DIFERENTES FASES DO DESENVOLVIMENTO DA PROLE

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de doutora em Bioquímica.

Orientadora: Profa. Dra. Cristiane Matté

Porto Alegre, junho de 2020

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Resumo

O ganho de peso excessivo é um problema de saúde crescente na população mundial, levando ao aumento do risco de desenvolvimento de doenças crônicas, bem como os custos em saúde no tratamento das mesmas. A prática de exercício físico e a suplementação com polifenóis tem se mostrado benéficas na melhora de diversos parâmetros relacionados ao excesso de peso em todos os períodos da vida, sendo essas intervenções durante a gestação ainda pouco estudadas. Alterações positivas no ambiente intrauterino buscam aproveitar a janela de oportunidade sobre a modulação do metabolismo fetal, afetando o desenvolvimento do feto no sentido de prevenir doenças futuras. Na presente tese foi avaliado o efeito o exercício materno sobre parâmetros bioquímicos e comportamentais avaliados em dois modelos de obesidade na prole. Inicialmente, avaliamos o efeito da natação materna, aliada ou não à suplementação com naringenina, sobre o modelo de superalimentação durante a lactação na prole. Analisamos parâmetros sorológicos, de homeostase redox encefálica e também o comportamento materno em resposta ao modelo de redução de ninhada. Posteriormente, avaliamos o efeito de dois tipos de natação materna, livre e com sobrecarga, sobre a prole exposta à dieta obesogênica por trinta dias na vida adulta. Avaliamos o consumo e a eficiência calórica dos animais, parâmetros sorológicos e também a homeostase redox encefálica. O exercício de natação foi mantido, em ambos os modelos, durante uma semana antes do acasalamento e durante toda a gestação, 5 dias na semana, 30 min ao dia. A suplementação com naringenina foi administrada por via oral, na dose de 50 mg/kg/dia, durante cinco dias na semana a partir do acasalamento até o final da gestação. Foi demonstrado que as intervenções gestacionais não causaram malefícios ao ganho de peso na gestação, peso ao nascer ou tamanho de ninhada. Tanto a suplementação com naringenina quanto o exercício materno trouxeram melhora nos níveis de glicose no soro da prole ao desmame. As intervenções gestacionais e a superalimentação durante a lactação trouxeram alterações na homeostase redox da prole aos 21 dias de vida, ocorrendo um aumento na capacidade antioxidante do hipocampo em resposta à redução de ninhada. O comportamento materno de lactação arqueada das ratas foi aumentado em resposta a esse modelo, e o exercício materno preveniu algumas das alterações. Quando a prole foi exposta à dieta rica em gordura na vida adulta, os filhotes expostos à natação materna com sobrecarga apresentaram maior ganho de peso, sem alteração nos demais grupos. Não houve prevenção mediada pelo exercício materno sobre o aumento de percentual de gordura induzido pela dieta. A natação materna sem sobrecarga preveniu o aumento da glicemia e de superóxido no hipocampo da prole, enquanto a natação com sobrecarga não demonstrou o mesmo efeito. Os dois tipos de exercício materno causaram aumento na capacidade antioxidante no hipocampo da prole aos 90 dias de vida. Os resultados apresentados ressaltam o potencial efeito benéfico da suplementação com naringenina e do exercício materno em modelo animal em parâmetros sorológicos e de homeostase redox encefálica. Maiores estudos são necessários para avaliar os mecanismos exatos dos efeitos de intervenções pré- e pós-natais, a fim de obter um impacto positivo na saúde da próxima geração sem trazer efeitos colaterais.

Abstract

Excessive weight gain is a growing health problem in the world population, leading to an increased risk of developing chronic diseases, as well as health costs in treatment. The practice of physical exercise and supplementation with polyphenols has been shown to be beneficial in the improvement of several parameters related to excess weight in all periods of life, and these interventions during pregnancy are still poorly studied. Positive changes in the intrauterine environment seek to take advantage of the window of opportunity on the modulation of fetal metabolism, affecting the development of the fetus in order to prevent future diseases. In the present thesis, the effect of maternal exercise on biochemical and behavioral parameters evaluated in two models of obesity in offspring was evaluated. Initially, we evaluated the effect of maternal swimming, combined or not with naringenina supplementation, on the overfeeding model during lactation in the offspring. We analyzed serological parameters, brain redox homeostasis and also maternal behavior in response to the litter reduction model. Subsequently, we evaluated the effect of two types of maternal swimming, free and overloaded, on the offspring exposed to the obesogenic diet for 30 days in adulthood. We evaluated the consumption and caloric efficiency of animals, serological parameters and also brain redox homeostasis. The swimming exercise was maintained, in both models, for one week before mating and during the entire pregnancy, 5 days a week, 30 min a day. Naringenin supplementation was administered orally, at a dose of 50 mg/kg/day, for five days a week from mating until the end of pregnancy. It has been shown that gestational interventions have not caused harm to pregnancy weight gain, birth weight or litter size. Both supplementation with naringenin and maternal exercise brought improvement in the glucose levels in the serum of the offspring at weaning. Gestational interventions and overfeeding during lactation brought changes in the offspring's redox homeostasis at 21 days of age, with an increase in the antioxidant capacity of the hippocampus in response to reduced litter size. The maternal behavior of arched lactation in rats was increased in response to this model, and maternal exercise prevented some of the changes. When the offspring were exposed to a high fat diet in adulthood, the exposure to maternal swimming with overload bring greater weight gain, without alteration in the other groups. There was no prevention mediated by maternal exercise on the increase in the percentage of fat induced by the diet. Maternal swimming without overload prevented an increase in blood glucose and superoxide in the offspring's hippocampus exposed to high fat diet, while swimming with overload did not show the same effect. Both types of maternal exercise caused an increase in antioxidant capacity in the offspring's hippocampus at 90 days of life. The results presented highlight the potential beneficial effect of supplementation with naringenin and maternal exercise in an animal model on serological parameters and brain redox homeostasis. Further studies are needed to assess the exact mechanisms of the effects of pre- and post-natal interventions, in order to have a positive impact on the health of the next generation without bringing side effects.

Lista de abreviaturas

Acetil-CoA - Acetil-Coenzima A

ATP - Adenosina trifosfato

BDNF - Fator neurotrófico derivado do encéfalo

CAT – Catalase

DCF - Diclorofluoresceína

DNA - Ácido desoxirribonucleico

EROs – Espécies reativas de oxigênio

ERNs – Espécies reativas de nitrogênio

$FADH_2$ - Flavina adenina dinucleotídeo reduzido

GPx - Glutationa-peroxidase

GSH - Glutationa reduzida

GTP - Guanosina trifosfato

HFD – Dieta rica em gordura

HPA - Hipotálamo-hipófise-adrenal

IL1 - Interleucina 1

IL6 – Interleucina 6

LDL - Lipoproteína de baixa densidade

MDA - Malondialdeído

mRNA - Ácido ribonucleico mensageiro

NAD^+ - Nicotinamida adenina dinucleotídeo

$NADH.H^+$ - Nicotinamida adenina dinucleotídeo reduzido

Nrf - Fator nuclear respiratório

PGC-1α - Coativador de transcrição 1α do receptor ativado por proliferação peroxissomal

SOD – Superóxido-dismutase

STEM – Sistema transportador de elétrons mitocondrial

CAC – Ciclo do ácido cítrico

TNFα - Fator de necrose tumoral α

Sumário

I. INTRODUÇÃO

1.1 Regulação metabólica cerebral

1.1.1 Metabolismo energético

Sabe-se que o cérebro requer uma alta demanda energética para a manutenção das funções, consumindo cerca de 20% da energia necessária pelo organismo apesar de representar apenas 2% do peso corporal em adultos (DIENEL, 2019; TOMASI; WANG; VOLKOW, 2013). Na infância, o consumo energético cerebral chega a 43% do total utilizado; entre os mamíferos, o desenvolvimento cerebral humano é o de maior duração, tendo o fornecimento de energia priorizado em relação ao crescimento dos demais órgãos (KUZAWA; CHUGANI; GROSSMAN; LIPOVICH *et al.*, 2014).

Desde os primeiros estudos envolvendo o metabolismo energético cerebral, sabe-se que a glicose é a maior fonte energética deste tecido (DIENEL, 2019) e sua utilização é crescente desde a vida intrauterina até cerca de 18 anos de vida, quando atinge o consumo máximo que se manterá até a vida adulta (CHUGANI, 1998; VANNUCCI; VANNUCCI, 2000). Em roedores a utilização de glicose é semelhante ao encontrado em humanos, chegando a fornecer 60% da energia cerebral nos primeiros dias de vida (VANNUCCI; YAGER; VANNUCCI, 1994). Sendo assim, para manutenção da homeostase energética cerebral a glicólise e a fosforilação oxidativa são as vias metabólicas que possibilitam a formação da adenosina trifosfato (ATP) (PELLERIN; MAGISTRETTI, 2004).

No citoplasma a molécula de glicose é oxidada a duas de piruvato por meio da glicólise aeróbica, formando também dois equivalentes reduzidos de nicotinamida

adenina dinucleotídeo ($NADH.H^+$) e dois ATPs. Na ausência de oxigênio o piruvato é reduzido a lactato na glicólise anaeróbica, pela oxidação do $NADH.H^+$ (NELSON; COX, 2014). A glicólise anaeróbica é favorecida em exercícios de longa duração ou de alta intensidade, em eritrócitos maduros devidos a ausência de mitocôndria e também em outros órgãos com baixo fornecimento de oxigênio, como a córnea (MELKONIAN; SCHURY, 2020).

Na matriz mitocondrial ocorre o ciclo do ácido cítrico (CAC), responsável pela oxidação da acetil-Coenzima A (acetil-CoA), proveniente da oxidação do piruvato, vindo da glicose, e também dos ácidos graxos e aminoácidos provenientes da dieta ou da degradação do pool celular. Neste processo de oito reações será formado CO_2, três moléculas $NADH.H^+$, uma de flavina adenina dinucleotídeo reduzido ($FADH_2$) e uma molécula de guanosina trifosfato (GTP) (NELSON; COX, 2014). Os agentes redutores podem então seguir para a o sistema de transporte de elétrons mitocondrial (STEM), que consiste em quatro complexos presentes na membrana mitocondrial interna. Lá serão reoxidados nos complexos I e II, formando uma molécula de água por meio da transferência dos elétrons para o oxigênio molecular. Cada transferência leva ao acúmulo de H^+ no espaço intermembranas, conservando energia em forma de um gradiente de prótons que será utilizada na fosforilação oxidativa para síntese de 36 moléculas de ATP pela reação da ATP sintase, sendo a forma mais eficiente para manutenção da energia celular (NELSON; COX, 2014).

A taxa de utilização da glicólise aeróbica varia dependendo da região encefálica e ocorre mesmo quando existe quantidade suficiente de oxigênio para a fosforilação oxidativa (GOYAL; HAWRYLYCZ; MILLER; SNYDER *et al.*, 2014;

VAISHNAVI; VLASSENKO; RUNDLE; SNYDER *et al.*, 2010), devido ao seu papel na regulação da morte celular, neuroproteção e fornecimento de substratos para a proliferação celular (LUNT; VANDER HEIDEN, 2011; MERGENTHALER; LINDAUER; DIENEL; MEISEL, 2013).

Sendo a organela mais importante no fornecimento de energia, a mitocôndria tem impacto importante no desenvolvimento cerebral e na homeostase celular (BENKHALIFA; FERREIRA; CHAHINE; LOUANJLI *et al.*, 2014; HARVEY; GIBSON; LONERGAN; BRENNER, 2011; SON; HAN, 2018). Alterações no tamanho e morfologia mitocondrial são essenciais em diversos processos fisiológicos, incluindo a ativação da fosforilação oxidativa neuronal (KHACHO; SLACK, 2018), e ocorrem por meio da sinalização dos reguladores como o coativador de transcrição 1α do receptor ativado por proliferação peroxissomal (PGC-1α), as mitofusinas (MFN) 1 e 2 e as proteínas dinâmicas relacionadas (DRP) (LARSSON; CLAYTON, 1995; ONYANGO; LU; RODOVA; LEZI *et al.*, 2010; TILOKANI; NAGASHIMA; PAUPE; PRUDENT, 2018). Sabe-se que em roedores, já durante a vida intrauterina, ocorre alta diferenciação e proliferação mitocondrial, enquanto após o nascimento as alterações seguem ocorrendo, mas em relação à densidade e ao volume mitocondrial (ALCOLEA; COLOM; LLADO; GIANOTTI *et al.*, 2006; HAGBERG; MALLARD; ROUSSET; THORNTON, 2014; PIKO; TAYLOR, 1987). Disfunções na regulação mitocondrial são relacionadas ao envelhecimento e ao aparecimento de doenças neurodegenerativas (KHACHO; SLACK, 2018).

1.1.2 Homeostase redox

Reações de oxidação e redução envolvem a troca de elétrons ou átomos de hidrogênio e são essenciais para o funcionamento celular de organismos vivos. Estas reações redox ocorrem por meio do recebimento de elétrons, tornando a molécula reduzida, ou pela transferência dos mesmos, tornando a molécula oxidada (BUETTNER, 1993; FORMAN; URSINI; MAIORINO, 2014; GUTOWSKI; KOWALCZYK, 2013; MCCORD, 2000; NORDBERG; ARNER, 2001).

Na manutenção do metabolismo energético as reações redox são essenciais para a captação de energia proveniente dos alimentos ingeridos para posterior formação de moléculas altamente energéticas que possam ser utilizadas no nosso metabolismo, como é o caso do ATP. Durante a transferência de elétrons na STEM é comum o vazamento de elétrons (1 a 3%), que podem causar a formação de moléculas instáveis com ao menos um elétron desemparelhado em seus orbitais externos, chamadas de espécies reativas (GUTTERIDGE; HALLIWELL, 2000). A formação das espécies reativas ocorre em todos os compartimentos celulares por diversos mecanismos, entretanto a mitocôndria atua de forma mais importante devido a ação da STEM (HALLIWELL; GUTTERIDGE, 2007; JONES, 2006; MAILLOUX, 2015).

A produção de espécies reativas é essencial em diversos processos celulares (DAN DUNN; ALVAREZ; ZHANG; SOLDATI, 2015; THANNICKAL; FANBURG, 2000), e elas são divididas entre as de oxigênio (ERO), que incluem o radical ânion superóxido ($O_2^{\bullet -}$), o peróxido de hidrogênio (H_2O_2), e o radical hidroxil ($^{\bullet}OH$); e as de nitrogênio (ERN), entre elas o óxido nítrico ($NO^{\bullet}$) e o peroxinitrito ($ONOO^-$) (DE TULLIO; ASARD, 2012; HALLIWELL, 2006b).

Considerado uma ERO primária, o ânion $O_2^{\bullet-}$ é produto principalmente da função mitocondrial e apresenta seletividade em reações com moléculas não radicais, entretanto interage mais rapidamente com outros radicais como o $NO^{\bullet}$, levando a formação de $ONOO^-$ que é um forte agente oxidante de aminoácidos (BECKMAN; KOPPENOL, 1996). O H_2O_2 é um não radical que, diferente do ânion $O_2^{\bullet-}$, pode se difundir pela membrana interna da mitocôndria. Por meio da reação com Fe^{2+} na reação de Fenton pode ocorrer a formação de $^{\bullet}OH$, que é a espécie mais danosa entre as EROs, tanto por ser a mais reativa, podendo causar dano oxidativo a quase todas as biomoléculas e também por não haver defesa antioxidante enzimática endógena capaz de causar sua eliminação (CADENAS; DAVIES, 2000; HALLIWELL, 2006b; HALLIWELL; GUTTERIDGE, 2007; MCCORD, 2000).

Devido ao dano que pode ser causado pelas espécies reativas, a sua formação é altamente regulada por meio das defesas antioxidantes, que mantém os níveis fisiológicos das mesmas utilizando um sistema enzimático e não enzimático. Se a formação das espécies reativas ocorre de forma exagerada e/ou as defesas antioxidantes estão reduzidas, ocorre um desequilíbrio na homeostase redox que é chamada de estresse oxidativo, estado onde pode ocorrer maior dano a biomoléculas e que está relacionado a diversas doenças (HALLIWELL, 2006a; MEI; THOMPSON; COHEN; TONG, 2015; NORDBERG; ARNER, 2001; THANAN; OIKAWA; HIRAKU; OHNISHI *et al.*, 2015).

Antioxidantes são as substâncias que, quando em baixas concentrações em relação ao substrato oxidável, podem atrasar ou impedir a oxidação do mesmo

(HALLIWELL, 2011). Entre os antioxidantes não enzimáticos estão as moléculas que podem doar elétrons diretamente para espécies reativas, assim reduzindo a sua possibilidade de reação com biomoléculas. Antioxidantes enzimáticos são moléculas endógenas que agem com maior eficiência, por meio da conversão de EROs em espécies menos reativas (HALLIWELL; GUTTERIDGE, 2007; TOKARZ; KAARNIRANTA; BLASIAK, 2013).

Entre os antioxidantes não enzimáticos está a glutationa reduzida (GSH), sintetizada no citoplasma e considerada o principal 'tampão redox' celular, agindo como substrato para antioxidantes enzimáticos e também diretamente na redução $^{\bullet}OH$ e $ONOO^{-}$ (HALLIWELL; GUTTERIDGE, 2007). Entre os antioxidantes provenientes da dieta estão as vitaminas C, E e do complexo B e também os carotenoides (HALLIWELL, 1999; HALLIWELL; GUTTERIDGE, 2007; MACHLIN; BENDICH, 1987; MAY, 2000).

Entre as enzimas antioxidantes podemos citar como principais a superóxido-dismutase (SOD), a catalase (CAT) e a glutationa-peroxidase (GPx), devido a sua ação nas espécies reativas primárias. A SOD atua na dismutação do $O_2^{\bullet -}$ formando H_2O_2 e O_2 e pode ser encontrada em três isoformas, que diferem na localização celular e cofator utilizado: MnSOD, encontrada em mitocôndrias e dependente de manganês; CuZnSOD, presente no citoplasma e em algumas organelas e dependente de cobre e zinco; além da EcSOD, semelhante a CuZnSOD e também dependente de cobre e zinco (HALLIWELL; GUTTERIDGE, 2007; MATTÉ, 2015; MCCORD; FRIDOVICH, 1969).

O H_2O_2 é eliminado pela ação de várias enzimas, entre elas a CAT, que utiliza o mesmo como substrato para formação de H_2O e O_2. A enzima é dependente de Fe^{2+} e é encontrada predominantemente em peroxissomos (HALLIWELL; GUTTERIDGE, 2007; MATTÉ, 2015; SIES, 2014). A redução do H_2O_2 também é catalisada pela GPx, que é dependente de selênio e utiliza duas moléculas de GSH na reação que terá como produto duas moléculas de H_2O e uma molécula de glutationa oxidada (GSSG). A GPx está presente em diversas regiões celulares em oito isoformas diferentes, também agindo na remoção de hidroperóxidos orgânicos (ROOH) e $ONOO^-$ (BRIGELIUS-FLOHE; MAIORINO, 2013; HALLIWELL; GUTTERIDGE, 2007; MATTÉ, 2015).

Entre outras defesas enzimáticas, estão as tiorredoxinas (Trx) e glutarredoxinas (Grx). O sistema Trx inclui a TrxR e as 3 isoformas de Trx, que estão presentes em diferentes locais na célula: Trx-1 no citosol, Trx-2 na mitocôndria e a Trx-3 apenas em células germinativas. A enzima age eliminando peróxidos e também regenerando outras enzimas antioxidantes, e sua regeneração é realizada pela enzima TrxR (HANSCHMANN; GODOY; BERNDT; HUDEMANN *et al.*, 2013; LU; HOLMGREN, 2014).

As Grx agem regenerando tiois oxidados em reação dependente de GSH, e estão presentes em quatro isoformas, localizadas na mitocôndria, citosol e núcleo. Fazem parte da família de Trx e também são extremamente importantes na reciclagem de enzimas ou tiois proteicos oxidados (BRIGELIUS-FLOHE; MAIORINO, 2013; HALLIWELL; GUTTERIDGE, 2007; MATTÉ, 2015).

Agindo na detoxificação do metilglioxal, produto da glicólise, ainda está o sistema glioxalase (GLO). Sua ação é importante para evitar a modificação a proteínas causada pelo metilgioxal e inclui três isoformas de GLO (1 a 3), sendo a GLO 1 com ação mais importante, está presente no citosol e tem sua reação dependente de GSH (DISTLER; PALMER, 2012; RABBANI; THORNALLEY, 2014).

Todos os tecidos estão suscetíveis ao dano oxidativo, entretanto o encéfalo é especialmente sensível ao mesmo por diversos fatores, tais como: a alta necessidade de ATP, a qual eleva a produção de espécies reativas (SOKOLOFF, 1999); a baixa capacidade para regeneração celular, levando a perdas que podem ser irreversíveis (STEWARD; SRIDHAR; MEYER, 2013; SUN, 2018); o grande conteúdo de íons de ferro, possibilitando a reação de Fenton (GERLACH; BEN-SHACHAR; RIEDERER; YOUDIM, 1994); o alto conteúdo de lipídios poli-insaturados de membrana, propensos à oxidação (AURELI; GRASSI; PRIONI; SONNINO *et al.*, 2015); a presença de neurotransmissores que podem reagir com O_2 e gerar espécies reativas (HALLIWELL, 2001), e, ainda, os baixos níveis de defesas antioxidantes em relação a outros tecidos (HALLIWELL; GUTTERIDGE, 2007; HO; MAGNENAT; BRONSON; CAO *et al.*, 1997).

O dano oxidativo ocorre em lipídios, proteínas, ácidos nucléicos e carboidratos. A peroxidação lipídica envolve a oxidação de ácidos graxos poli-insaturados, componentes importantes das membranas plasmáticas, podendo haver perda de suas funções. Sua avaliação pode ser realizada por meio dos produtos gerados na peroxidação lipídica, sendo o malondialdeido o mais estudado. A oxidação de proteínas afeta diretamente receptores, enzimas e outras proteínas

importantes no funcionamento celular, e pode ser mensurado pelo conteúdo de carbonilas, um dos produtos do ataque de espécies reativas a proteínas. A oxidação de ácidos nucléicos pode levar a quebra da dupla fita do DNA e a mutações, sendo o 8-hidroxi-2'-desoxiguanosina (8-OHdG) o marcador mais estudado de dano ao DNA. Por fim, a oxidação a carboidratos pode gerar compostos reativos, como o glioxal, metilglioxal e 3-deoxiglicosona, sendo intermediários na formação dos produtos finais de glicação avançada (AGEs), que também levam a danos em biomoléculas (HALLIWELL; GUTTERIDGE, 2007; MATTÉ, 2015).

1.1.3 Consequências do sobrepeso e obesidade

A prevalência de sobrepeso e obesidade vêm aumentado em ritmo alarmante, sendo considerados uma epidemia mundial (JAMES; RIGBY; LEACH; INTERNATIONAL OBESITY TASK, 2004; SMITH; SMITH, 2016). Globalmente, entre crianças e adolescentes a obesidade teve um aumento de quase 10 vezes nas últimas quatro décadas, saltando de menos de 1% em 1975 para 5,6% em meninas e 7,8% em meninos no ano de 2016 (COLLABORATION, 2017). Em adultos, nos últimos 30 anos a prevalência aumentou em quase 40% (NG; FLEMING; ROBINSON; THOMSON *et al.*, 2014). Seguindo a tendência mundial, dados coletados na última Vigitel de 2018, que compõe o sistema de Vigilância de Fatores de Risco para doenças crônicas não transmissíveis (DCNT) do Ministério da Saúde, indicaram que praticamente 55,7% da população com mais de 20 anos encontra-se com sobrepeso, enquanto uma em cada três crianças encontra-se acima do peso indicado para a idade (IBGE, 2010; VIGITEL, 2014; 2018).

Mudanças no estilo de vida têm levado a um ambiente obesogênico. Fatores genéticos assim como ambientais, por meio do maior consumo e menor gasto calórico devido ao aumento do comportamento sedentário e maior acesso e ingestão de alimentos hipercalóricos, principalmente vindo de gorduras (CANELLA; LEVY; MARTINS; CLARO *et al.*, 2014; HARIRI; THIBAULT, 2010; POPKIN; ADAIR; NG, 2012; THAKER, 2017), causam o desequilíbrio energético e consequentemente, alterações negativas na composição corporal que podem ocorrer antes mesmo do aumento de peso efetivamente (ANDRICH; MELBOUCI; OU; LEDUC-GAUDET *et al.*, 2018). Dietas ricas em gordura (HFD, do inglês *high fat diet*) já demonstraram serem efetivos indutores de obesidade tanto em humanos quanto em modelo animal (HARIRI; THIBAULT, 2010).

O aumento excessivo de peso causa diversas alterações metabólicas negativas, elevando os custos em saúde pública (TREMMEL; GERDTHAM; NILSSON; SAHA, 2017) e também sendo fortemente relacionado às maiores causas de morte no mundo, tais como doenças cardiovasculares, diabetes mellitus e outras doenças metabólicas crônicas (DAS, 2015; MCGAVOCK; ANDERSON; LEWANCZUK, 2006; MYLES, 2014; WELLBURN; RYAN; AZEVEDO; ELLS *et al.*, 2015; WILMOT; EDWARDSON; ACHANA; DAVIES *et al.*, 2012; YE; CHACKO; CHOU; KUGIZAKI *et al.*, 2012), de acordo com a Organização Mundial da Saúde (WHO, 2014).

Com o aumento no conteúdo de tecido adiposo ocorre a reação de inflamação sistêmica, por meio da redução na produção de interleucina (IL) 10, que tem ação anti-inflamatória, e aumento na liberação de marcadores inflamatórios, tais como IL-

1, IL-6 e fator de necrose tumoral α (TNF-α, do inglês *tumor necrosis factor α*) (LOPATEGI; LOPEZ-VICARIO; ALCARAZ-QUILES; GARCIA-ALONSO *et al.*, 2016; VAN GAAL; MERTENS; DE BLOCK, 2006). O aumento da inflamação leva a alterações importantes no metabolismo da glicose e de lipídios (FRUHBECK; CATALAN; RODRIGUEZ; RAMIREZ *et al.*, 2017; SHOELSON; LEE; GOLDFINE, 2006), levando a maiores níveis plasmáticos de triglicerídeos, colesterol total, glicose e insulina (KLOP; ELTE; CABEZAS, 2013).

O sobrepeso e consequentemente o excesso de nutrientes também causam disfunção mitocondrial (LV; BHATIA; WANG, 2017). Alterações negativas na sua funcionalidade estão associadas com as causas da obesidade e doenças associadas (WANG; YUAN; DUAN; LI *et al.*, 2018), principalmente devido ao aumento na produção de espécies reativas. Devido ao seu papel no metabolismo energético, a mitocôndria é facilmente afetada pela dieta, e sua disfunção também está relacionada à resistência à insulina e ao desenvolvimento de diabetes tipo 2 induzidos pela obesidade, sendo relacionada principalmente à redução no número de mitocôndrias e da biogênese mitocondrial (CHENG; SCHMELZ; LIU; HULVER, 2014; CRUNKHORN; DEARIE; MANTZOROS; GAMI *et al.*, 2007; JHENG; HUANG; KUO; HUGHES *et al.*, 2015; ZAMORA; VILLENA, 2014). Além do aumento da produção das espécies reativas, também é demonstrada uma redução na atividade de enzimas antioxidantes como a SOD, CAT e GPx em tecido adiposo e plasma de obesos, desregulando a homeostase redox (MARSEGLIA; MANTI; D'ANGELO; NICOTERA *et al.*, 2015; MATSUDA; SHIMOMURA, 2013).

Indivíduos com excesso de peso corporal apresentam maiores níveis de cortisol (em humanos, corticosterona em ratos) (BAUDRAND; VAIDYA, 2015; MUSSIG; REMER; MASER-GLUTH, 2010) e de pressão sanguínea, resultando em hipertensão (JONES; MILLER; WOFFORD; ANDERSON *et al.*, 1999; STEVENS; OBARZANEK; COOK; LEE *et al.*, 2001). Todas estas alterações citadas estão ligadas ao aumento do risco cardiovascular, que são diretamente relacionados ao aumento no conteúdo de tecido adiposo (GRUNDY, 2015; O'NEILL; O'DRISCOLL, 2015).

As alterações causadas pelo excesso de peso causam dano a diversos tecidos (UNGER, 2003) e também afetam negativamente o funcionamento cerebral, sendo relacionadas ao aparecimento de doenças tais como depressão, ansiedade, Alzheimer e Parkinson (TAN; NORHAIZAN, 2019). O estresse oxidativo cerebral leva a danos importantes a biomoléculas e também desregula a função mitocondrial (SALIM, 2017), efeitos fortemente relacionadas ao aparecimento e ao progresso de doenças neurológicas (SALIM, 2017; SINGH; KUKRETI; SASO; KUKRETI, 2019).

Alterações em regiões envolvidas no controle do apetite como córtex parietal, hipotálamo, hipocampo e amigdala levam a uma maior resposta a estímulos alimentares, maior recompensa e maior consumo, gerando um ciclo vicioso que resulta no aumento ou manutenção do ganho de peso (FARR; LI; MANTZOROS, 2016). O aumento no índice de massa corporal (IMC) em humanos já foi relacionado com a atrofia em diversas regiões cerebrais e aumento no risco para o declínio cognitivo, apesar da dificuldade em isolar o efeito do sobrepeso das demais

comorbidades associadas (MONDA; LA MARRA; PERRELLA; CAVIGLIA *et al.*, 2017).

Em modelo animal, dietas ricas em gordura já demonstraram causar no encéfalo o aumento de parâmetros inflamatórios e de estresse oxidativo, bem como resistência à insulina, diminuição na plasticidade sináptica, perda neuronal, alterações na morfologia, entre outros (KOTHARI; LUO; TORNABENE; O'NEILL *et al.*, 2017; LIU; FU; LAN; LI *et al.*, 2014; NAKANDAKARI; MUNOZ; KUGA; GASPAR *et al.*, 2019; WU; LIU; KALAVAGUNTA; HUANG *et al.*, 2018).

1.2 Origens desenvolvimentistas da saúde e da doença

1.2.1 Histórico da programação metabólica

Recentemente o estudo de WARD; LONG; RESCH; GILES *et al.* (2017) trouxe o dado alarmante de que 75% das crianças obesas aos 2 anos de idade seguirão obesas na idade adulta, renovando a importância de intervenções ao início da vida buscando reduzir os níveis de sobrepeso mundial e trazer melhora na saúde em geral. O estudo da relação de fatores gestacionais e genéticos na saúde inicia nos anos 1960 (NEEL, 1962) quando pela primeira vez relacionou-se o nascimento de crianças macrossômicas a mães diabéticas, gerando a teoria de que o diabetes passaria aos filhos o genótipo da maior eficiência calórica.

Alguns anos mais tarde os estudos de Ravelli, Stein e Susser (RAVELLI; STEIN; SUSSER, 1976) sobre os filhos de mulheres que estavam grávidas durante o período chamado de “Inverno da Fome” na Holanda ao final da II Guerra Mundial demonstraram que o baixo fornecimento de alimento durante o os primeiros meses de gestação causou aumento na prevalência de obesidade nos filhos aos 19 anos

de idade, enquanto a mesma exposição no final da gestação ou nos primeiros meses de vida causou o efeito contrário. Este período foi foco de diversos trabalhos que posteriormente demonstraram que a fome durante qualquer período da gestação causou nos filhos intolerância à glicose e maior risco para diabetes mellitus tipo 2, e quando ocorrida no início da gestação aumentou o risco para doença cardiovascular, depressão, esquizofrenia, obesidade, câncer de mama, entre outros (ROSEBOOM; DE ROOIJ; PAINTER, 2006; ROSEBOOM; PAINTER; VAN ABEELEN; VEENENDAAL *et al.*, 2011). Mulheres que tinham entre 2 e 6 anos durante a exposição a fome também apresentaram menopausa precocemente (ELIAS; VAN NOORD; PEETERS; TONKELAAR *et al.*, 2018).

Barker e colaboradores ao final dos anos 1980 demonstraram que o baixo peso ao nascer está relacionado à hiperglicemia em homens jovens (ROBINSON; WALTON; CLARK; BARKER *et al.*, 1992), resistência à insulina em idosos (ERIKSSON; FORSEN; TUOMILEHTO; JADDOE *et al.*, 2002), um risco 10 vezes maior de desenvolver síndrome metabólica (BARKER; HALES; FALL; OSMOND *et al.*, 1993) e também uma maior taxa de mortalidade por doenças cardíacas (BARKER; WINTER; OSMOND; MARGETTS *et al.*, 1989). Estes trabalhos levaram à Hipótese de Barker, que relacionou um ambiente intrauterino desfavorável a alterações negativas permanentes no feto, que irão predizer a forma que o mesmo reage ao ambiente pós-natal (BARKER, 1990; BARKER; WINTER; OSMOND; MARGETTS *et al.*, 1989; HALES; BARKER, 1992).

Mais recentemente o estudo da programação metabólica fetal ganhou maior atenção por meio do estudo das Origens Desenvolvimentistas da Saúde e da

Doença (DOHaD, do inglês *Developmental Origins of Health and Disease*) (HANSON, 2016), que define os primeiros 1000 dias de vida, iniciando na concepção, como essenciais na programação do metabolismo do indivíduo.

Apesar de não ser completamente esclarecido como ocorre essa programação, já se sabe que o ambiente pré-natal pode causar alterações placentárias (JANSSEN; KERTES; MCNAMARA; BRAITHWAITE *et al.*, 2016; NUNEZ ESTEVEZ; RONDON-ORTIZ; NGUYEN; KENTNER, 2020), desenvolvimento anormal de alguns tecidos do feto (FERNANDEZ-TWINN; OZANNE, 2010) e inclusive alterar o cuidado materno (JOHN, 2019). Também já foi demonstrado que ocorre a passagem de células maternas tanto através da placenta quanto posteriormente pelo leite na amamentação (ZHOU; YOSHIMURA; HUANG; SUZUKI *et al.*, 2000). Os mecanismos epigenéticos são indicados como fator fundamental para as modulações causadas pelo ambiente materno à prole, e ocorre por meio de modificações covalentes em histonas e em bases do DNA, entretanto sem modificar a sua sequência (BALE, 2015; BALE; BARAM; BROWN; GOLDSTEIN *et al.*, 2010; FRANKLIN; MANSUY, 2010).

1.2.2 Superalimentação durante a lactação

Com as mudanças no padrão de alimentação mundial os estudos em DOHaD mudam também o foco. Inicialmente a preocupação visava o baixo fornecimento de energia, entretanto atualmente a má nutrição vem justamente do excesso de alimento. Nas últimas décadas as mudanças no padrão alimentar levaram a uma redução de 35% no número de crianças com baixo peso antes dos 5 anos de idade em comparação aos anos 1990, enquanto ocorre um aumento de 54% nos índices

de obesidade neste mesmo período (BLACK; VICTORA; WALKER; BHUTTA *et al.*, 2013). A obesidade infantil é considerada um risco a saúde pública global (KARNIK; KANEKAR, 2012).

O ambiente de exposição no período pós natal também pode influenciar o metabolismo durante toda a vida (PATEL; SRINIVASAN, 2011). Durante a infância a obesidade é relacionada a maiores danos metabólicos e cardiovasculares e também a problemas gastrointestinais, esqueléticos, endócrinos, psicossociais e neurológicos (KUMAR; KELLY, 2017). Na vida adulta uma infância com excesso de peso aumenta o risco para obesidade e as possíveis comorbidades relacionadas como doenças cardiovasculares, câncer, diabetes e doenças neurológicas, além do maior risco de morte em adultos jovens (BARTON, 2012; BIRO; WIEN, 2010; WEIHRAUCH-BLUHER; SCHWARZ; KLUSMANN, 2019).

Para obtermos um melhor entendimento da obesidade infantil e dos mecanismos envolvidos, modelos animais são aliados essenciais. Em roedores a superalimentação durante a lactação pode ser induzida por método indireto, pela redução no tamanho da ninhada, geralmente entre 2 a 4 filhotes, que leva ao aumento no ganho de peso e percentual de gordura em resposta a menor competição na lactação, aumento nos lipídios do leite, maior cuidado materno e também a imaturidade no controle do apetite (ENES-MARQUES; GIUSTI-PAIVA, 2018; KENNEDY, 1957; MOZES; SEFCIKOVA; RACEK, 2014; SEFCIKOVA; RACEK, 2015).

Ao desmame os animais apresentam desregulação da homeostase redox no plasma, coração e fígado (CONCEICAO; MOURA; CARVALHO; OLIVEIRA *et al.*,

2015; HABBOUT; GUENANCIA; LORIN; RIGAL *et al.*, 2013), demonstrada pelo aumento nos níveis de espécies reativas, dano oxidativo e redução na atividade de enzimas antioxidantes. A redução de ninhada causa nos filhotes o aumento no ganho de peso e desenvolve hiperfagia durante toda a vida, aliado ao maior percentual de gordura, hiperglicemia, hiperinsulinemia e hiperlipidemia, aumentando o risco para doenças crônicas (ACHARD; SANCHEZ; TASSISTRO; VERDIER *et al.*, 2015; BOULLU-CIOCCA; DUTOUR; GUILLAUME; ACHARD *et al.*, 2005; DU; HOSODA; UMEKAWA; KINOUCHI *et al.*, 2015; HABBOUT; LI; ROCHETTE; VERGELY, 2013; PATEL; SRINIVASAN, 2011; PLAGEMANN; HARDER; RAKE; VOITS *et al.*, 1999). A superalimentação durante a lactação também causa alterações cerebrais. Aos 14 dias de vida os animais já apresentam maiores níveis de inflamação no hipotálamo (ZIKO; DE LUCA; DINAN; BARWOOD *et al.*, 2014) e aos 3 meses ocorre desregulação na sinalização neuronal da mesma estrutura, causando a desregulação no consumo (HABBOUT; LI; ROCHETTE; VERGELY, 2013; MUSRATI; KOLLAROVA; MERNIK; MIKULASOVA, 1998; PLAGEMANN; HARDER; RAKE; VOITS *et al.*, 1999; RODRIGUES; DE MOURA; PASSOS; TREVENZOLI *et al.*, 2011; YANKOVSKAYA; HORSEFIELD; TORNROTH; LUNA-CHAVEZ *et al.*, 2003). Na vida adulta apresentam alteração na sinalização da insulina cerebral e também diminuição nos receptores de dopamina (PORTELLA; SILVEIRA; LAUREANO; CARDOSO *et al.*, 2015), além de maior inflamação no hipocampo aliada a alterações comportamentais (SALARI; SAMADI; HOMBERG; KOSARI-NASAB, 2018).

1.2.3 Exercício físico materno

Um ambiente saudável durante o início da vida pode modular positivamente o metabolismo, e entre as intervenções benéficas na programação metabólica está a prática de exercício materno gestacional. Sabe-se que a prática de exercício físico em qualquer momento da vida está relacionado a uma melhora na qualidade de vida, já sendo indicado como tratamento para 26 doenças crônicas, entre elas doenças metabólicas, cardiovasculares, psiquiátricas e neurológicas (PEDERSEN; SALTIN, 2015). Desde 2002 a manutenção de exercício físico durante a gestação é indicada pelo Colégio Americano de Obstetras e Ginecologistas por ao menos 150 minutos semanais em casos de gestações sem complicações (ACOG, 2002; 2015b). A prática deve se manter em intensidade moderada, que pode ser mensurada de várias formas como por meio da frequência cardíaca máxima (FCmáx), que deve se manter entre 60 e 90%, ou ser realizado enquanto a mulher conseguir manter uma conversa normalmente, o chamado teste da fala (ACOG, 2015a).

Uma gestação ativa reduz o risco para diabetes gestacional para mulheres saudáveis e obesas, sendo efetivo na redução em 75% dos casos em gestantes com sobrepeso (GARNAES; MORKVED; SALVESEN; MOHOLDT, 2016; MAGRO-MALOSSO; SACCONE; DI MASCIO; DI TOMMASO *et al.*, 2017; WANG; WEI; ZHANG; ZHANG *et al.*, 2017), além de reduzir o percentual de gordura, aliado ou não ao menor ganho de peso gestacional (CLAPP; LITTLE, 1995; FERRARI; BAE-GARTZ; BAUER; JANOSCHEK *et al.*, 2018). Além de trazer melhora no bem-estar da gestante e maior tolerância a dor, também leva a partos mais rápidos e com menor risco de parto prematuro ou cesariana (DI MASCIO; MAGRO-MALOSSO; SACCONE; MARHEFKA *et al.*, 2016; HUANG; FAN; DING; HE *et al.*, 2019; TINLOY;

CHUANG; ZHU; PAULI *et al.*, 2014; ZAVORSKY; LONGO, 2011). Filhos de mulheres exercitadas durante a gestação apresentam menor risco para nascimento com macrossomia (CURRIE; WOOLCOTT; FELL; ARMSON *et al.*, 2014; SIEBEL; CAREY; KINGWELL, 2012; WIEBE; BOULE; CHARI; DAVENPORT, 2015), importante fator de risco associado a maior morbidade e mortalidade perinatal (MCGUIRE, 2017), também apresentando menor percentual de gordura ao nascer (DAHLY; LI; SMITH; KHASHAN *et al.*, 2018). O efeito benéfico sobre a composição corporal segue ao menos até a infância, como demonstrado com o menor peso e percentual de gordura até os cinco anos de idade (CLAPP, 1996).

Estudos clínicos mostram que o exercício materno traz um melhor neurodesenvolvimento às crianças, quando analisado por meio de testes específicos para auto orientação e regulação nos primeiros dias de vida (CLAPP; LOPEZ; HARCAR-SEVCIK, 1999), desenvolvimento neuromotor aos 12 meses (CLAPP; SIMONIAN; LOPEZ; APPLEBY-WINEBERG *et al.*, 1998), e inteligência geral e habilidades linguísticas aos 5 anos de idade (CLAPP, 1996). Duas revisões abordam os efeitos do exercício materno em humanos e em grande parte dos trabalhos são encontrados benefícios, entretanto com a dificuldade de isolar as variáveis em humanos, deve-se ter cuidado ao analisar os resultados e também recorrer aos modelos animais para melhor entendimento do fenômeno molecularmente (ALVAREZ-BUENO; CAVERO-REDONDO; SANCHEZ-LOPEZ; GARRIDO-MIGUEL *et al.*, 2018; NINO CRUZ; RAMIREZ VARELA; DA SILVA; HALLAL *et al.*, 2018).

Em roedores a prática de exercício materno demonstrou reduzir o percentual de gordura na prole adulta (SHELDON; NICOLE BLAIZE; FLETCHER; PEARSON *et*

al., 2015), além de trazer benefícios metabólicos como melhora na sensibilidade a insulina (CARTER; QI; DE CABO; PEARSON, 2013) e redução na frequência cardíaca (BAHLS; SHELDON; TAHERIPOUR; CLIFFORD *et al.*, 2014). O exercício materno também causou prevenção de danos induzidos por dieta obesogênica tanto durante a gestação (VEGA; REYES-CASTRO; BAUTISTA; LARREA *et al.*, 2015) quanto no período pós natal (SHELDON; NICOLE BLAIZE; FLETCHER; PEARSON *et al.*, 2015; WASINSKI; BACURAU; ESTRELA; KLEMPIN *et al.*, 2015). Os benefícios podem ocorrer por meio da manutenção da expressão dos receptores de transportador de glicose 4 (GLUT4) (RAIPURIA; BAHARI; MORRIS, 2015) e também por mecanismos epigenéticos, com prevenção da hipermetilação do gene responsável pela transcrição de PGC-1α (JORNAYVAZ; SHULMAN, 2010).

Já demonstramos em trabalhos anteriores que a prática de natação durante a gestação traz aumento na capacidade antioxidante cerebral da prole, aliada ao aumento na biogênese mitocondrial (MARCELINO; LONGONI; KUDO; STONE *et al.*, 2013). Quando exposto ao modelo de doença de Alzheimer na vida adulta, os filhotes apresentaram melhora contra o déficit de memória e aprendizado, aliado à prevenção dos danos da doença no metabolismo energético cerebral (KLEIN; HOPPE; SACCOMORI; DOS SANTOS *et al.*, 2019). Apesar de ainda pouco estudado, o exercício materno já demonstrou em roedores o aumento na neurogênese e ativação neuronal, diminuição do comportamento do tipo ansioso e melhora na aprendizagem e memória (AKHAVAN; EMAMI-ABARGHOIE; SAFARI; SADIGHI-MOGHADDAM *et al.*, 2008; GOMES DA SILVA; DE ALMEIDA; FERNANDES; LOPIM *et al.*, 2016; M; MILADI-GORJI; EMAMI-ABARGHOIE;

SAFARI *et al.*, 2013; RAHIMI; AKHAVAN; KAMYAB; EBRAHIMI, 2018; ROBINSON; BUCCI, 2014; YAU; LEE; FORMOLO; LEE *et al.*, 2019).

1.2.4 Consumo de polifenóis na gestação

Durante o período gestacional e pós-parto ocorre um aumento na demanda de nutrientes, e um desequilíbrio entre a demanda e o fornecimento dos mesmos pode trazer malefícios tanto para a mãe quanto ao feto. Devido a fatores econômicos ou sociais, em grande parte das gestações no mundo ocorre deficiência alimentar (BLACK; VICTORA; WALKER; BHUTTA *et al.*, 2013). Diversos estudos têm buscado entender o efeito da nutrição materna sobre a saúde da mãe e da prole por meio da análise de dietas já consolidadas quando aplicadas na vida adulta, como vegetarianas e veganas (SEBASTIANI; HERRANZ BARBERO; BORRAS-NOVELL; ALSINA CASANOVA *et al.*, 2019) e dieta mediterrânea (AMATI; HASSOUNAH; SWAKA, 2019), ou com desordens alimentares (DORSAM; PREISSL; MICALI; LORCHER *et al.*, 2019) e dietas ricas em gordura (LIMA; PEREZ; MORAIS; SANTOS *et al.*, 2018).

O consumo de polifenóis para a saúde humana tem sido amplamente estudado e já demonstrou trazer benefícios quando consumido em longo prazo por meio de sua ação anti-inflamatória e antioxidante, reduzindo riscos cardiovasculares e neurológicos e havendo relação direta com o seu consumo e a redução no risco para diabetes mellitus tipo 2 (CORY; PASSARELLI; SZETO; TAMEZ *et al.*, 2018; WILLIAMSON, 2017). Polifenóis são compostos encontrados em frutas e vegetais, conferindo cor e proteção contra raios solares e infecções nas plantas. São divididos entre duas classes, os ácidos fenólicos e flavonoides, sendo os últimos ainda

subdivididos entre: flavonas, flavonóis, catequinas ou flavanóis, flavanonas, antocianinas e isoflavonas (MUNAWAR ABBAS; FARHAN SAEED; FAQIR MUHAMMAD ANJUM; MUHAMMAD AFZAAL *et al.*, 2016).

Entre as flavanonas está a naringenina, que é encontrada principalmente em frutas cítricas, onde as flavanonas representam a maior parte dos flavonoides encontrados (PETERSON; DWYER; BEECHER; BHAGWAT *et al.*, 2006). Em trabalhos realizados em modelo animal, a suplementação com naringenina demonstra trazer benefícios à saúde, trazendo melhora na sensibilidade à insulina e tolerância à glicose em resposta a dieta hiperlipídica (MULVIHILL; ALLISTER; SUTHERLAND; TELFORD *et al.*, 2009) e demonstrando efeito semelhante ao fármaco gliclazida, já utilizado no tratamento para diabetes, no aumento dos níveis de insulina e redução dos níveis de glicose no plasma (ANNADURAI; MURALIDHARAN; JOSEPH; HSU *et al.*, 2012).

A suplementação com polifenóis durante a vida adulta tem sido questionada recentemente, devido a alguns trabalhos que demonstram efeitos negativos com o seu consumo (CORY; PASSARELLI; SZETO; TAMEZ *et al.*, 2018; WILLIAMSON, 2017). O mesmo tem acontecido durante o período gestacional (LY; YOCKELL-LELIEVRE; FERRARO; ARNASON *et al.*, 2015). Enquanto alguns trabalhos demonstram um efeito positivo do consumo materno em modelo animal, sendo a suplementação com quercetina capaz de prevenir o dano causado por estresse materno pré-natal (TOUMI; MERZOUG; BAUDIN; TAHRAOUI, 2013) e também capaz de aumentar a capacidade antioxidante em fígado e pulmão de filhotes de ratas suplementadas durante a gestação (VANHEES; VAN SCHOOTEN; VAN

WAALWIJK VAN DOORN-KHOSROVANI; VAN HELDEN *et al.*, 2013), os estudos de Zielinsky e colaboradores encontraram em humanos que o alto consumo polifenóis ao final da gestação causam a constrição do canal arterial coronário fetal (ZIELINSKY; BUSATO, 2013; ZIELINSKY; PICCOLI; MANICA; NICOLOSO *et al.*, 2010; ZIELINSKY; PICCOLI; MANICA; NICOLOSO *et al.*, 2012), demonstrando a necessidade de maiores estudos sobre o tema.

II. OBJETIVOS

2.1 Objetivo geral

Avaliar se a prática de exercício materno aliada à suplementação pré-natal com naringenina pode prevenir as alterações bioquímicas na prole submetida ao modelo de obesidade pós-natal.

2.2 Objetivos específicos

- Avaliar, na prole superalimentada durante a lactação, o efeito do exercício físico materno e da suplementação com naringenina durante a gestação sobre os seguintes parâmetros:

- peso, percentual de gordura corporal;

- Avaliar, no plasma da prole:

- níveis de glicose, triglicerídeos e colesterol total;
- atividade da alanina aminotransferase e da aspartato aminotransferase;

- Avaliar, em cerebelo, hipocampo e hipotálamo da prole:

- parâmetros de homeostase redox, por meio do conteúdo de espécies reativas, conteúdo de GSH e atividade de enzimas antioxidantes; além de marcadores de dano proteico.
- Avaliar o comportamento materno em resposta à redução de ninhada.
    - Avaliar, na prole exposta a modelo de obesidade na vida adulta, o efeito do exercício físico materno durante a gestação sobre os seguintes parâmetros:
- consumo e eficiência calórica;
- peso, percentual de gordura corporal;
    - Avaliar, no plasma da prole:
- níveis de glicose, triglicerídeos e colesterol total;
- atividade da alanina aminotransferase e da aspartato aminotransferase;
    - Avaliar, em cerebelo, hipocampo e hipotálamo da prole:
- parâmetros de homeostase redox, por meio do conteúdo de espécies reativas, conteúdo de GSH e atividade de enzimas antioxidantes; além de marcadores de dano proteico.

III. RESULTADOS

Capítulo I: Effect of maternal antioxidant supplementation and/or exercise practice during pregnancy on postnatal overnutrition induced by litter size reduction: Brain redox homeostasis at weaning

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Effect of Maternal Antioxidant Supplementation and/or Exercise Practice during Pregnancy on Postnatal Overnutrition Induced by Litter Size Reduction: Brain Redox Homeostasis at Weaning

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Abstract

Prenatal and early postnatal environments can permanently influence health throughout life. Early overnutrition increases the risk to develop chronic diseases. Conversely, the intake of flavonoids and exercise practice during pregnancy seem to promote long-term benefits to offspring. We hypothesized that beneficial interventions during pregnancy could protect against possible postnatal metabolic insults, like overnutrition induced by reduced litter size. Female Wistar rats were divided into four groups: (1) sedentary + vehicle, (2) sedentary + naringenin, (3) swimming exercise + vehicle, and (4) swimming exercise + naringenin. One day after birth, the litter was culled to 8 pups (control) or 3 pups (overfed) per dam, yielding control and overfed subgroups for each maternal group. Serum of 21-days-old pups was collected, also the cerebellum, hippocampus, and hypothalamus were dissected. Litter size reduction increased fat mass and enhanced body weight. Maternal interventions, when isolated, caused reduced glucose serum levels in offspring nurtured in control litters. In cerebellum, reducing the litter size decreased the activity of thyoredoxin reductase, which was prevented by maternal supplementation with naringenin. Hippocampus and hypothalamus have shown altered antioxidant enzymes activities in response to litter size reduction. Interestingly, when maternal exercise and naringenin supplementation were allied, the effect disappeared, suggesting a concurrent effect of the two maternal interventions. In conclusion, exercise performance or naringenin supplementation during pregnancy can be important interventions for combating the increasing rates of overweight and related disorders, especially when applied isolated.

Keywords: metabolic programming, redox status, obesity, polyphenol

1. Introduction

The prevalence of obesity in children and adolescents has increased in recent years, reaching 17% [1, 2]. Overweight in early ages is highly related to obesity at adulthood [3-6], besides increased cardiovascular disease risk increment appearing at pediatric age [7, 8]. The raised obesity worldwide has led to the worrying theory that increasing life expectancy tends to end soon [9]. In rodents, the reduction of litter size during suckling, firstly demonstrated by Kennedy [10], has been used to study postnatal excessive weight gain due to the enhanced milk intake caused by greater availability, and also increased milk fat concentration about 7 days after litter reduction [11, 12]. The overweight is maintained through life, leading to negative effects that enhance the risk for the development of chronic diseases, such as vascular damage [13] and increased blood pressure from infancy to adulthood [14, 15].

Strategies against excessive weight gain have been studied. In this context, physical exercise raised as an important alternative to increase the caloric expenditure, reduce the content of adipose tissue and improve several markers found in chronic diseases [16]. Positive interventions can also bring health benefits when applied during pregnancy. Maternal exercise promotes positive adaptations to the offspring, from birth to adulthood [17-20]. Five-years-old children born to active pregnancies present lower weight and lower body fat [19]. Other benefits are demonstrated in animal models, including improvement on brain development, cognition, and neurogenesis [21, 22]. We also demonstrated increased antioxidant

defenses and mitochondrial biogenesis in the offspring's brain of rats, when exposed to maternal practice of swimming exercise before and during [23].

Similarly, dietary polyphenols seems to improve glucose and insulin metabolism, facilitating weight loss through several pathways [24, 25]. In addition, polyphenol frequent consumption is associated with decreased risk for diabetes and cardiovascular diseases development [26-28]. In addition, polyphenol supplementation during pregnancy has been associated with reduced oxidative damage, and enhanced antioxidant activity in the offspring [29, 30]. Despite present several benefits when supplemented during pregnancy, the safety of its consumption in this period has been questioned after showing harmful effects when ingested in the third trimester in humans [31, 32].

In view of the lack of studies investigating the combination of exercise and polyphenol intake during pregnancy, and also the effect of the isolated interventions on offspring, our objective was to evaluate whether these two redox-active strategies, allied or not, might interfere in rat offspring's redox homeostasis exposed to overnutrition during suckling. Our hypothesis is based on the Developmental Origin of Health and Disease (DOHaD) concept, which correlates the metabolic effect of prenatal interventions with the adult risk of chronic non-transmissible disease development.

2. Experimental Procedures

2.1 Animals and reagents

Forty-eight adult female (90 days of age), and 24 adult male Wistar rats (60 days of age), with an average weight of 200 and 250 g respectively, were obtained

from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained in a 12/12-h light/dark cycle in an air-conditioned constant temperature (22 ± 1°C) colony room. The animals had free access to water and a 20% (w/w) protein commercial chow.

The experiments were approved by the local Ethics Commission (Comissão de Ética no Uso de Animais - Universidade Federal do Rio Grande do Sul, CEUA/UFRGS) under the number 31307, and followed national animal rights regulations (Law 11.794/2008), the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and Directive 2010/63/EU. We further attest that all efforts were made to minimize the number of animals used and their suffering.

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.2 Experimental design

2.2.1 Pregnancy

Female rats were randomly divided into four groups (n= 12 each): 1) sedentary rats receiving vehicle (1 mL/Kg p.o.); 2) sedentary rats receiving naringenin (50 mg/Kg p.o.); 3) swimming exercised rats receiving vehicle (1 mL/Kg p.o.); 4) swimming exercised rats receiving naringenin (50 mg/Kg p.o.).

The administration of naringenin and/or vehicle was started after mating, while the maternal exercise began one week previous to mating, in order to adapt the animals to the aquatic environment. During the exercise protocol four animals were kept in each cage, except for mating (one male per two female rats). Pregnancy was

diagnosed by the presence of a vaginal plug. From the 20th day after the onset of pregnancy, we isolated the pregnant dams (one per cage), and the rats were observed twice a day (at 9 a.m. and 6 p.m.), to verify the litter's birth. The day corresponding to the offspring's birth is defined as postnatal day 0 (PND0).

2.2.1.1 Naringenin supplementation

Naringenin (50 mg/Kg) was suspended in sunflower oil (1 mL/kg), which was used as vehicle. One week before mating the female rats were exposed to manipulation and insertion of the oral gavage needle to adapt to manipulation and gavage. The oral treatments, administered by gavage, were given just before the swimming exercise, according the weight of each animal (measured daily), and initiated with the onset of pregnancy in the second week of swimming protocol. The scheme of naringenin administration is due its increasing availability in plasma immediately after it is ingested [33, 34]. The dose was defined according to its neuroprotective action reported in literature [35, 36].

2.2.1.2 Swimming exercise protocol

The maternal exercise protocol was adapted from Lee, Kim [22], as described in Marcelino, Longoni [23]. The rats were divided into control and exercised groups. In the exercised group, rats were submitted to swimming in a pool filled with 32±1 ºC water on 5 days/week for 4 weeks. Each swimming session lasted for 30 minutes, and always took place between 9 and 12 a.m. Each rat was isolated for the swim, which was conducted using an apparatus designed specifically for rat swimming. Within this apparatus, each room measures 30x30x90 cm (width x length x depth), preventing the animals from touching the bottom of the tank. The animals were left

free to swim, without any extra weight, and were gently stimulated to swimming when it was necessary. This protocol has moderate intensity. Control rats were immersed in water, carefully dried, and returned to the housing boxes.

2.2.2 Overnutrition model

One day after birth (PND1), to induce early postnatal overnutrition, litter sizes were manipulated to overfed or control groups, with 3 and 8 pups, respectively. The overfed litter was maintained with only male pups, and the control litter with at least 3 male pups. This yielded eight experimental groups: sedentary mother with control litter (control group), sedentary mother with overfed litter (SO), exercised mother with control litter (EC), exercised mother with overfed litter (EO), naringenin supplemented mother with control litter (NC), naringenin supplemented with overfed litter (NO), exercised and naringenin supplemented mother with control litter (ENC), and exercised and naringenin supplemented mother with overfed litter (ENO). Body weight of pups was monitored daily, from PND1 until PND21.

The offspring was left with the mother up to PND21, when offspring was euthanized by decapitation without anesthesia. Cerebellum, hippocampus, and hypothalamus were dissected, and used freshly to flow cytometry or stored at -80 °C to the remaining biochemical assays. Blood samples were collected by decapitation. Body fat of pups (retroperitoneal and mesenteric) were dissected and weighed, and fat mass calculated as a percentage of wet tissue per whole body weight. One pup from each offspring was used for each assay, in order to eliminate the litter effect.

2.3 Biochemical assays

2.3.1 Sample preparation

For flow cytometry, 100 mg of fresh tissue were dissociated with a Pasteur pipette in phosphate buffered saline (PBS) solution pH 7.4, containing 1 mg% of collagenase IV and 0.5 mg% DNAse. Dissociated tissue was filtered and then incubated with fluorescent probes.

For biochemical analysis, each brain structure was individually homogenized in 10 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 1,000 x *g* for 10 min at 4 ºC, to discard nuclei and cell debris. The pellet was discarded and the supernatant was taken to biochemical assays.

For plasma measurements, blood was obtained on decapitation and then quickly centrifuged (1000*g*, 20 °C, 10 min) and plasma stored at −20 ºC until assayed.

2.3.2 Oxidant levels measurement

On cerebellum and hypothalamus, reactive species levels were measured by flow cytometry. The samples were incubated at 37 ºC with the fluorescent probe dichloro-dihydro-fluorescein diacetate (DCFH-DA) (H2DCF-DA; Sigma Aldrich Co., St. Louis, MO, USA). Cells were gated based on the FSC and SSC pattern of the sample cells and 20,000 events were acquired per sample in a FACScalibur flow cytometer (BD Biosciences). Data were analyzed using the software FlowJo®.

On hippocampus, reactive species levels were measured fluorimetrically, through the 2',7'-dichlorofluorescein (DCFH) oxidation method [37]. In a 96-well plate, 50 µL of diluted sample was incubated at 37 °C/ 30 min, in the dark, with the addition of 200 µL of DCFH diacetate (H_2DCF-DA). H_2DCF-DA is cleaved by cellular

esterases and form DCFH, a non-fluorescent compound, that is oxidized by reactive species present in the sample, producing a fluorescent compound, DCF. DCFH oxidation was measured fluorimetrically, using a 488 nm excitation and 525 nm emission wavelength. A standard curve of DCF (0.25-10 mM) was performed in parallel with the samples. The results were expressed as nmol/mg protein.

2.3.3 Biomolecule oxidative parameters

Protein carbonyl content, a marker of protein oxidative damage, was assayed by a method based on the reaction of protein carbonyls with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370nm [38]. Briefly, 1 mg of sample protein was treated with 20% trichloroacetic acid, and centrifuged at 4000 x g, 4ºC for 5 min. The pellet was dissolved in 0.2 M NaOH, and was added of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). This was kept in the dark during 1h, and vortexed each 15 min. Samples were added of 20% thiobarbituric acid), and centrifuged at 20.000 x g, 4ºC for 5 min. The pellet was washed three times with ethanol:ethyl acetate (1:1, v/v). The supernatant was discarded and the pellet was ressuspended in 8M urea pH 2.3. The sample was vortexed and incubated at 60ºC for 15 min. After that, it was centrifuged at 20.000 x g for 3 min and the absorbance was measured at 370 nm. Protein carbonyl content was expressed as nmol/mg protein.

The lipid peroxidation was assessed using the methodology described by Yagi [39], which measures the thiobarbituric acid reactive substances (TBARS) levels with slight adaptations. Briefly, 200 µL of 10% trichloroacetic acid and 300 µL of 0.67% thiobarbituric acid in 7.1% sodium sulfate were added to 150 µL of tissue

supernatants containing 0.3 mg of protein and incubated for 2 h in a boiling water bath. The mixture was allowed to cool on running tap water for 5 min. The resulting pink-stained complex was extracted with 400 µL of butanol. Fluorescence of the organic phase was read at 515 nm and 553 nm as excitation and emission wavelengths, respectively. Calibration curve was performed using 1,1,3,3-tetramethoxypropane and subjected to the same treatment as supernatants. TBARS levels were calculated as nmol/mg protein.

2.3.4 Antioxidant enzymes activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was evaluated by quantifying the inhibition superoxide-dependent autoxidation of epinephrine, verifying the absorbance of the samples at 480 nm [40]. SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. The data were expressed as units/mg protein.

Catalase (CAT, EC 1.11.1.6) activity was assayed according to Aebi [41] by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100 and 10 mM potassium phosphate buffer, pH 7.0. One CAT unit is defined as 1 µmol of hydrogen peroxide consumed per minute and the specific activity is reported as units/mg protein.

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was measured according to the method described by Wendel [42] using *tert*-butyl hydroperoxide as substrate. NADPH disappearance was monitored spectrophotometrically at 340 nm in a medium containing 2 mM reduced glutathione (GSH), 0.15 U/mL glutathione reductase (GR, EC 1.8.1.7), 0.4 mM azide, 0.5 mM *tert*-butyl hydroperoxide and

0.1 mM NADPH. One GPx unit is defined as 1 μmol of NADPH consumed per minute and the specific activity is represented as units/mg protein.

Glutaredoxin (Grx, EC1.20.4.1) activity was measured according to the method described by Holmgren and Aslund [43] using hydroxyethyl disulfide (HED) as substrate. NADPH disappearance was monitored spectrophotometrically at 340 nm in a medium containing 2.5 mM GSH, 454 U/mL GR (EC 1.8.1.7), 2 mM HED and 5 mM NADPH. One Grx unit is defined as 1 μmol of NADPH consumed per minute and the specific activity is represented as units/mg protein.

Thyoredoxin reductase (TrxR, EC 1.8.1.9) activity is measured by following the oxidation of NADPH along with reduction of one molecule of DTNB to 2TNB molecules at 412 nm. One unit of TrxR is defined as the amount of enzyme needed to catalyze the oxidation of 1 μmol of NADPH per minute, and the specific activity is represented as units/mg protein [44].

Glyoxalase 1 (GLO1, EC 4.4.1.5) activity was assessed by the initial velocity of formation of S-D-lactoylglutathione from methylglyoxal and GSH at 240 nm [45]. One GLO1 unit is defined as the amount of enzyme that catalyzes the formation of 1 μmol of S-D-lactoylglutathione per minute.

2.3.5 Protein concentration assay

Protein concentration was measured by the method of Lowry et al. [46], using bovine serum albumin as standard.

2.3.6 Serum glucose, triglyceride, and total cholesterol measurements

Serum glucose, triglyceride (TGL), and total cholesterol (CL) concentrations were measured using commercially available kits (*Labtest* Diagnóstica S.A, Lagoa Santa, *Brazil)*.

2.3.7 Statistical analysis

GraphPad Prism 6.0 software was used for data analysis. All the data presented are expressed as the mean±S.E.M. We used two-way ANOVA to compare only the interest groups, analyzing the effect of maternal interventions related to offspring overfeeding. Statistical significance was designated at $p<0.05$.

3. Results

3.1 Pregnancy interventions did not alter maternal and fetus outcome

Gestational outcomes of dams exposed to exercise and/or naringenin supplementation during pregnancy were found unchanged (Table 1). Dams pregnancy rate ($F(1,16)=0.2604;p=0.6168$), and weight gain were not altered ($F(1,153)=0.2070;p>0.9999$), as well as litter size ($F(1,142)=0.4039;p=0.9984$) and litter weight on PND1 ($F(1,143)=2.950;p=0.0880$).

Table 1. Effect of maternal interventions on litter's weight and size, pregnancy rate and weight gain

	Sedentary	Exercise	Naringenin	NE	p value
Pregnancy rate (%)	61.6±9.4	68.3±8.9	66.6±2.6	65±9.60	0.6168
Weight gain during pregnancy (%)	28.2±1.2	28.8±1.2	28.6±1.2	29.2±1.5	>0.999
Litter size (number of pups)	8.40±0.5	8.00±0.5	9.20±0.6	8.80±0.4	0.9984
Litter weight on PND1 (weight/pup) (g)	6.60±0.1	6.50±0.1	6.40±0.1	6.8±0.08	0.0880

Data was expressed as mean ± S.E.M (two-way ANOVA) for n=34-41.

3.2 Litter size reduction had a substantial effect on offspring weight gain and body fat

Up to PND7, litter groups have similar body weights. On PND 14, animals from SO (34.2 g vs. 26.6 g, $p<0.001$) and ENO (34.5 g vs. 26.6 g, $p<0.001$) groups were

significant heavier than control pups. At 21 days of age, SO pups were 21% heavier ($p<0.01$), EO pups were 16% heavier ($p<0.05$), NO pups were 17% heavier ($p<0.05$), and ENO pups were 21.5% heavier ($p<0.001$), when compared to control group ($F(1,72)=51.52;p<0.0001$). The weight gain during lactation is presented in Fig 1A, and the weight on PND21 in Fig 1B.

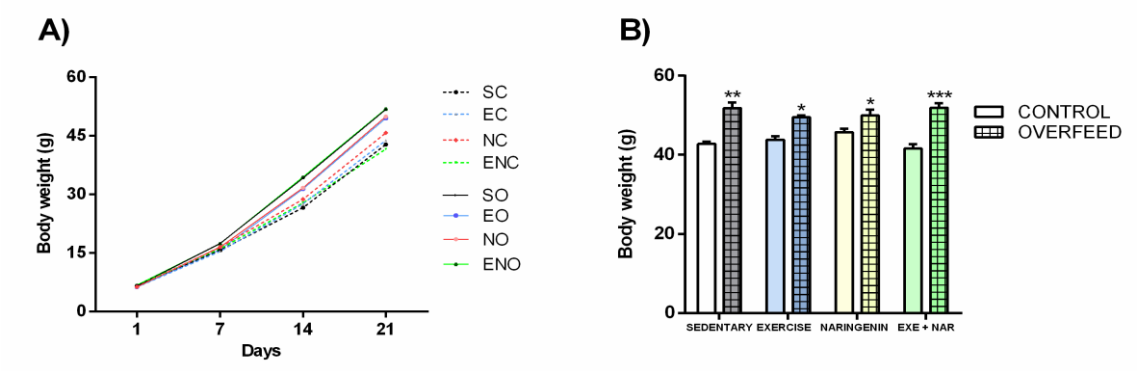

Fig. 1. Pups body weight from PND1 to PND21 (A). Pups body weight on PND21 (B). Results are expressed as mean + SEM for n=9-39. Results were analyzed by two-way ANOVA.*p<0.05; **p<0.01; ***p<0.001

In addition to the greater weight gain, reduced litter pups also presented an increment of almost 3-fold in retroperitoneal fat compared to control group (Fig. 2A) ($F(1,87)=103,0;p<0.0001$) , while mesenteric fat was not significantly different between groups (Fig. 2B) ($F(1,87)=1.021;p=0.3151$).

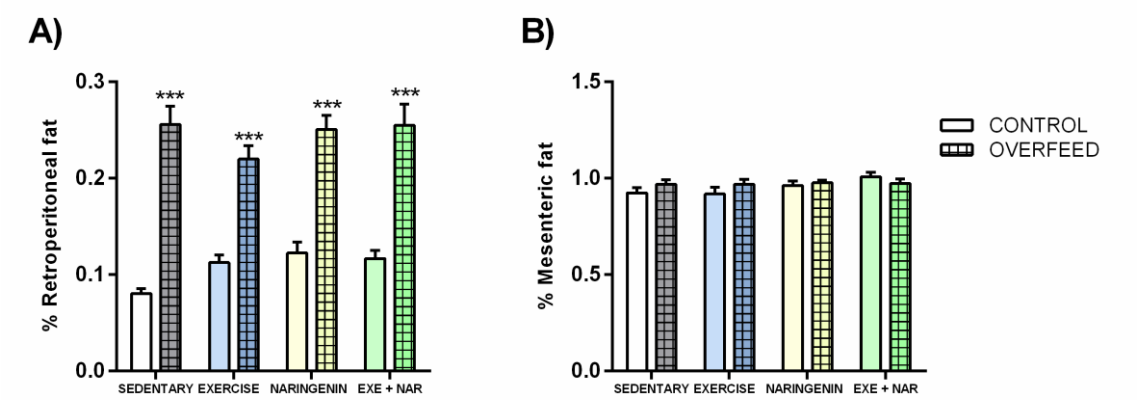

Fig. 2. Effect of postnatal overfed on retroperitoneal (2A) and mesenteric fat (2B) on PND21. Results are expressed as mean with SEM for n=10-33. Results were analyzed by two-way ANOVA. ***p<0.001

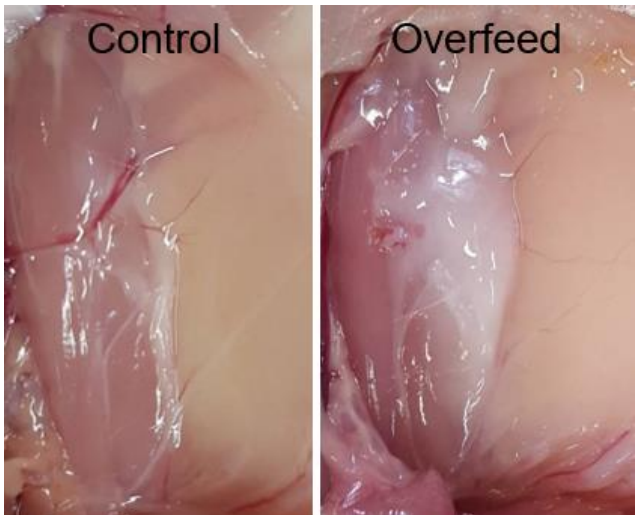

Fig. 3. Representative image of retroperitoneal fat comparing animals on PND21 maintained in a control litter size and in a reduced litter size.

3.3 Maternal exercise or naringenin supplementation improves glycaemia in offspring

Table 2 shows the effect of maternal treatments and postnatal overfed on serum markers at pups on PND21. Glucose levels were reduced by maternal exercise, and also by naringenin supplementation during pregnancy ($F(1,18)=13.52;p=0.0017$), but not when the both interventions were allied

(F(1,19)=0.3651;p=5529). Total cholesterol (F(1,20)=0.114;p=0.7383) and triglycerides levels (F(1,19)=3.569;p=0.0710) were not affected.

	CONTROL GROUP	SO	EC	EO	NC	NO	ENC	ENO	
Glucose (mg/dL)	131.2±3.4	137.3±3.4	113.8±3.3[a]	125.2±4.2	114.7±3.3[a]	126.1±4.4	118.0±5.6	129.2±3.5	0
Total cholesterol (mg/dL)	122.8±4.9	133.0±7.3	132.8±3.6	133.9±4.0	123.0±3.0	137.9±3.2	130.2±4.0	137.0±4.0	0
TGL (mg/dL)	127.7±3.7	142.2±10.8	133.8±7.3	117.9±4.73	117.4±3.6	121.1±8.9	145.8±7.9	132.1±6.4	0

Table 2. Effect of maternal treatments and postnatal overfed on serum glucose, cholesterol, and triglycerides levels measured in offspring

[a]= different of control group. Data was expressed as mean ± S.E.M (two-way ANOVA) for n=5-7.

3.4 Maternal interventions improves redox homeostasis in pups' cerebellum

In offspring's cerebellum, maternal exercise could decrease protein oxidation, demonstrated through the carbonyl levels (F(1,19)=5.128;p=0.0354); Fig. 4B), whiles was no changes on DCFH oxidation (F(1,26)=1.870;p=0.1832; Fig. 4A).

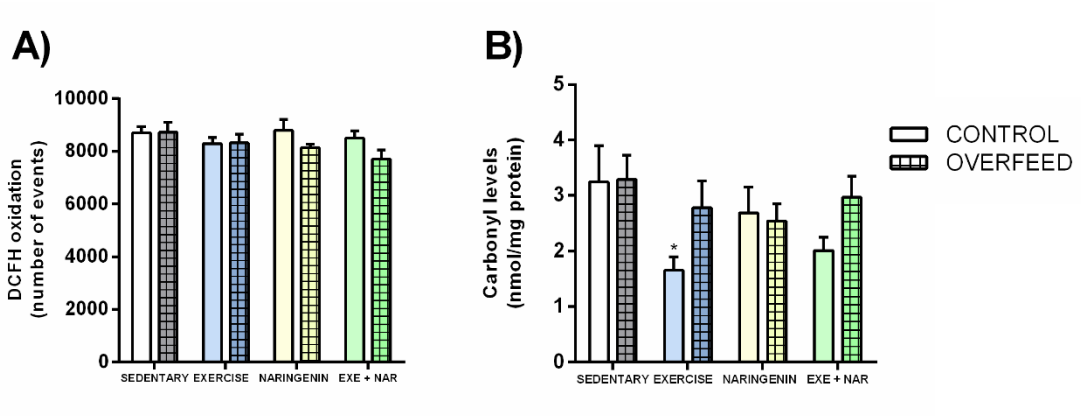

Fig. 4. Effect of maternal treatments and postnatal overfed in cerebellum of pups on PND21: (A) DCFH oxidation levels, (B) Carbonyl levels. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05

TrxR activity was impaired by litter size reduction, and maternal supplementation with naringenin was able to prevent the effect completely ($F(1,22)=7.730;p=0.0109$); Fig. 5E). The activities of SOD ($F(1,20)=1.186;p=0.2891$)), CAT ($F(1,22)=0.04581;p=0.8325$), GPx ($F(1,22)=0.1348;p=0.7170$), Grx ($F(1,23)=0.04406;p=0.8356$), and GLO 1 ($F(1,23)=0.01684;p=0.8979$) were not altered (Fig. 5A-F).

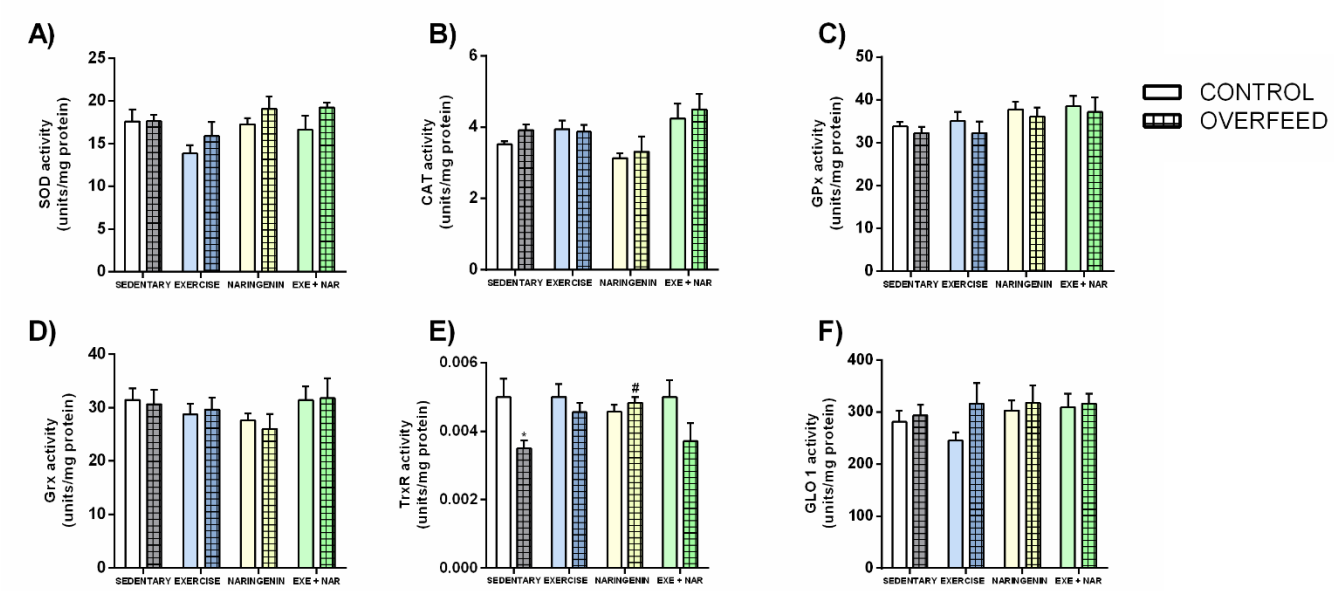

Fig. 5. Effect of maternal treatments and postnatal overfed in cerebellum of pups on PND21: (A) SOD activity, (B) CAT activity, (C) GPx activity, (D) Grx activity, (E) TrxR activity, and (F) GLO1 activity. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05 Significant difference between SO and NO is indicated: #p<0.05

3.5 The offspring's hippocampus was affected in several parameters by litter size reduction

As illustrated in Fig. 6, maternal exercise cause increased carbonyl levels, allied or not to postnatal litter size reduction ($F(1,20)=23.58;p<0.0001$), while DCFH oxidation ($F(1,32)=0.7391;p=0.3963$) and TBARS levels ($F(1,21)=0.1396;p=0.7125$) were not altered.

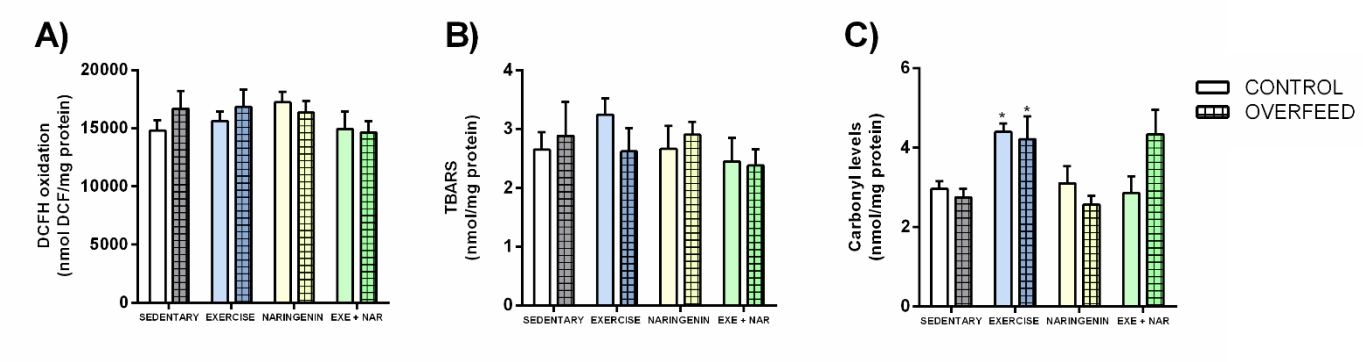

Fig. 6. Effect of maternal treatments and postnatal overfed in hippocampus of pups on PND21: (A) DCFH oxidation levels, (B) TBARS content, and (C) Carbonyl levels. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05

Related to antioxidant enzymes, as demonstrated in Fig. 7 postnatal overnutrition per se enhances SOD activity, and the effect is maintained when allied to maternal naringenin supplementation ($F(1,25)=4.390;p=0.0464$)). The litter size reduction also increased GPx activity, allied or not to maternal exercise ($F(1,31)=11.64;p=0,0018$), and GLO 1 activity, that was completely restored by maternal interventions when allied ($F(1,28)=23.58;p<0.0001$).

Maternal naringenin supplementation could increase TrxR activity when applied postnatal overnutrition ($F(1,27)=7.930;p=0.0090$), and activities of CAT ($F(1,25)=0.1076;p=0.7456$) and Grx ($F(1,32)=1.344;p=0.2549$) were not altered.

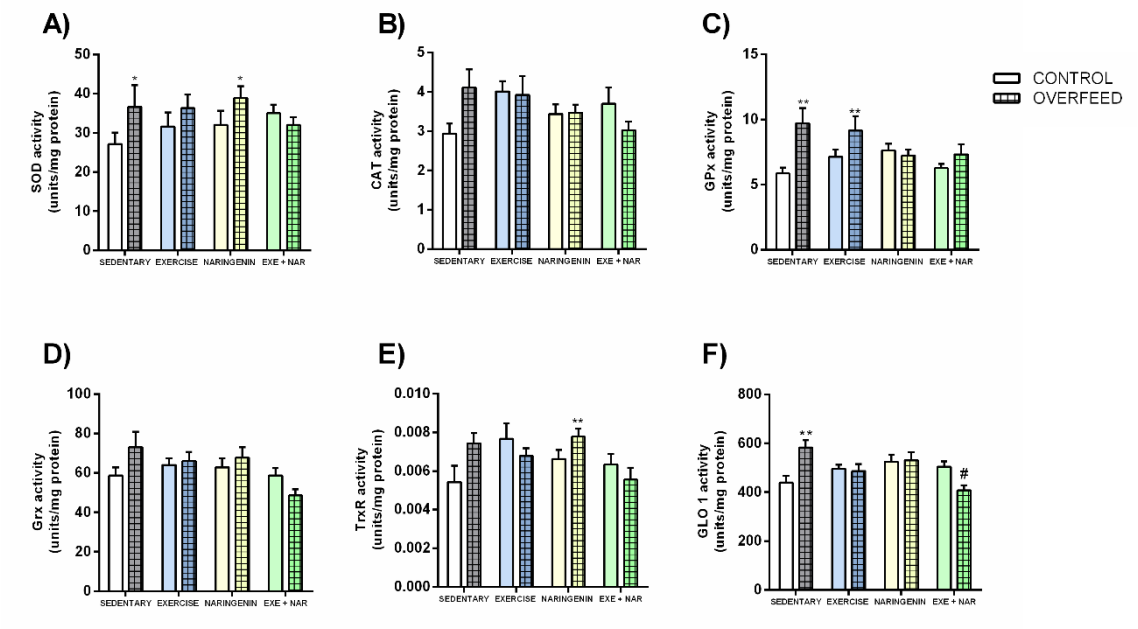

Fig. 7. Effect of maternal treatments and postnatal overfed in hippocampus of pups on PND21: (A) SOD activity, (B) CAT activity, (C) GPx activity, (D) Grx activity, (E) TrxR activity, and (F) GLO1 activity. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05 **p<0.01 Significat difference between SO and ENO is indicated: #p<0.05

3.6 The offspring's hypothalamus was affected by maternal and postnatal environment

Figure 8 shows that maternal exercise increased DCFH oxidation ($F(1,28)=5.249;p=0.0297$), and allied naringenin supplementation abolished the effect ($F(1,24)=0.01616;p=0.8999$), while lipid peroxidation was not altered

(F(1,22)=0.02529;p=0.8756).

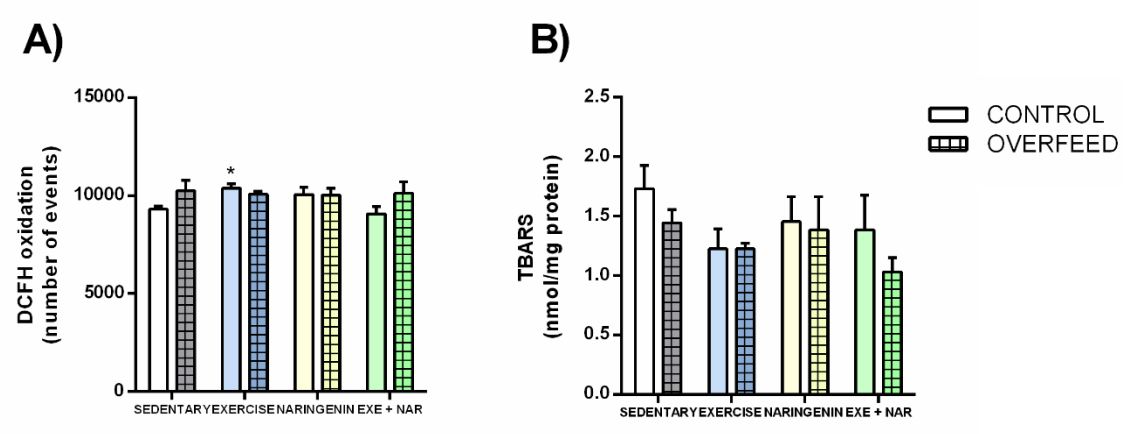

Fig. 8. Effect of maternal treatments and postnatal overfed in hypothalamus of pups on PND21: (A) DCFH oxidation levels and (B) TBARS content. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05

SOD (F(1,23)=10.32;p=0.0039) and GLO 1 activities (F(1,24)=7.157;p=0.0132) were enhanced by maternal exercise allied to postnatal overnutrition, and the effects does not persist when maternal naringenin supplementation was allied. GPx activity was reduced by postnatal overnutrition and maternal exercise practice (F(1,20)=20.36;p=0.0002), and also when naringenin supplementation was allied (F(1,19)=15.51;p=0.0009). CAT (F(1,23)=0.02564;p=0.8742), Grx (F(1,18)=0.6640;p=0.4258), and TrxR activities were not altered (F(1,18)=0.03038;p=0.8636). Antioxidant enzymes activities were

illustrated in Figure 9.

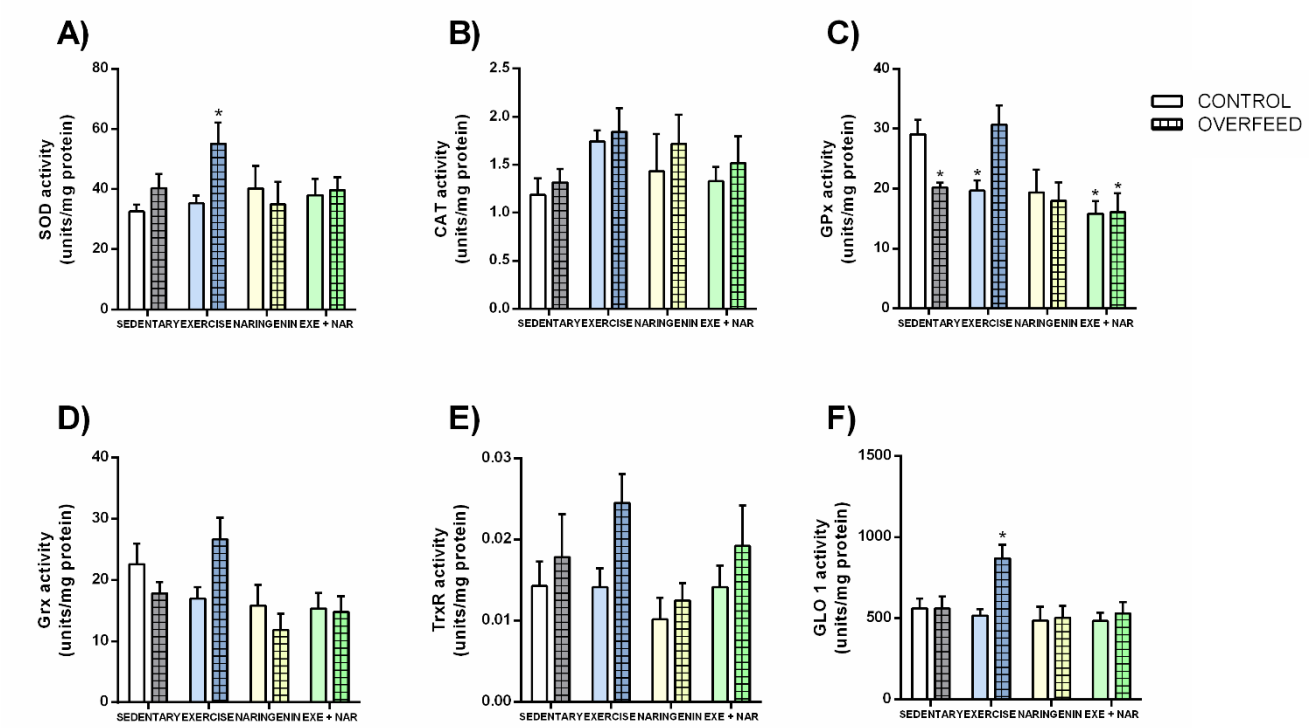

Fig. 9. Effect of maternal treatments and postnatal overfed in hypothalamus of pups on PND21: (A) SOD activity, (B) CAT activity, (C) GPx activity, (D) Grx activity, (E) TrxR activity, and (F) GLO1 activity. Results are expressed as mean + SEM for n=7-8. Results were analyzed by two-way ANOVA followed by Tukey post-hoc. *p<0.05

4. Discussion

This study is the first to (A) show the effects of maternal exercise and naringenin supplementation on early overfeeding at brain redox homeostasis; and (B) demonstrate that the annulation of effects when exercise practice and antioxidant intake were applied concomitantly, already demonstrated in adult animals [47, 48], also occurs through generation.

In this context, the maternal treatments applied in our study, swimming exercise and/or naringenin supplementation, did not alter maternal and fetus outcome. In humans, exercise during pregnancy also did not change the birth weight

[19, 49], and polyphenol consumption during pregnancy did not affect litter size or birth weight in animal model [50].

The litter size reduction from eight (control) to three pups (overfed) on PND1 leads to increased offspring's weight gain. In agreement with our data, Conceição, Kaezer [51] also found about 20% increased weight gain in this model. On EO and NO animals, maternal interventions delayed the increase on pups body weight induced by small litter size. Interestingly, on ENO pups the effect of isolated treatments in the weight gain disappears, being equal to SO. The increment in retroperitoneal fat was similar on overfed pups regardless the maternal environment. Increased body fat at weaning after the litter reduction was demonstrated elsewhere [52, 53].

At the same time, EC and NC pups present lower glucose levels, but again the effect disappears when maternal interventions were allied. Maternal exercise improves offspring glucose metabolism at weaning and adulthood, increasing insulin sensitivity [54, 55]. Polyphenol consumption also has an important effect on the adult animal glycaemia reduction through several mechanisms, including improved insulin sensitivity [25]. Neither alteration was found in overfeeding-rats serum parameters, agreeing with other studies that used this model [14, 15, 52].

We also evaluated parameters of redox homeostasis in several brain areas involved with memory, satiety, and motor control. Brain regions were differentially affected by maternal and postnatal interventions, and these effects may be related to the formation of the cerebral structures, which occurs at different stages of development [56].

Cerebellum has been beneficially affected by maternal interventions, being the exercise during pregnancy able to reduce the oxidative damage to proteins. The effect of exercise training on carbonyl levels reduction in the adult rat brain has already been demonstrated by Casuso, Martinez-Amat [57]. An important enzyme in protein denitrosilation [58], TrxR had its activity reduced in the cerebellum of SO animals, and the naringenin supplementation during pregnancy could prevent this effect completely, preventing redox imbalance. Polyphenol supplementation on adult mice already shows increased expression of this enzyme [59].

On offspring's hippocampus, EC and EO animals present increased carbonyl levels. The practice of continuous exercise leads to reduction in oxidative damage to proteins, while the acute exercise causes an increase in this marker in plasma [60], demonstrating that offspring's hippocampus can respond differently to maternal exercise. On the other hand, overfeeding during lactation cause a positive response on the hippocampus antioxidant enzymes activities, with an increase in SOD, GPx, and GLO 1, maybe in response to the pro-inflammatory environment found in this structure in the litter size reduction model [61, 62].

In the hypothalamus, EC pups show increased content of reactive species, allied to a decreased activity of GPx, and the enzyme effect remains in ENC and ENO groups. Although there are no studies evaluating the response of the offspring's hypothalamus to maternal exercise, it has already been shown that the gestational exercise causes benefits in the maternal rat hypothalamus preventing the effects of prenatal stress [63]. The effect of metabolic programming on a brain structure that is more formed postnatally needs to be further clarified.

The reduced litter size also decreased GPx activity on hypothalamus. On whole adult rat brain, 12 weeks of high-fat diet decreased GPx activity [64], showing a negative effect of overnutrition. SOD and GLO1 activities were improved on EO pups, demonstrating a positive response, which may be caused by adapting effect of exercise over the pro-inflammatory environment caused by litter reduction [65].

The effects on weight gain, serum and the majority of encephalic alterations caused by maternal treatments when isolated were abolished by the combined maternal exercise and naringenin supplementation, as previously hypothesized. The use of antioxidants counteracts the establishment of metabolic adaptations promoted by exercise in adult animals [47, 48, 66], and now we have shown that the effect can also occur in the next generation.

5. Conclusion

In summary, postnatal early overnutrition leads to higher weigh gain and fat mass at weaning, which was delayed by the maternal exercise practice or naringenin supplementation. The positive effect of these maternal interventions was also demonstrated by the reduced pups' glycaemia. In cerebellum, maternal interventions seem to cause positive effects on redox balance, while hippocampus and hypothalamus present several parameters of positive adaptation to postnatal overnutrition, as increased antioxidant enzymes activities. When maternal exercise was allied to naringenin supplementation, many of the effects disappear, demonstrating a clear concurrent effect. More in-depth studies are required to assess the exact mechanisms behind the effects of pre and postnatal interventions,

however, we found evidence that antioxidant strategies must be applied isolated in the pregnancy, in order to obtain a positive health impact in the next generation.

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Conflict of interest

The authors declare no conflicts of interest or competing interest.

References

1. Ogden, C.L., et al., *Prevalence of Obesity Among Adults and Youth: United States, 2011–2014*, N.C.f.H. Statistics, Editor. 2015: NCHS Data Brief.
2. Skinner, A.C., et al., *Prevalence of Obesity and Severe Obesity in US Children, 1999-2016.* Pediatrics, 2018.
3. McCloskey, K., et al., *Infant adiposity at birth and early postnatal weight gain predict increased aortic intima-media thickness at 6 weeks of age: a population-derived cohort study.* Clin Sci (Lond), 2016. **130**(6): p. 443-50.
4. Araujo de Franca, G.V., et al., *Associations of birth weight, linear growth and relative weight gain throughout life with abdominal fat depots in adulthood: the 1982 Pelotas (Brazil) birth cohort study.* Int J Obes (Lond), 2016. **40**(1): p. 14-21.
5. Prioreschi, A., et al., *The associations between adult body composition and abdominal adiposity outcomes, and relative weight gain and linear growth from birth to age 22 in the Birth to Twenty Plus cohort, South Africa.* PLoS One, 2018. **13**(1): p. e0190483.
6. Stettler, N., et al., *Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula.* Circulation, 2005. **111**(15): p. 1897-903.
7. Genovesi, S., et al., *Hypertension, prehypertension, and transient elevated blood pressure in children: association with weight excess and waist circumference.* Am J Hypertens, 2010. **23**(7): p. 756-61.
8. Ayer, J., et al., *Lifetime risk: childhood obesity and cardiovascular risk.* Eur Heart J, 2015. **36**(22): p. 1371-6.
9. Olshansky, S.J., et al., *A potential decline in life expectancy in the United States in the 21st century.* N Engl J Med, 2005. **352**(11): p. 1138-45.
10. Kennedy, G.C., *The development with age of hypothalamic restraint upon the appetite of the rat.* J Endocrinol, 1957. **16**(1): p. 9-17.
11. Sefcikova, Z. and L. Racek, *Effect of neonatal beta(3)-adrenoceptor agonist CL 316,243 treatment on body fat accumulation and intestinal alkaline phosphatase activity in rats from reduced nests.* Folia Histochem Cytobiol, 2015. **53**(4): p. 307-13.
12. Mozes, S., Z. Sefcikova, and L. Racek, *Long-term effect of altered nutrition induced by litter size manipulation and cross-fostering in suckling male rats on development of obesity risk and health complications.* Eur J Nutr, 2014. **53**(5): p. 1273-80.
13. Achard, V., et al., *Immediate Postnatal Overfeeding in Rats Programs Aortic Wall Structure Alterations and Metalloproteinases Dysregulation in Adulthood.* Am J Hypertens, 2015.
14. Plagemann, A., et al., *Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats.* Brain Res, 1999. **836**(1-2): p. 146-55.
15. Boullu-Ciocca, S., et al., *Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome.* Diabetes, 2005. **54**(1): p. 197-203.
16. Vina, J., et al., *Exercise acts as a drug; the pharmacological benefits of exercise.* Br J Pharmacol, 2012. **167**(1): p. 1-12.
17. Schlussel, M.M., et al., *Physical activity during pregnancy and maternal-child health outcomes: a systematic literature review.* Cad Saude Publica, 2008. **24 Suppl 4**: p. s531-44.
18. Prather, H., T. Spitznagle, and D. Hunt, *Benefits of exercise during pregnancy.* PM R, 2012. **4**(11): p. 845-50; quiz 850.

19. Clapp, J.F., 3rd, *Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy.* J Pediatr, 1996. **129**(6): p. 856-63.
20. Hochner, H., et al., *Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors: the Jerusalem Perinatal Family Follow-up Study.* Circulation, 2012. **125**(11): p. 1381-9.
21. Dayi, A., et al., *Maternal Aerobic Exercise during Pregnancy Can Increase Spatial Learning by Affecting Leptin Expression on Offspring's Early and Late Period in Life Depending on Gender.* ScientificWorldJournal, 2012. **2012**: p. 429803.
22. Lee, H.H., et al., *Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups.* Brain Dev, 2006. **28**(3): p. 147-54.
23. Marcelino, T.B., et al., *Evidences that maternal swimming exercise improves antioxidant defenses and induces mitochondrial biogenesis in the brain of young Wistar rats.* Neuroscience, 2013. **246**: p. 28-39.
24. Wang, S., et al., *Novel insights of dietary polyphenols and obesity.* J Nutr Biochem, 2014. **25**(1): p. 1-18.
25. Kim, Y., J.B. Keogh, and P.M. Clifton, *Polyphenols and Glycemic Control.* Nutrients, 2016. **8**(1).
26. Yamada, T., et al., *Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: the Jichi Medical School cohort study.* J Epidemiol, 2011. **21**(3): p. 169-75.
27. Yang, X., et al., *Antidiabetic effects of flavonoids from Sophora flavescens EtOAc extract in type 2 diabetic KK-ay mice.* J Ethnopharmacol, 2015. **171**: p. 161-70.
28. Ponzo, V., et al., *Dietary flavonoid intake and cardiovascular risk: a population-based cohort study.* J Transl Med, 2015. **13**: p. 218.
29. Vanhees, K., et al., *Intrauterine exposure to flavonoids modifies antioxidant status at adulthood and decreases oxidative stress-induced DNA damage.* Free Radic Biol Med, 2013. **57**: p. 154-61.
30. Toumi, M.L., et al., *Quercetin alleviates predator stress-induced anxiety-like and brain oxidative signs in pregnant rats and immune count disturbance in their offspring.* Pharmacol Biochem Behav, 2013. **107**: p. 1-10.
31. Hahn, M., et al., *Polyphenol-rich food general and on pregnancy effects: a review.* Drug Chem Toxicol, 2017. **40**(3): p. 368-374.
32. Barenys, M., S. Masjosthusmann, and E. Fritsche, *Is Intake of Flavonoid-Based Food Supplements During Pregnancy Safe for the Developing Child? A Literature Review.* Curr Drug Targets, 2017. **18**(2): p. 196-231.
33. Wang, X., et al., *Simultaneous determination of five free and total flavonoids in rat plasma by ultra HPLC-MS/MS and its application to a comparative pharmacokinetic study in normal and hyperlipidemic rats.* J Chromatogr B Analyt Technol Biomed Life Sci, 2014. **953-954**: p. 1-10.
34. Mata-Bilbao Mde, L., et al., *Absorption and pharmacokinetics of grapefruit flavanones in beagles.* Br J Nutr, 2007. **98**(1): p. 86-92.
35. Raza, S.S., et al., *Neuroprotective effect of naringenin is mediated through suppression of NF-kappaB signaling pathway in experimental stroke.* Neuroscience, 2013. **230**: p. 157-71.
36. Sabarinathan, D., P. Mahalakshmi, and A.J. Vanisree, *Naringenin, a flavanone inhibits the proliferation of cerebrally implanted C6 glioma cells in rats.* Chem Biol Interact, 2011. **189**(1-2): p. 26-36.
37. LeBel, C.P., H. Ischiropoulos, and S.C. Bondy, *Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress.* Chem Res Toxicol, 1992. **5**(2): p. 227-31.

38. Reznick, A.Z. and L. Packer, *Oxidative damage to proteins: spectrophotometric method for carbonyl assay.* Methods Enzymol, 1994. **233**: p. 357-63.
39. Yagi, K., *Simple procedure for specific assay of lipid hydroperoxides in serum or plasma.* Methods Mol Biol, 1998. **108**: p. 107-10.
40. Misra, H.P. and I. Fridovich, *The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase.* J Biol Chem, 1972. **247**(10): p. 3170-5.
41. Aebi, H., *Catalase in vitro.* Methods Enzymol, 1984. **105**: p. 121-126.
42. Wendel, A., *Glutathione peroxidase.* Methods Enzymol, 1981. **77**: p. 325-33.
43. Holmgren, A. and F. Aslund, *Glutaredoxin.* Methods Enzymol, 1995. **252**: p. 283-92.
44. Arner, E.S. and A. Holmgren, *Measurement of thioredoxin and thioredoxin reductase.* Curr Protoc Toxicol, 2001. **Chapter 7**: p. Unit 7 4.
45. McLellan, A.C. and P.J. Thornalley, *Glyoxalase activity in human red blood cells fractioned by age.* Mech Ageing Dev, 1989. **48**(1): p. 63-71.
46. Lowry, O.H., et al., *Protein measurement with the Folin phenol reagent.* J Biol Chem, 1951. **193**(1): p. 265-75.
47. Gomez-Cabrera, M.C., E. Domenech, and J. Vina, *Moderate exercise is an antioxidant: upregulation of antioxidant genes by training.* Free Radic Biol Med, 2008. **44**(2): p. 126-31.
48. Casuso, R.A., et al., *Oral quercetin supplementation hampers skeletal muscle adaptations in response to exercise training.* Scand J Med Sci Sports, 2013.
49. Mudd, L.M., et al., *Leisure-time physical activity in pregnancy and the birth weight distribution: where is the effect?* J Phys Act Health, 2012. **9**(8): p. 1168-77.
50. Ros, P., et al., *Resveratrol Intake During Pregnancy and Lactation Modulates the Early Metabolic Effects of Maternal Nutrition Differently in Male and Female Offspring.* Endocrinology, 2018. **159**(2): p. 810-825.
51. Conceição, E.P.S., et al., *Effects of Ilex paraguariensis (yerba mate) on the hypothalamic signalling of insulin and leptin and liver dysfunction in adult rats overfed during lactation.* Journal of Developmental Origins of Health and Disease, 2016. **8**(01): p. 123-132.
52. Chen, H., et al., *Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism.* Endocrinology, 2008. **149**(11): p. 5348-56.
53. Rodrigues, A.L., et al., *Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats.* J Physiol, 2009. **587**(Pt 11): p. 2647-61.
54. Quiclet, C., et al., *Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function.* Am J Physiol Endocrinol Metab, 2016. **311**(2): p. E508-18.
55. Stanford, K.I., et al., *Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring.* Diabetes, 2015. **64**(2): p. 427-33.
56. Bekkedal, M.Y., D. Arfsten, and D. Mattie, *An evaluation of neurobehavioral tests used to assess the neurodevelopmental effects of early ammonium perchlorate exposure.* J Toxicol Environ Health A, 2004. **67**(8-10): p. 835-44.
57. Casuso, R.A., et al., *Quercetin supplementation does not enhance cerebellar mitochondrial biogenesis and oxidative status in exercised rats.* Nutr Res, 2015. **35**(7): p. 585-91.
58. Benhar, M., *Roles of mammalian glutathione peroxidase and thioredoxin reductase enzymes in the cellular response to nitrosative stress.* Free Radic Biol Med, 2018.
59. Ji, L., et al., *Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism.* J Nutr Biochem, 2013. **24**(11): p. 1911-9.

60. Wadley, A.J., J.E. Turner, and S. Aldred, *Factors influencing post-exercise plasma protein carbonyl concentration.* Free Radic Res, 2016. **50**(4): p. 375-84.
61. De Luca, S.N., et al., *Neonatal overfeeding by small-litter rearing sensitises hippocampal microglial responses to immune challenge: Reversal with neonatal repeated injections of saline or minocycline.* J Neuroendocrinol, 2017. **29**(11).
62. De Luca, S.N., et al., *Early life overfeeding impairs spatial memory performance by reducing microglial sensitivity to learning.* J Neuroinflammation, 2016. **13**(1): p. 112.
63. Seo, J.H., et al., *Treadmill exercise during pregnancy ameliorates posttraumatic stress disorderinduced anxietylike responses in maternal rats.* Mol Med Rep, 2013. **7**(2): p. 389-95.
64. Amri, Z., et al., *Effect of pomegranate extracts on brain antioxidant markers and cholinesterase activity in high fat-high fructose diet induced obesity in rat model.* BMC Complement Altern Med, 2017. **17**(1): p. 339.
65. Ziko, I., et al., *Neonatal overfeeding alters hypothalamic microglial profiles and central responses to immune challenge long-term.* Brain Behav Immun, 2014. **41**: p. 32-43.
66. Casuso, R.A., et al., *The combination of oral quercetin supplementation and exercise prevents brain mitochondrial biogenesis.* Genes Nutr, 2014. **9**(5): p. 420.

Capítulo II: Influence of Gestational Exercise Practice and Litter Size Reduction on Maternal Care

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Abstract

The first thousand days of life are of utmost importance in defining the future health of the individual. As a healthy intervention, physical exercise during pregnancy has been shown to bring benefits to the mother and the offspring. On the other side, childhood overweight has been associated with negative outcomes over the lifespan. In this context, the litter size reduction model has been widely used to study early overfeeding in rats. The goal of this study was to evaluate the influence of prenatal and neonatal interventions on maternal care, due to its importance on offspring development. Female Wistar rats were divided into two groups: 1) sedentary rats, and 2) swimming exercise. One day after birth, the litter was culled to 8 pups (normal) or 3 pups (small) per dam, yielding control and overfed subgroups for each maternal group, respectively. From postnatal days 2 to 9 the litter was observed 5 periods a day, to evaluate maternal behavior, and the pups were observed to evaluate developmental signs from day 4 to 15. Litter reduction caused important alterations in maternal behavior, reducing the total time out of the nest and increasing the frequency of maternal care and lactation in several observation periods, justifying the increased pup's weight gain already demonstrated by this animal model. The practice of maternal exercise was able to prevent some maternal behavior changes caused by the reduction of litter size. On the other hand, perinatal interventions did not alter eye opening or incisor eruption in the pups. These data demonstrated that small litter size can significantly alter maternal behavior, and gestational exercise can positively impact these changes.
Keywords: metabolic programming, obesity, maternal exercise, DOHaD, behaviour

Introduction

A large body of evidence demonstrates how early life environment can have a long-term impact on the individual's health. Since the 1960s the fetal metabolic programming has been investigated through a series of studies that gave rise to the Barker's hypothesis, which firstly described the relationship between an unfavorable intrauterine environment and the negative adaptation of the fetus to the postnatal environment [1-4]. This area has gained greater notoriety in recent years through the study of the Developmental Origins of Health and Disease (DOHaD), which highlights the importance of the first thousand days of life since conception up to 24 months, in predicting lifelong health [5]. The programming of the metabolism and behavior of the offspring either during intrauterine or postnatal life are influenced by several factors, such as maternal lifestyle during pregnancy and lactation, and early life habits of the individual [5, 6] .

In this scenery, the maternal environment is associated with the offspring's outcomes. A healthy lifestyle, such as being physically active during pregnancy, has been related to better offspring' memory and learning index, insulin sensitivity, oxidative capacity, and body mass adiposity [7-12]. Conversely, an unhealthy lifestyle, such as gestational under or overnutrition, has been related to impaired function of several organs in the progeny, leading to enhanced risk to insulin resistance and metabolic syndrome in adulthood [13, 14].

The maternal care early in life is also of the utmost importance and an essential component to survival, growth, and behavior building of the offspring [15, 16]. Maternal care is inherent in many species, presenting even hormonal changes

that activate greater nursing for offspring at birth [17]. In rats, maternal care after birth is naturally stimulated by several factors that combine somatosensory factors and tactile sensations, such as pups' sound and smell, and suckling stimulation during mother-pup interaction, respectively [18]. Maternal behavior can influence offspring development [19] as well as the early life nutrition [6], once both the malnutrition or overfeeding during lactation represent a higher risk for the development of non-communicable chronic diseases [20-25]. Over the last decades, infant overweight and obesity prevalence increased exponentially and thereby the consequently related comorbidities [26, 27]. Reducing the number of pups per litter is a well-established model of lactational overfeeding in rodents, in which there is an increase in the consumption of milk per pup due the less competition for milk, allied to the lack of complete maturation in satiety control at first days of life [28, 29]. In this model is possible to identify increased weight gain, dysregulation in adipose tissue redox homeostasis [30], increased release of proinflammatory mediators [31], and resistance to leptin, causing hyperphagia [23, 25, 32, 33]. These factors are important in the development of obesity and maintenance of overweight until adulthood.

Prevention of excess weight gain early in life is a public health commitment. In this context, the modification of maternal lifestyle during pregnancy might be a central factor preventing childhood obesity. The exercise practice during pregnancy has been linked to benefits over detrimental effects of overweight, such as reduced adipose tissue [34, 35], and improved glucose and insulin metabolism [12, 36], despite it fails to prevent the weight gain induced by reduced litter size in rats at age

of 21 days [37]. On basis of DOHAD concept with regard to fetal and early postnatal metabolic programming, we sought to verify how maternal care interferes with induced weight gain in the litter reduction model, and whether exercise practice during pregnancy can cause benefic alterations on maternal behavior.

2. Experimental Procedures

2.1 Animals and reagents

Twenty-four adult female (90 days of age), and twelve adult male (60 days of age) Wistar rats, with an average weight of 200 and 250 g, respectively, were obtained from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. All animals were maintained in a 12/12-h light/dark cycle in an air-conditioned constant temperature (22±1 °C) colony room. The animals had free access to water and 20% (w/w) protein commercial chow.

The experiments were approved by the local Ethics Commission (Comissão de Ética no Uso de Animais - Universidade Federal do Rio Grande do Sul, CEUA/UFRGS) under the number 31307, and followed national animal rights regulations (Law 11.794/2008), the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and Directive 2010/63/EU. We further attest that all efforts were made to minimize the number of animals used and their suffering.

2.2 Experimental design

2.2.1 Pregnancy

Female rats were randomly divided into two groups (n= 18 each): 1) sedentary rats, and 2) swimming exercise. The maternal exercise protocol began one week

previous to mating, in order to adapt the animals to the aquatic environment and reduce the stress response. During the experiment, four animals were kept in each cage, except for mating (one male per two female rats). The pregnancy was diagnosed by the presence of a vaginal plug. From the 20th day after the onset of pregnancy, we reallocated the pregnant dams (one per cage), and the rats were observed twice a day (at 9:00 and 18:00 h) to verify the litter's birth. The day corresponding to the offspring's birth was defined as postnatal day 0 (PND0).

2.2.2 Swimming exercise protocol

The maternal exercise protocol was adapted from Lee et al. [38], as described in Marcelino et al. [39]. In the exercised group, rats were submitted to swimming in a pool filled with 32±1 ºC water on 5 days/week for 4 weeks. Each swimming session lasted for 30 minutes, and always took place between 9:00 and 12:00 h. Each rat was isolated for the swim, which was conducted using an apparatus designed specifically for rat swimming. Within this apparatus, each room measures 30x30x90 cm (width x length x depth), preventing the animals from touching the bottom of the tank. The animals were left free to swim, without any extra weight, and were gently stimulated to swimming when it was necessary. This protocol has moderate intensity. Rats in the control group were immersed in water, carefully dried, and returned to the housing boxes. The litter's weight and size, pregnancy rate and weight gain are described at August et al. 2018 [37].

2.2.3 Overnutrition model

One day after birth (PND1), to induce early postnatal overnutrition, litter sizes were manipulated to small or normal groups, with 3 and 8 pups, respectively. The

small litter was maintained with only male pups and the normal litter with at least 3 male pups. This yielded four experimental groups: sedentary mother with normal litter (SN), sedentary mother with small litter (SS), exercised mother with normal litter (EN), exercised mother with small litter (ES). The offspring was left with the mother up to PND21 to induce overfeeding during lactation. The offspring evaluation at weaning is described at August et al.[37]

2.3 Maternal care

Maternal behavior observation was scored for five periods of 72 min, daily, from PND2 to PND9 at regular times (6:00, 10:00, 13:00, 17:00, and 20:00 h), as described by Klein et al. [40], and adapted from Champagne et al. [19]. Within each observation period, the dams were monitored in sequence every 3 min, and the observer recorded the ongoing behavior at the instant of the observation. The schedule is resumed as follows: 25 observations/period × 5 periods per day, resulting in 125 observations/dam/day yielding 1125 observations/dam during the experiment (n=5 to 9 litters/group).

The following behaviors were scored by trained observers: a) dam in or out of the nest, b) dam licking any pup, c) dam nursing pups in arched back, blanket (in which the dam lays over the pups) or supine (in which the dam is lying either on her back or side while the pups nurse) posture, d) nest building, retrieving pups, and e) dam drinking/eating. The quantitative measure of maternal behavior was analyzed as the frequency (in percentage) of observations in which animals engaged in the target behavior. Reduced duration of nurturing bouts results in a more fragmented pattern of care and in an increased behavioral inconsistency. Herein, we used the behavioral

inconsistency score as a qualitative measure of maternal behavior as described by Couto-Pereira et al. [41] and Klein et al. [40]. Maternal behavior was analyzed for each period to provide a behavioral inconsistency score, which varies between 0 and 1. The transition between one to other behavior in two sequential observations was scored as 1, and if there was no transition, the score was 0. The sum of scores was divided by 24 (the possible number of behavioral transitions per period). The higher the score, the more fragmented and inconsistent the maternal care. Transitions behaviors considered: nursing, licking, retrieving pups, nest building, away from pups, and dam drinking/eating.

2.4 Offspring development

From PND 4, one male of each litter was observed for developmental signs, evaluated daily at the same hour until the appearance of the incisor teeth eruption (the first appearance of the upper and lower incisors), and eye opening (both eyes fully open) 42].

2.5 Statistical analysis

GraphPad Prism 6.0 software was used for data analysis. All the data presented are expressed as the mean ± standard error mean (S.E.M.), and were analyzed through two-way ANOVA followed by Tukey's post-test, or repeated measures two-way ANOVA and Bonferroni post-test. Statistical significance was designated at $p<0.05$.

3. Results

3.1 Maternal behavior

To ascertain whether gestational exercise practice and/or litter size reduction alters the maternal care of dams, we observed maternal behavior from PND2 to PND9. When analyzing the total time in which mothers spent with non-maternal behavior, the reduced litter clearly caused a shorter time away from the offspring without interference of gestational intervention, as demonstrated in figure 1. In response to the small litter, sedentary dams demonstrated less time away from the pups at 6:00, 17:00 and 20:00 h, while exercised dams at 17:00 and 20:00 h only [$F(3,182)=18.72$; $p<0.0001$], when compared to SN group.

The higher frequency for non-maternal behavior occurred for all the groups at 20:00 h in dark cycle (60.9% in SN group, 39.8% at SS group, 53.7% in the EN group and 41.9% in the ES group), while the lowest frequency occurred at different periods between groups: at 13:00 h in the SN and EN groups (15.3 and 11.4%, respectively), at 6:00 h in the SS group (4.9%) and at 10:00 h in the ES group (6.4%).

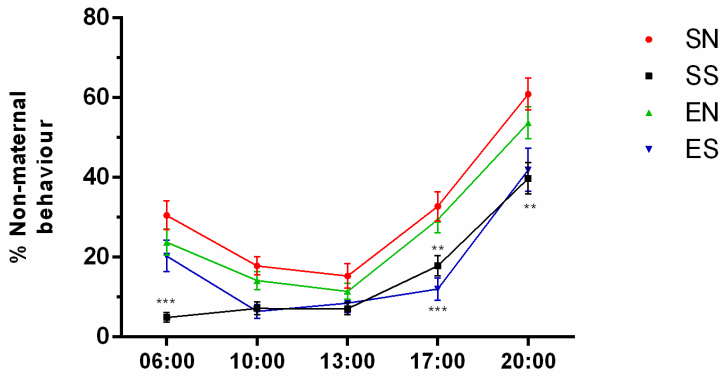

Figure 1. Frequency of non-maternal behavior at different hours from the postnatal days 2 to 9. Data were analyzed through two-way ANOVA repeated measures followed by Bonferroni post-test and presented as mean ± SEM (n=5-9).

When analyzing the frequency in contact with their offspring each day, the two-way ANOVA repeated measures indicated that the maternal-behavior decreased significantly from postnatal days 2 to 9 (Fig. 2) [F(7,128)=2.778; p=0.0101]. The frequency in contact with pups also differs between groups [F(3,128)=8.225; p=0.001].

To increase the understanding of maternal contact with the pups we analyzed the frequency of each maternal behavior according to the different times of observation (6:00, 10:00, 13:00, 17:00 and 20:00 h), as demonstrated in figure 3.

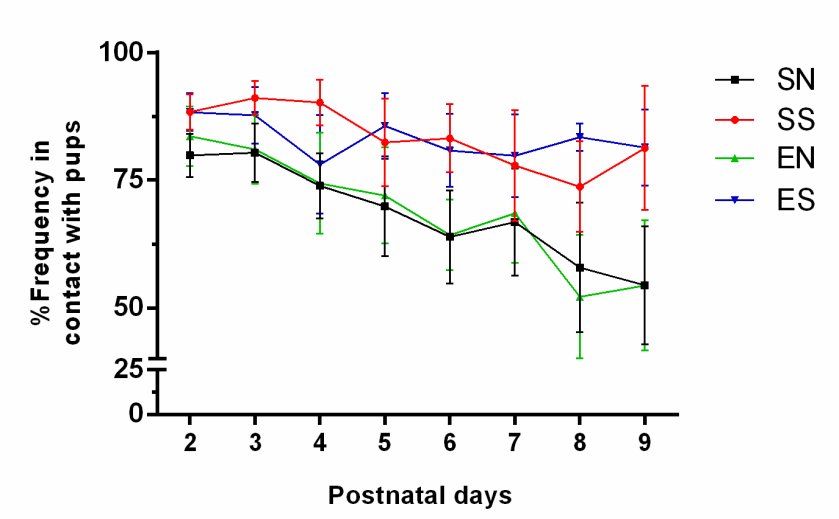

Figure 2. Frequency of maternal interaction with the pups over the postnatal days 2 to 9. Data were analyzed through two-way ANOVA repeated measures followed by Bonferroni post-test and presented as mean ± SEM (n=5-9).

The frequency spent with pups without lactating (in the nest and/or licking) (Fig. 3A) was increased at 06:00 h in the ES group and at 20:00 h in the SS and ES groups [F(12,724)=4.177; p<0.0001)]. The frequency of arched-back nursing (Fig. 3B), most common position used for lactation in this study, was increased in the SS

group at 06:00 and 10:00, and 17:00 h, and also in the ES group at 17:00 h [F(12,270)=2.242; p=0.0088)]. The frequency of arched-back and licking nursing behavior (Fig. 3C) did not differ between groups [F(12,716)=0.8130; p=0.6372)], while the frequency of passive nursing (Fig. 3D) was increased in the SS group at 06:00 h and at ES group at 20:00 h [F(12,720)=2.785; p=0.0010)]

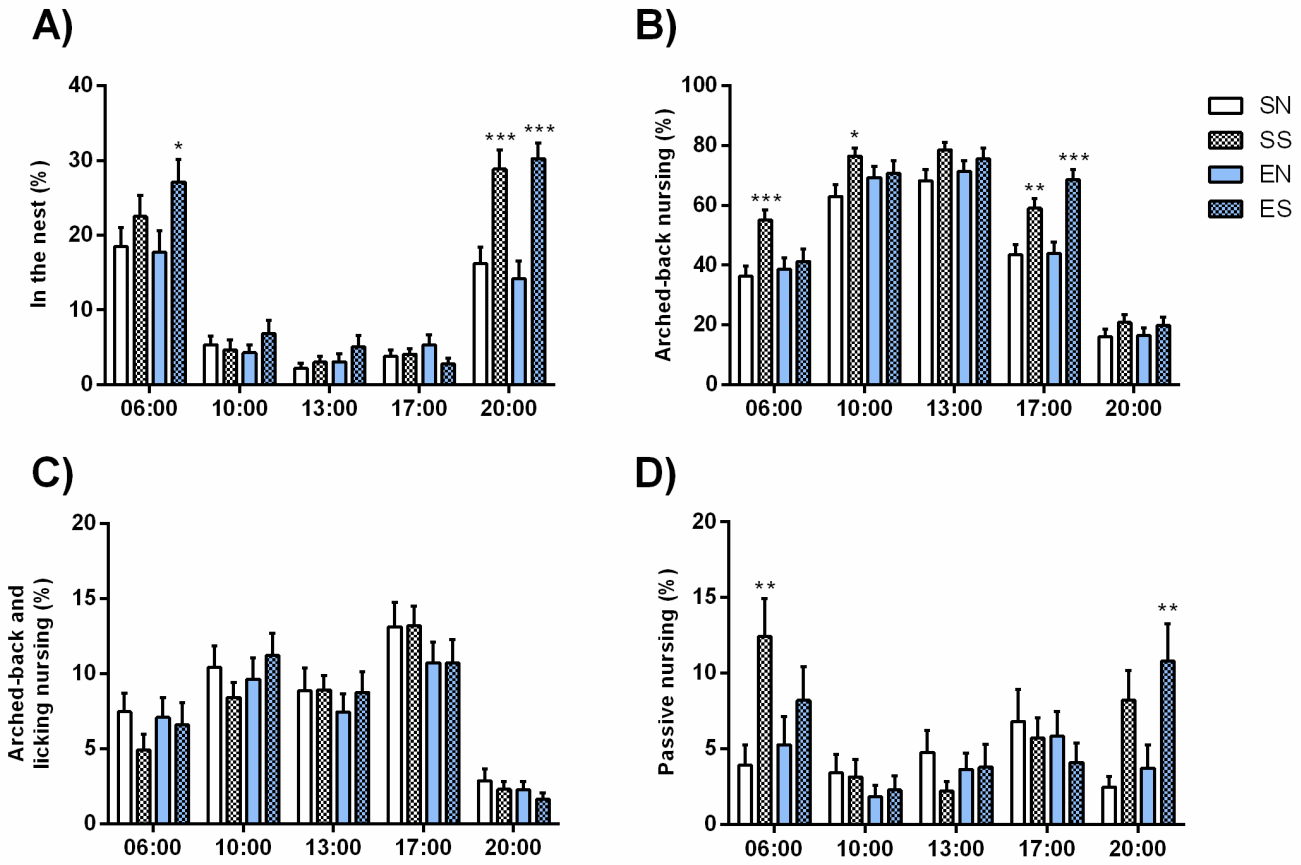

Figure 3. Cumulative frequency of maternal presence in the nest without lactating *(A)*, arched-back nursing behavior *(B),* arched-back and licking nursing behavior *(C)* and passive (supine) nursing behavior *(D)*. Data were analyzed through two-way ANOVA repeated measures followed by Bonferroni post-test and presented as mean ± SEM (n=5-9).

The higher frequency of dams in contact with their pups without lactating (Fig. 3A) differ between groups, occurring at 06:00 h in the SN and EN groups (18.5 and 17.7%, respectively), and at 20:00 h in the SS and ES groups (28.8 and 30.3%, respectively), but always in the dark cycle. The lowest frequency occurred at 13:00 h in the SN, SS and EN groups (2.2, 3 and 3.1%, respectively), and at 17:00 h in the ES group (2.8%).

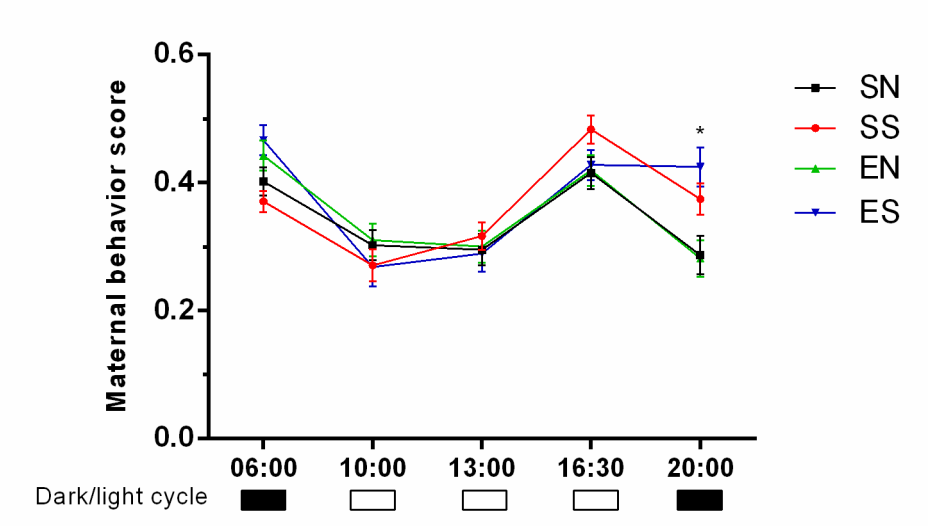

Figure 4. Maternal behavior inconsistency score. Data were analyzed through two-way ANOVA repeated measures followed by Bonferroni post-test and presented as mean ± SEM (n=5-9).

Cumulative behavioral inconsistency scores (Fig 4) had variation between observation times [$F(4,700)=31.86$; $p<0.0001$], the higher inconsistency score occurred at 17:00 h to sedentary dams (0.41 in the SN group, and 0.48 in the SS group), and at 6:00 h to exercised dams (0.44 to EN group, and 0.46 to ES group). Despite these differences, only at 20:00 h the ES group presented statistically higher

inconsistency score when compared to SN and EN groups [F(12,700)=3.040; p=0.0003].

3.2 Offspring development

Neither gestational interventions or litter size reduction cause any alterations on incisor eruption [F(1,12)=0.4762; p=0.5033] and eye opening [F(1,12)=1.333; p=0.2707], as demonstrated on table 1.

Table 1. Effect of maternal intervention and litter size reduction on offspring development parameters

	SN	SS	EN	ES	p value
Incisor eruption day	9.5±0.70	9.0±0.0	10.0±1.22	10.2±0.75	0.5033
Eye opening day	14.5±0.70	14.0±0.0	14.8±0.44	14.83±0.41	0.2707

Data was expressed as mean ± SD (two-way ANOVA followed by Tukey post-test) for n=2-6.

4. Discussion

Gestational and early life environment can influence the offspring's health throughout life, improving the life quality or increasing the risk of development for several diseases [2, 3, 5, 14]. Exercise during pregnancy has been proven to be safe and to promote positive adaptation for the mother and offspring [43-47]. On the other side, childhood overweight prevalence has increased worldwide and is related to negative health outcomes, being the litter size reduction model widely used to study early overfeeding in animal model [23-25, 48]. This paper seeks to clarify the role of the prenatal and neonatal interventions on maternal behavior, also very important in establishing the future health of the offspring [15], bringing alterations that may even be transgenerational [49].

When we analyze the time spent on each type of maternal behavior, the dams of reduced litters presented decreased non-maternal behavior at different times of the day, regardless of gestational sedentarism or exercise practice. This finding is in accordance with the data of Enes-Marques and Giusti-Paiva [50], that have already demonstrated similar maternal behavior in a model of litter size reduction to three pups per litter. In addition to the accentuated maternal care, the authors demonstrated reduced eating and explorative behavior by dams of reduced litter. During the dark cycle, the dams are commonly involved with their own food and water consumption, and at this period, we found that reduced litter size group had practically doubled the frequency at the nest, caring and licking the pups. Maternal licking is important to stimulate pup urination but also to maintain the mother's fluid balance through water recycling [51]. The increased licking behavior was probably important to prevent maternal dehydration since the mother spends less time for self-care.

The total frequency of mother-pup interaction was in accordance with Stern [18], demonstrating approximately 80% of the maternal behavior is focused on contact with their pups during the first days in all groups. Regarding lactation, the most important behavior for the offspring nutrition is the arched-back nursing, which naturally occurs after the pup's stimulus to the dam' ventral area, which adapts her position to arched, facilitating lactation [18]. In three of the five periods of the day evaluated here, the sedentary dams with small litters have increased frequency of arched-back nursing, even in one of the dark phase moments, where the frequency was almost double compared to the other groups. Gestational exercise prevented the

increase in arched-back nursing frequency induced by litter size reduction at 2 periods of the day, maintaining the same pattern as sedentary dams only in one period of the day. At the dark cycle, the reduced litter also increased the passive nursing, where the rats are lying on their side, with free access to the nipples. The increase in maternal care frequency is shown in different models of early life stress, such as early handling, maternal separation or deprivation, that when the mother rat is replaced in contact with the pups the time spent licking and lactating is increased, probably to compensate the stress induced by the separation [52]. Litter size manipulation already is shown to intensively modify dam's metabolism and body fat [53-55], however, present a positive correlation with dam plasma corticosterone [56], demonstrating that the increased maternal care probably is not caused by maternal stress, needing further studies to understand the maternal motivation.

As for the qualitative analysis of maternal care assessed through the maternal inconsistency score, we observed a difference between the groups only in one of the dark cycle analysis moments, with higher behavioral alteration in the exercised mothers with reduced litter, that present a less consistent maternal care at this period of the day. Considering that the frequency of permanence in each evaluated positions was very similar with the other groups, the score increase may have occurred due to the nocturnal period, where the dam had conflicts between her own care, that occurs in higher frequency at night, and the care of the offspring, since at this same period, the frequency of passive nursing is increased, in which the mother rat remains lying down.

Several factors have already demonstrated to influence the weight gain in puppies kept in reduced litters, such as less competition between puppies and higher fat content in milk [57, 58]. In addition, our results showed that mothers with reduced litters spend less time outside the nest, giving more care for the young pups and keeping longer lactation periods, which justifies the weight gain demonstrated in the model, regardless the exercise during pregnancy [37]. The quality of maternal care has been cited as worsened [18] or improved [59] in small litters, but in the present study, analyzing litters maintained with 3 male pups, the result was similar to Enes-Marques and Giusti-Paiva [50], with improved nursing care that could result in enhanced offspring's weight gain and body fat at weaning, as demonstrated previously in details in August et al. [37]. Maternal swimming exercise was not able to prevent the weight gain induced by lactational overfeeding, but it was able to delay the onset of excessive weight gain [37], which can be explained by the prevention of increased frequency of lactation induced by litter size reduction in some periods evaluated.

When exposed to reduced litter size, at adulthood the rat offspring commonly present increased risk for several diseases, trough enhanced body weight and fat, and increased glucose, triglycerides, total cholesterol, and insulin levels, allied to decreased HDL, leptin resistance and deleterious effect on several tissues, such as heart, liver, kidney, and brain [25, 50].

Although increased maternal contact with offspring could cause metabolic dysregulation, it seems to improve behavioral skills in the adult offspring. Litter size reduction caused less anxiety-like behavior in male and female rat offspring at 60

days of age, evaluated in open field test, and decreased anxiety-like symptoms and stress-induced corticosterone in C57BL/6 male mice at 90 days of age the, evaluated in light-dark box and elevated plus maze tests [50, 60]. In Norway rats the higher frequency of maternal licking and arched-back nursing caused lower levels of plasma corticosterone and adrenocorticotropic hormone in response to restraint stress, also lowering the hypothalamic-pituitary-adrenal responses to adulthood stress in the offspring that received more maternal care [61]. Long-Evans rats also present several alterations in the neural system related to fearfulness, improving anxiolytic action [62]. Considering those studies, it seems that dams who spend more time caring for puppies in the first days of life generate less anxious adult rats. Conversely, the effect seems to differ depending on the animal strain, since NMRI male mice present increased anxiety-like behavior and stress-induced at 90 days of age, allied to decreased spatial memory [60].

Gestational and neonatal interventions did not cause alteration in offspring developmental parameters, evaluated through incisor appearance and eye opening. Maternal swimming exercise also did not alter sensorimotor reflexes development, but present more mature motor development [40]. Small litters appear to positively affect the onset of these factors, however when evaluated in born-small litters and not in reduced litters after birth [63].

5. Conclusion

In summary, the litter size reduction causes important alterations in maternal behavior in the first days of life, increasing the care and lactation periods and also reducing the frequency of time outside the nest. The maternal exercise was able to

decrease some of these behaviors, especially during lactation periods. Neither maternal or postnatal interventions alter offspring developmental parameters evaluated.

Our results corroborate with the already demonstrated increased weight gain with the litter reduction model, and maternal physical exercise was unable to completely prevent the alterations on maternal care induced by the model.

Further studies are essential to assess the influence of these factors throughout the offspring's life, and what mechanism motivates the increased maternal care when the litter is reduced.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. Hales, C.N. and D.J. Barker, *Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis.* Diabetologia, 1992. **35**(7): p. 595-601.
2. Barker, D.J., et al., *Weight in infancy and death from ischaemic heart disease.* Lancet, 1989. **2**(8663): p. 577-80.
3. Barker, D.J., *The fetal and infant origins of adult disease.* BMJ, 1990. **301**(6761): p. 1111.
4. Neel, J.V., *Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"?* Am J Hum Genet, 1962. **14**: p. 353-62.
5. Hanson, M.A., *Background to the Cape Town Manifesto: harnessing the power of the normal.* J Dev Orig Health Dis, 2016. **7**(5): p. 498-500.
6. Koletzko, B., et al., *Long-Term Health Impact of Early Nutrition: The Power of Programming.* Ann Nutr Metab, 2017. **70**(3): p. 161-169.
7. Akhavan, M.M., et al., *Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups.* Neuroscience, 2008. **151**(4): p. 1173-83.
8. Gomes da Silva, S., et al., *Maternal Exercise during Pregnancy Increases BDNF Levels and Cell Numbers in the Hippocampal Formation but Not in the Cerebral Cortex of Adult Rat Offspring.* PLoS One, 2016. **11**(1): p. e0147200.
9. M, M.A., et al., *Maternal Voluntary Exercise during Pregnancy Enhances the Spatial Learning Acquisition but not the Retention of Memory in Rat Pups via a TrkB-mediated Mechanism: The Role of Hippocampal BDNF Expression.* Iran J Basic Med Sci, 2013. **16**(9): p. 955-61.
10. Yau, S.Y., et al., *Effects of Maternal Voluntary Wheel Running During Pregnancy on Adult Hippocampal Neurogenesis, Temporal Order Memory, and Depression-Like Behavior in Adult Female and Male Offspring.* Front Neurosci, 2019. **13**: p. 470.
11. Rahimi, R., et al., *Maternal voluntary exercise ameliorates learning deficit in rat pups exposed, in utero, to valproic acid; role of BDNF and VEGF and their receptors.* Neuropeptides, 2018. **71**: p. 43-53.
12. Carter, L.G., et al., *Maternal exercise improves insulin sensitivity in mature rat offspring.* Med Sci Sports Exerc, 2013. **45**(5): p. 832-40.
13. Wentzel, P., U.J. Eriksson, and E. Herrera, *High-fat diet in pregnant rats and adverse fetal outcome.* Ups J Med Sci, 2019. **124**(2): p. 125-134.
14. Fernandez-Twinn, D.S. and S.E. Ozanne, *Early life nutrition and metabolic programming.* Ann N Y Acad Sci, 2010. **1212**: p. 78-96.
15. Gonzalez-Mariscal, G. and A.I. Melo, *Bidirectional Effects of Mother-Young Contact on the Maternal and Neonatal Brains.* Adv Exp Med Biol, 2017. **1015**: p. 97-116.
16. Potter, H.G., D.G. Ashbrook, and R. Hager, *Offspring genetic effects on maternal care.* Front Neuroendocrinol, 2019. **52**: p. 195-205.
17. Levy, F., *Neuroendocrine control of maternal behavior in non-human and human mammals.* Ann Endocrinol (Paris), 2016. **77**(2): p. 114-25.
18. Stern, J.M., *Offspring-induced nurturance: animal-human parallels.* Dev Psychobiol, 1997. **31**(1): p. 19-37.
19. Champagne, F.A., et al., *Variations in maternal care in the rat as a mediating influence for the effects of environment on development.* Physiol Behav, 2003. **79**(3): p. 359-71.
20. Lind, M.V., et al., *Dietary protein intake and quality in early life: impact on growth and obesity.* Curr Opin Clin Nutr Metab Care, 2017. **20**(1): p. 71-76.
21. Achard, V., et al., *Immediate Postnatal Overfeeding in Rats Programs Aortic Wall Structure Alterations and Metalloproteinases Dysregulation in Adulthood.* Am J Hypertens, 2015.

22. Du, Q., et al., *Postnatal weight gain induced by overfeeding pups and maternal high-fat diet during the lactation period modulates glucose metabolism and the production of pancreatic and gastrointestinal peptides.* Peptides, 2015. **70**: p. 23-31.
23. Plagemann, A., et al., *Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats.* Brain Res, 1999. **836**(1-2): p. 146-55.
24. Boullu-Ciocca, S., et al., *Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome.* Diabetes, 2005. **54**(1): p. 197-203.
25. Habbout, A., et al., *Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences.* J Nutr, 2013. **143**(5): p. 553-62.
26. Gungor, N.K., *Overweight and obesity in children and adolescents.* J Clin Res Pediatr Endocrinol, 2014. **6**(3): p. 129-43.
27. Kartiosuo, N., et al., *Predicting overweight and obesity in young adulthood from childhood body-mass index: comparison of cutoffs derived from longitudinal and cross-sectional data.* Lancet Child Adolesc Health, 2019. **3**(11): p. 795-802.
28. Hall, M.D., D.G. Levitt, and L.J. Banaszak, *Crystal structure of Escherichia coli malate dehydrogenase. A complex of the apoenzyme and citrate at 1.87 A resolution.* J Mol Biol, 1992. **226**(3): p. 867-82.
29. Sabarinathan, D., P. Mahalakshmi, and A.J. Vanisree, *Naringenin, a flavanone inhibits the proliferation of cerebrally implanted C6 glioma cells in rats.* Chem Biol Interact, 2011. **189**(1-2): p. 26-36.
30. Conceicao, E.P., et al., *Early redox imbalance is associated with liver dysfunction at weaning in overfed rats.* J Physiol, 2015. **593**(21): p. 4799-811.
31. Ziko, I., et al., *Neonatal overfeeding alters hypothalamic microglial profiles and central responses to immune challenge long-term.* Brain Behav Immun, 2014. **41**: p. 32-43.
32. Rodrigues, A.L., et al., *Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats.* J Nutr Biochem, 2011. **22**(2): p. 109-17.
33. Yankovskaya, V., et al., *Architecture of succinate dehydrogenase and reactive oxygen species generation.* Science, 2003. **299**(5607): p. 700-4.
34. Ribeiro, T.A., et al., *Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition.* Sci Rep, 2017. **7**(1): p. 7634.
35. Carter, L.G., et al., *Perinatal exercise improves glucose homeostasis in adult offspring.* Am J Physiol Endocrinol Metab, 2012. **303**(8): p. E1061-8.
36. Raipuria, M., H. Bahari, and M.J. Morris, *Effects of maternal diet and exercise during pregnancy on glucose metabolism in skeletal muscle and fat of weanling rats.* PLoS One, 2015. **10**(4): p. e0120980.
37. August, P.M., et al., *Effect of maternal antioxidant supplementation and/or exercise practice during pregnancy on postnatal overnutrition induced by litter size reduction: Brain redox homeostasis at weaning.* Int J Dev Neurosci, 2018. **71**: p. 146-155.
38. Lee, H.H., et al., *Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups.* Brain Dev, 2006. **28**(3): p. 147-54.
39. Marcelino, T.B., et al., *Evidences that maternal swimming exercise improves antioxidant defenses and induces mitochondrial biogenesis in the brain of young Wistar rats.* Neuroscience, 2013. **246**: p. 28-39.
40. Klein, C.P., et al., *Swimming exercise before and during pregnancy: Promising preventive approach to impact offspring s health.* Int J Dev Neurosci, 2018. **71**: p. 83-93.

41. Couto-Pereira, N.S., et al., *Neonatal interventions differently affect maternal care quality and have sexually dimorphic developmental effects on corticosterone secretion.* Int J Dev Neurosci, 2016. **55**: p. 72-81.
42. Heyser, C.J., *Assessment of developmental milestones in rodents.* Curr Protoc Neurosci, 2004. **Chapter 8**: p. Unit 8 18.
43. Schlussel, M.M., et al., *Physical activity during pregnancy and maternal-child health outcomes: a systematic literature review.* Cad Saude Publica, 2008. **24 Suppl 4**: p. s531-44.
44. Prather, H., T. Spitznagle, and D. Hunt, *Benefits of exercise during pregnancy.* PM R, 2012. **4**(11): p. 845-50; quiz 850.
45. Clapp, J.F., 3rd, *Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy.* J Pediatr, 1996. **129**(6): p. 856-63.
46. Hochner, H., et al., *Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors: the Jerusalem Perinatal Family Follow-up Study.* Circulation, 2012. **125**(11): p. 1381-9.
47. Klein, C.P., et al., *Physical Exercise During Pregnancy Prevents Cognitive Impairment Induced by Amyloid-beta in Adult Offspring Rats.* Mol Neurobiol, 2019. **56**(3): p. 2022-2038.
48. Kennedy, G.C., *The development with age of hypothalamic restraint upon the appetite of the rat.* J Endocrinol, 1957. **16**(1): p. 9-17.
49. Ward, I.D., et al., *Transgenerational programming of maternal behaviour by prenatal stress.* BMC Pregnancy Childbirth, 2013. **13 Suppl 1**: p. S9.
50. Enes-Marques, S. and A. Giusti-Paiva, *Litter size reduction accentuates maternal care and alters behavioral and physiological phenotypes in rat adult offspring.* J Physiol Sci, 2018. **68**(6): p. 789-798.
51. Friedman, M.I., J.P. Bruno, and J.R. Alberts, *Physiological and behavioral consequences in rats of water recycling during lactation.* J Comp Physiol Psychol, 1981. **95**(1): p. 26-35.
52. Orso, R., et al., *How Early Life Stress Impact Maternal Care: A Systematic Review of Rodent Studies.* Front Behav Neurosci, 2019. **13**: p. 197.
53. Plumel, M.I., et al., *Litter size manipulation in laboratory mice: an example of how proteomic analysis can uncover new mechanisms underlying the cost of reproduction.* Front Zool, 2014. **11**: p. 41.
54. Capriglioni Cancian, C.R., et al., *Histological and Metabolic State of Dams Suckling Small Litter or MSG-Treated Pups.* ScientificWorldJournal, 2016. **2016**: p. 1678541.
55. Xavier, J.L.P., et al., *Litter Size Reduction Induces Metabolic and Histological Adjustments in Dams throughout Lactation with Early Effects on Offspring.* An Acad Bras Cienc, 2019. **91**(1): p. e20170971.
56. van Haasteren, G.A., et al., *Studies on the role of TRH and corticosterone in the regulation of prolactin and thyrotrophin secretion during lactation.* J Endocrinol, 1996. **148**(2): p. 325-36.
57. Sefcikova, Z. and L. Racek, *Effect of neonatal beta(3)-adrenoceptor agonist CL 316,243 treatment on body fat accumulation and intestinal alkaline phosphatase activity in rats from reduced nests.* Folia Histochem Cytobiol, 2015. **53**(4): p. 307-13.
58. Mozes, S., Z. Sefcikova, and L. Racek, *Long-term effect of altered nutrition induced by litter size manipulation and cross-fostering in suckling male rats on development of obesity risk and health complications.* Eur J Nutr, 2014. **53**(5): p. 1273-80.
59. Bester-Meredith, J.K., et al., *Peromyscus as a model system for understanding the regulation of maternal behavior.* Semin Cell Dev Biol, 2017. **61**: p. 99-106.
60. Salari, A.A., et al., *Small litter size impairs spatial memory and increases anxiety- like behavior in a strain-dependent manner in male mice.* Sci Rep, 2018. **8**(1): p. 11281.

61. Liu, D., et al., *Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress.* Science, 1997. **277**(5332): p. 1659-62.
62. Caldji, C., et al., *Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat.* Proc Natl Acad Sci U S A, 1998. **95**(9): p. 5335-40.
63. Chahoud, I. and F.J. Paumgartten, *Influence of litter size on the postnatal growth of rat pups: is there a rationale for litter-size standardization in toxicity studies?* Environ Res, 2009. **109**(8): p. 1021-7.

Capítulo III: Effect of maternal exercise on diet-induced redox imbalance in hippocampus of adult offspring

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Effect of maternal exercise on diet-induced redox imbalance in hippocampus of adult offspring

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Abstract

Physical exercise practice has been increasingly recommended in the prevention and treatment of chronic diseases, causing a positive effect from body weight/fat loss to improved cognitive function. Maternal exercise seems to induce the same positive lifelong adaptations to the offspring. We hypothesized that maternal exercise can prevent redox imbalance in adult offspring's hippocampus exposed to a high-fat diet (HFD). Female Wistar rats were divided into three groups before and during pregnancy: (1) sedentary, (2) swimming exercise, and (3) swimming exercise with overload. On 60 days of age, the male pups were divided into standard diet or HFD for one month, yielding normal and HFD subgroups for each maternal condition. Maternal interventions did not alter gestational parameters, birth outcomes, and offspring weight gain from weaning to 90 days of age. The HFD consumption increased body fat, which was not prevented by maternal exercise. Serum glucose levels were increased by HFD, an effect that was prevented by unload maternal exercise. In the hippocampus, both maternal exercise intensities could increase antioxidant defense. Hippocampal redox homeostasis was impaired by HFD, causing increased superoxide levels, which was prevented by exercise without load, while overload caused only a reduction of the effect. In summary, the practice of swimming exercise without overload during pregnancy seems to be more beneficial when evaluated in animal model, preventing HFD induced redox imbalance and increasing antioxidant defense while overload swimming exercise during pregnancy demonstrated a negative effect on offspring submitted to HFD consumption.

Keywords: metabolic programming, DOHaD, brain redox status, obesity, maternal exercise.

Introduction

Over the past three decades, the prevalence of worldwide obesity in adults has increased by almost 40% (Ng et al. 2014). The expressive increase in the population's weight gain is related to high caloric consumption, mainly from fat, combined with other factors such as sedentarism and genetic predisposition (Hariri and Thibault 2010, Popkin et al. 2012, Thaker 2017). A high-fat diet (HFD) is an effective obesity inducer in both humans and animals, demonstrating a positive correlation between increased fat intake and higher prevalence of obesity (Hariri and Thibault 2010).

The health problems caused by obesity are already well reported. Overweight individuals present an increased risk to several comorbidities such as hypertension, type 2 diabetes, dyslipidemia, and cardiovascular disease (De Lorenzo et al. 2019). Notably, these conditions are related to the major causes of death worldwide (Collaborators 2017, Organization 2018). Additionally, with the progress of life expectancy (Crimmins 2015), obesity also negatively affect an increasingly frequent health problem: neurological dysfunctions (O'Brien et al. 2017).

Depression, anxiety, Alzheimer's and Parkinson's diseases are some of the diseases related to obesity (Tan and Norhaizan 2019). From a cellular perspective, brain redox imbalance is considered a key factor in their appearance and progress (Salim 2017, Singh et al. 2019). The excessive increase of reactive species production and its insufficient detoxification by antioxidant defenses can lead to biomolecule damage and mitochondrial dysfunction, which occurs with excessive weight gain and drastically affect normal brain function (Salim 2017, de Mello et al. 2018). An important region related to learning, memory, and food control (Opitz 2014, Kanoski and Grill 2017), the hippocampus is highly affected by fat-rich diets. It has been observed, in an adult animal model feed with HFD, altered morphology and decreased synaptic protein related (Liu et al. 2014), neuronal loss (Wu et al. 2018), and enhanced apoptosis, inflammatory and stress markers (Wu et al. 2018, Nakandakari et al. 2019).

As a strategy for health promotion, the exercise practice appears as a non-pharmacological option, with low-cost and low side effects (Warburton et al. 2006,

Gaesser 2007, Kruk 2007). Regular and moderate physical activity leads to systemic adaptations that are responsible for increased caloric expenditure and weight loss, decreasing the risk for chronic diseases and improving cognitive function (Hamer and Chida 2009, Vina et al. 2012, Barha et al. 2017). In an adult animal models, HFD-induced adipose tissue expansion, increased inflammation (Kawanishi et al. 2010, Kolahdouzi et al. 2019), and hippocampal dysfunction (Park et al. 2019), which were prevented by exercise practice. When applied during pregnancy, maternal exercise can bring benefits to healthy and overweight women, decreasing gestational weight gain and gestational diabetes incidence (Garnaes et al. 2016, Magro-Malosso et al. 2017, Wang et al. 2017). Additionally, children born from physically active women present lower body fat (Clapp 1996, Dahly et al. 2018), and improved cognitive performance (Clapp 1996, Clapp et al. 1999, Labonte-Lemoyne et al. 2017). In rats, maternal exercise promotes increased hippocampal neurogenesis, mitochondrial biogenesis, and antioxidant defenses in the offspring (Marcelino et al. 2013, Gomes da Silva et al. 2016, Yau et al. 2019).

Furthermore, the Developmental origins of Health and Disease (DOHaD) concept, established that interventions in the first one thousand days of life, from conception to the second year of life, can interfere with the health of the individual throughout lifespan (Hanson 2016). Thus, the purpose of this study was to examine the effects of HFD on offspring hippocampal redox homeostasis, and how does the maternal swimming exercise practice on two different intensities can prevent redox alterations. We hypothesize that maternal exercise during pregnancy can prevent a redox imbalance in adult offspring exposed to HFD.

Experimental Procedures

Animals and reagents

Thirty-six adult female (90 days of age), and 18 adult male Wistar rats (60 days of age), with an average weight of 200 and 250 g respectively, were obtained from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained in a 12/12-h light/dark cycle in an air-conditioned

constant temperature (22±1 °C) colony room. The animals had free access to water and a 20% (w/w) protein commercial chow.

The experiments were approved by the local Ethics Commission (Comissão de Ética no Uso de Animais - Universidade Federal do Rio Grande do Sul, CEUA/UFRGS) under the number 31307, and followed national animal rights regulations (Law 11.794/2008), the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and Directive 2010/63/EU. We further attest that all efforts were made to minimize the number of animals used and their suffering.

The chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA), *Labtest* Diagnóstica S.A. (Lagoa Santa, *Brazil)* and Invitrogen by Thermo Fischer Scientific (Carlsbad, CA, USA).

Experimental design

Pregnancy

Female rats were randomly divided into three groups (n= 12 each): 1) sedentary rats; 2) swimming exercised rats; and 3) swimming exercised rats with overload (2% body weight).

The maternal exercise began one week previous to mating, to adapt the animals to the aquatic environment. During the exercise protocol, four animals were kept in each cage, except for mating (one male per two female rats). Pregnancy was diagnosed by the presence of a vaginal plug. From the 20th day after the onset of pregnancy, we isolated the pregnant dams (one per cage), and the rats were observed twice a day (at 9 a.m. and 4 p.m.), to verify the litter's birth. The day corresponding to the offspring's birth is defined as postnatal day 0 (PD0).

Swimming exercise protocol

The experimental design of pregnancy interventions was demonstrated in figure 1. The maternal exercise protocol was adapted from Lee et al. (2006), as described in Marcelino et al. (2013). The rats were divided into sedentary, swimming exercised and swimming exercised with overload. The sedentary rats were immersed in water, carefully dried, and returned to the housing boxes. In the exercised groups,

rats were submitted to swimming in a pool filled with 32±1 ºC water on 5 days/week for 4 weeks. Each swimming session lasted for 30 minutes, and always took place between 9 and 12 a.m. Each rat was isolated for the swim, which was conducted using an apparatus designed specifically for rat swimming. Within this apparatus, each room measures 30x30x90 cm (width x length x depth), preventing the animals from touching the bottom of the tank. In group 2, the animals were left free to swim, without any extra weight, and were gently stimulated to swimming when it was necessary. In the group 3, the rats were stimulated with an overload (2% body weight attached to the tail), adapted from Wasinski et al. (2015) and Chinkin (2013), that was used from day 2 to 5 of adaptation and during pregnancy, except in the first two days after pregnancy detection, when they were exposed to swimming without the extra weight. This protocol is considered moderate intensity.

High-fat diet

After weaning, male pups were separated and maintained in house boxes, receiving a standard diet. On 60 days of life, 1 to 2 male pups per dam were assigned into one of the diets: (1) standard diet (normal), with a caloric density of approximately 3.8 kcal/g (% of calories: 20,8 protein, 9,4 fat and 69,8 carbohydrates); or (2) high-fat diet, with a caloric density of approximately 5.4 kcal/g (% of calories: 14,8 protein, 58,7 fat and 26,5 carbohydrates), as described by Barella et al. (2012). This yielded six experimental groups: sedentary mother with control diet, as the control group (SN), sedentary mother with HFD (SH), exercised mother with normal diet (EN), exercised mother with HFD (EH), exercise with overload with normal diet (ON), exercised with overload with HFD (OH). Body weight was collected from PD21 until PD90 weekly. Food intake and caloric efficiency (body weight gain/ caloric intake) were estimated by weighing leftover feed.

The male offspring were euthanized by decapitation without anesthesia on PD90, after 30 days of the diet. Hippocampus was dissected and used freshly to flow cytometry or stored at -80°C to the remaining biochemical assays. Blood samples were collected by decapitation, after fasting for 4 hours. Body fat of pups (retroperitoneal and mesenteric) was dissected and weighed, and fat mass calculated as a percentage of wet tissue per whole body weight. One pup from each

offspring was used for each assay, to eliminate the litter effect. The surplus male animals were euthanized on PD60 when was collected mesenteric and retroperitoneal fat. Although there are sex-dependent differences in response to maternal exercise, we chose to use only males due to the fact that they are more highly affected by high-fat diets in the rodent model (Garcia-Carrizo et al. 2017).

Biochemical assays

Sample preparation

For flow cytometry, 50 mg of fresh tissue was dissociated with a Pasteur pipette in phosphate buffered saline (PBS) solution pH 7.4, containing 1 mg% of collagenase IV and 0.5 mg% DNAse. Dissociated tissue was centrifuged at 250 x g for 5 minutes to remove the excess of collagenase, resuspended on PBS, filtered and then incubated with fluorescent probes.

For biochemical analysis, each brain structure was individually homogenized in 10 volumes (1:10, w/v) of 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl. Homogenates were centrifuged at 1,000 x *g* for 10 min at 4 ºC, to discard nuclei and cell debris. The pellet was discarded and the supernatant was taken to biochemical assays.

For plasma measurements, blood was obtained on decapitation and then quickly centrifuged (1000*g*, 20 °C, 10 min) and plasma stored at −20 ºC until assayed.

Mitochondrial mass and membrane potential

Mitochondrial mass was analyzed using the probe MitoTracker® green, while mitochondrial membrane potential was measured using the probe MitoTracker® red, both purchased from Invitrogen®. Both analyzes above were performed in a FACScalibur flow cytometer (BD Biosciences®).

Fifty microliters of each sample were incubated at 37 ºC during 45 min in the presence of MitoTracker® green and red in a final concentration of 1 µM each. After

that, 20.000 cells were evaluated per sample in the flow cytometer. Negative control was evaluated without probe addition, and the fluorescence was discounted from the samples. Data were analyzed using the software FlowJo®

Oxidants measurement

Mitochondrial superoxide content was measured using the probe MitoSOX® red, purchased from Invitrogen®. Analyzes were performed in a FACScalibur flow cytometer (BD Biosciences®).

Fifty microliters of each sample were incubated at 37 ºC during 30 min in the presence of MitoSox® red in a final concentration of 1 µM. After that, 20.000 cells were evaluated per sample in the flow cytometer. Negative control was evaluated without probe addition, and the fluorescence was discounted from the samples. Data were analyzed using the software FlowJo®

Reactive species levels were evaluated by dichloro-dihydro-fluorescein diacetate oxidation (DCF) (H2DCF-DA; Sigma Aldrich Co., St. Louis, MO, USA). Fifty microliters of each sample were incubated at 37 ºC during 30 min in the presence of the fluorescent probe DCF in a final concentration of 1 µM.

Cells were gated based on the FSC and SSC pattern of the sample cells and 20,000 events were acquired per sample in a FACScalibur flow cytometer (BD Biosciences). Negative control was evaluated without probe addition, and the fluorescence was discounted from the samples. Data were analyzed using the software FlowJo®.

Biomolecule oxidative parameters

Protein carbonyl content, a marker of protein oxidative damage, was assayed by a method based on the reaction of protein carbonyls with dinitrophenylhydrazine forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm (Reznick and Packer 1994). Briefly, 1 mg of sample

protein was treated with 20% trichloroacetic acid and centrifuged at 4000 x g, 4 ºC for 5 min. The pellet was dissolved in 0.2 M NaOH and was added to 10 mM dinitrophenylhydrazine (prepared in 2 M HCl). This was kept in the dark during 1 h, and vortexed each 15 min. Samples were added of 20% thiobarbituric acid), and centrifuged at 20.000 x g, 4 ºC for 5 min. The pellet was washed three times with ethanol:ethyl acetate (1:1, v/v). The supernatant was discarded and the pellet was resuspended in 8 M urea pH 2.3. The sample was vortexed and incubated at 60 ºC for 15 min. After that, it was centrifuged at 20.000 x g for 3 min and the absorbance was measured at 370 nm. Protein carbonyl content was expressed as nmol/mg protein.

Antioxidant enzymes activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was evaluated by quantifying the inhibition superoxide-dependent autoxidation of epinephrine, verifying the absorbance of the samples at 480 nm (Misra and Fridovich 1972). The SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit. The data were expressed as units/mg protein.

Catalase (CAT, EC 1.11.1.6) activity was assayed according to Aebi (1984) by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100 and 10 mM potassium phosphate buffer, pH 7.0. One CAT unit is defined as 1 µmol of hydrogen peroxide consumed per minute and the specific activity is reported as units/mg protein.

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was measured according to the method described by Wendel (1981) using *tert*-butyl hydroperoxide as a substrate. NADPH disappearance was monitored spectrophotometrically at 340 nm in a medium containing 2 mM reduced glutathione (GSH), 0.15 U/mL glutathione reductase (GR, EC 1.8.1.7), 0.4 mM azide, 0.5 mM *tert*-butyl hydroperoxide and 0.1 mM NADPH. One GPx unit is defined as 1 µmol of NADPH consumed per minute and the specific activity is represented as units/mg protein.

Non-enzymatic antioxidant

The content of reduced glutathione (GSH) was measured according to the method described by Browne and Armstrong (1998). Initially, the proteins were precipitated with meta-phosphoric acid (1:1, v:v), after centrifugation (5.000 x g at 25 ºC/ 10 min), 40 µL of supernatant was incubated with 15 µL of 7.5 mM o-phthaldialdehyde and 185 µL of 120 mM sodium phosphate buffer pH 8.0, containing 5 mM ethylene diaminetetraacetic acid (EDTA), at room temperature during 15 min. GSH reacts with the fluorophore o-phthaldialdehyde. Fluorescence was measured using excitation and emission wavelengths of 350 nm and 420 nm, respectively. A blank sample and a standard curve of GSH (0.001–1 mM) were performed in parallel. The data are expressed as nmol/mg protein.

Protein concentration assay

Protein concentration was measured by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Serum glucose, total cholesterol, triglyceride, aspartate *aminotransferase,* **alanine** *aminotransferase, and lactate dehydrogenase* **measurements**

Serum glucose, total cholesterol (CL), and triglyceride (TGL) concentrations, aspartate *aminotransferase (AST),* alanine *aminotransferase (ALT), and lactate dehydrogenase* (LDH) activities were measured using commercially available kits (*Labtest* Diagnóstica S.A., Lagoa Santa, *Brazil)*.

Statistical analysis

GraphPad Prism 6.0 software was used for data analysis. Normal distribution was assessed by the Kolmogorov and Smirnov or Shapiro-Wilk method when the number of data was sufficient, and calculated by excel when it was not sufficient. Parameters with normal distribution were analyzed using one-way or two-way ANOVA followed by Tukey's post-test. Parameters with non-normal distribution were analyzed by the Kruskal-Wallis test with Dunn's post-hoc test. All data were expressed as mean±S.E.M. Statistical significance was designated at $p<0.05$.

Results

Maternal exercise did not alter the gestational and fetal outcome

Gestational outcomes of dams exposed to exercise with or without overload were found unchanged (Table 1). Dams pregnancy rate [F(2,69)=0.7769;p=0.4638], and weight gain were not altered [F(2,41)=0.4036;p=0.6705], as well as litter size (H(97) = 4.574, p=0.1016).

Sex ratio was not influenced by maternal interventions [F(2,40)=1.380;p=0.2633]. Male pups did not differ on PD1 [F(2,21)=1.700;p=0.2069] and PD8 weight [F(2,19)=0.3607;p=0.7019], as well as female pups on PD1 [F(2,21)=1.421;p=0.2638] and PD8 also [F(2,17)=0.4354;p=0.6541].

Table 1. Effect of maternal interventions on gestational and litter's parameters

	Sedentary	Exercise	Overload	p value
Pregnancy rate (%)	83.30±0.0	75.00±0.0	70.80±12.5	0.4638
Weight gain during pregnancy (%)	28.65±2.07	30.91±1.72	30.53±2.12	0.6705
Litter size (number of pups)	10 (5-13)	9 (5-12)	9 (1-13)	0.1016
Sex ratio	0.43±0.03	0.34±0.06	0.43±0.03	0.2633
Male weight on PD1 (g)	6.87±0.27	7.33±0.15	7.25±0.10	0.2069
Female weight on PD1 (g)	6.55±0.32	7.06±0.22	7.03±0.12	0.2638
Male weight on PD8 (g)	18.41±0.68	18.99±0.48	18.31±0.56	0.7019
Female weight on PD8 (g)	17.51±0.80	18.47±0.88	17.97±0.42	0.6541

Data was expressed as mean ± S.E.M. (one-way ANOVA with Tukey post-test) or median (min-max) (Kruskall-Wallis with Dunn's post-test) for n=7-16.

Maternal swimming exercise did not alter offspring weight gain from weaning to 60 days of age

Maternal exercise did not alter the weight gain of male pups from weaning to 60 days of life [$F(10,247)=0.3770;p=0.9558$], as demonstrated in figure 2. The percentage of mesenteric [$F(2,21)=2.949;p=0.0743$] and retroperitoneal fat [$F(2,20)=1.072;p=0.3612$] on PD60 was not altered by gestational exercise (data not shown).

High-fat diet increased the offspring caloric efficiency regardless of maternal intervention

From 60 to 90 days of age, the male offspring were exposed to an HFD, and the consumption profile can be seen in figure 3. The food intake (Fig. 3A), calculated as food intake per 100 g of animal, was decreased by HFD throughout the diet period [$F(3,129)=47.16;p<0.0001$].

Regarding caloric consumption (Fig. 3B), there was a difference between the weeks [$F(3,129)=41.55;p<0.0001$], however, the post-test did not find any significance related to the SN group in each week isolated. As expected, the caloric efficiency, that shows how much calories ingested were converted to grams of body weight, was increased in the HFD groups [$F(5,130)=31.89;p<0.0001$], demonstrating significant difference on PD67 in the EH and OH groups, and on PD81 in the SH, EH and OH groups (Fig. 3C).

Overloaded maternal exercise negatively affects diet-induced weight gain in the offspring

The weight gain during 30 days of HFD is demonstrated in Figure 4. The offspring exposed to overloaded maternal exercise present increased body weight on 81 and 90 days of age when compared to the control group [F(5,430)=19.07;p<0.0001]. The average body weight on PD90 in the groups was 402.7 g in the SN, 381.7 g in the EN, 399.8 g in the ON, 429.6 g in the SH, 418.3 g in the EH, and 443.1 g in the OH group.

Despite weight gain is not statistically significant in all the HFD groups, the body fat was drastically affected independent of maternal exercise, as demonstrated in Figure 5. Mesenteric fat was almost doubled after thirty days of HFD [F(1,84)=147.6;p<0.0001], as well as retroperitoneal fat [F(1,86)=126.5;p<0.0001], as shown in Figure 5A and 5B, respectively. The adrenal weight, an indirect method of assessing serum corticosterone levels (Akana et al. 1983, Ulrich-Lai et al. 2006), was not altered [F(2,86)=0.3956;p=0.6745] (data not shown).

Maternal exercise prevents diet-induced increased glycaemia in the offspring

Table 2 shows the effect of maternal treatments and HFD on serum markers at male offspring on PD90. Glucose levels were increased by HFD on maternal sedentarism or overloaded exercise [F(1,37)=53.33;p<0.0001], while maternal exercise prevents this effect. Total cholesterol [F(1,42)=6.570;p=0.0140], triglycerides levels [F(1,39)=11.80;p=0.0014], ALT activity [F(1,41)=5.257;p=0.0271], and LDH levels [F(1,42)=9.278;p=0.0040] were affected by the diet, however, the post-test did not find any significance. AST activity was not affected [F(1,42)=0.2679;p=0.6075].

Table 2. Effect of maternal treatments and postnatal high-fat diet on offspring' serum parameters

	SN	EN	ON	SH	EH	OH	p value
Glucose (mg/dL)	94.5±2.9	90.6±3.3	93.4±3.8	114.2±3.6***	106±3.4	115.3±1.8***	<0.001
CL (mg/dL)	105.9 ±2.1	106.7±2.1	106.1±2.0	108.7±2.0	108.3±1.7	113.5±1.2	0.0140
TGL (mg/dL)	147.5±3.3	152.8±5.8	148.5±4.9	168.3±4.1	165.4±7.4	170±9.5	0.0014
AST activity	48.2±3.1	41±4.8	47.2±4.7	47.4±5.6	47±5.2	48±4.5	0.6075
ALT activity	17.5±0.7	18.3±1.3	23±1.3	17.1±3.4	14.7±0.8	17.4±1.8	0.0271
LDH (U/L)	383.7±27.3	385.2±47.9	406.3±31.4	301±29.0	343.8±35.7	283.8±20.4	0.0040

Data was expressed as mean ± S.E.M. (two-way ANOVA with Tukey post-test) for n=8. ***p<0.001, different from the SN group.

Offspring hippocampus present altered redox homeostasis in response to maternal and postnatal interventions

In the offspring's hippocampus, the DCF oxidation was not significantly altered (H(44) = 3.316, p=0.6514), presenting the following median (min-max) values: SN= 103.4 (75.8-121.6), EN= 104.5 (77.4-128.4), ON= 98.6 (70.7-110.1), SH= 112.5 (80.4-210), EH= 105.1 (80.6-131.0), and OH= 101.9 (58.4-155.8 (Fig. 6A). Mitochondrial superoxide was increased by HFD consumption [F(1,38)=16.43;p=0.0002] (Fig. 6B), an effect that was reduced by maternal overloaded exercise and inhibited by the unloaded maternal exercise. Mitochondrial function, evaluated by Mitotracker Green and Red, was not altered [F(2,38)=0.3740;p=0.6905] (data not shown).

The effect of maternal and postnatal interventions on hippocampal antioxidant defense was demonstrated in Fig. 7. SOD activity (Fig. 7A) was increased by maternal overloaded exercise and HFD when isolated [F(2,37)=8.332;p=0.0010], while CAT [F(2,42)=5.569;p=0.0072] and GPx activities [F(2,39)=9.016;p=0.0006] were increased by both types of maternal exercise, as showed on Figure 7B and 7C, respectively. GSH content (Fig. 7D) was increased in EN, ON, SH, and EH groups [F(2,45)=6.525;p=0.0032].

Maternal and postnatal interventions did not alter protein oxidation, evaluated through carbonyl level in the hippocampus [F(2,39)=1.644;p=0.2064] (data not shown).

Discussion

This study evaluated the effect of free or loaded maternal swimming exercise on parameters of redox homeostasis in the hippocampus of high-fat diet-feed adult offspring. Weight gain, body composition, food intake and serum profile were also evaluated.

The maternal exercise practice did not impair the gestational and birth parameters, as already demonstrated in humans (Clapp 1996, Mudd et al. 2012) and other animal models (Marcelino et al. 2013, Raipuria et al. 2015, Ribeiro et al. 2017, August et al. 2018). The male young adult pups showed no difference in body weight or fat, regardless of maternal exercise practice, on 60 days of age. Although maternal

treadmill or running wheel exercise has already been shown to cause a reduction in the rat offspring body weight and fat during the life (Carter et al. 2012, Stanford et al. 2015), swimming exercise with or without overload seems to not present the same pattern (Wasinski et al. 2015, Wasinski et al. 2016, August et al. 2018, Klein et al. 2018).

When the offspring were exposed to the HFD, the food intake was decreased and caloric efficiency was increased, as expected. After the first few days of the diet, the animals tend to adjust their intake in response to higher dietary energy density (Hariri and Thibault 2010), and this effect was already demonstrated in several studies (Boitard et al. 2012, Maciejczyk et al. 2018, Wu et al. 2018). No significant effect of HFD on animal weight gain was found. In the animal model, the HFD already demonstrates increase weight gain from 3 days (Nakandakari et al. 2019) to several weeks of consumption (Boitard et al. 2012, Maciejczyk et al. 2018), but the absence of weight gain after 4 weeks was also already shown (Della Vedova et al. 2016).

Moreover, it has been demonstrated that body composition alterations may be found before any weight alterations (Andrich et al. 2018). Despite little change in weight gain, our data demonstrated that body fat was drastically affected, with a significant increase in retroperitoneal and mesenteric fat, as shown elsewhere (Morrison et al. 2010, Tomiga et al. 2017). When exposed to the HFD the male offspring of overloaded maternal exercise presented a higher body weight at 81 and 90 days of age. Both intensities of maternal exercise could not prevent alterations in body fat. Despite maternal treadmill (Quiclet et al. 2017, Ribeiro et al. 2017) or running wheel exercise (Sheldon et al. 2016) during pregnancy could prevent the increased body fat on offspring exposed to overfed models, swimming exercise did not prevent those effects on offspring exposed to overfed during lactation (August et al. 2018). Also, overloaded maternal swimming exercise with 2 weeks of adaptation before pregnancy, 60 minutes of exercise a day and load of 3% of BW decreased the diet-induced body fat in the offspring [40].

HFD consumption during 4 weeks cause increased serum glucose levels, as already demonstrated in other studies using the HFD model for 3 days (Nakandakari

et al. 2019) or several weeks (Morrison et al. 2010, Liu et al. 2014, Maciejczyk et al. 2018). Maternal exercise caused an intensity-dependent effect on glucose levels in offspring exposed to diet; while unload swimming could prevent it, overloaded exercise had no positive effect. The absence of an overload exercise effect on the offspring exposed to the HFD was also demonstrated by Wasinski et al. (2015), whilst maternal unload swimming could decrease offspring glucose levels at weaning (August et al. 2018). Moreover, maternal exercise using a treadmill or running wheel was already shown to ameliorate rat offspring glucose metabolism (Stanford et al. 2015, Quiclet et al. 2016).

The hippocampal redox homeostasis was highly affected by HFD, which cause increased superoxide levels, SOD activity, and GSH content, without alterations in protein oxidative damage or mitochondrial function. As the diet was kept for a short time, we believe that there was an onset of imbalance in redox homeostasis, with increased oxidant levels that promote the activation of the antioxidant response, avoiding significant oxidative damage to hippocampal tissue (Lushchak 2014). Alterations on hippocampal redox parameters by HFD were already described on different rodent models and periods of consumption, generally demonstrating an increase in the oxidant parameters, and reduced antioxidant defenses, when consumed for longer periods, usually between 7 and 16 weeks (Morrison et al. 2010, Hajiluian et al. 2018, Kaur et al. 2018, Si et al. 2019).

Maternal exercise could cause alterations on the redox homeostasis with or without the HFD consumption. In our experimental conditions, both intensities of swimming during pregnancy improved the antioxidant network in the adult male hippocampus, through increased CAT and GPx activities, as well as GSH content. We also observed increased SOD activity as a result of maternal overload exercise, without alterations on oxidative and damage parameters, as well as mitochondrial function. In response to HFD, maternal exercise also caused type-dependent effects in the offspring hippocampus. Unload swimming during pregnancy fully prevented the diet-induced superoxide increase, while exercise with overload only partially prevented the increase in mitochondrial superoxide levels.

Exercise positively impacts brain function (Voss et al. 2013, Alkadhi 2018) and its effect during pregnancy in the offspring's brain metabolic programming seems to act similarly. Maternal swimming exercise was already demonstrated to influence offspring brain redox homeostasis from the neonatal period (Marcelino et al. 2013) to weaning (Marcelino et al. 2015, August et al. 2018), and now we demonstrated that it also occurs in adulthood. Moreover, when applied the same maternal unloaded swimming model, the mitochondrial function is shown to be improved in offspring's hippocampus on 74 days of life (Klein et al. 2019), however, we did not find the same effect on 3 months old animals.

Despite the positive effect of maternal exercise in the offspring brain redox homeostasis is not well understood, it is hypothesized that it can occur through activation of important regulators. It has been demonstrated that PGC1α and BDNF levels are increased in the offspring's hippocampus with 8 weeks of life, when prenatally exposed to maternal exercise (Venezia et al. 2015, Gomes da Silva et al. 2016). These positive effects on redox homeostasis, coupled with greater neurogenesis (Dayi et al. 2012, Gomes da Silva et al. 2016) and neuronal activation (Robinson and Bucci 2014) can bring benefits to the cognitive function of the offspring, since a physical active pregnancy has already demonstrated to improve learning and memory in animal models (Kim et al. 2007, Akhavan et al. 2008, Dayi et al. 2012, Robinson and Bucci 2014, Gomes da Silva et al. 2016) and improve some parameters in the rat brain when exposed to prenatal stress (Bustamante et al. 2013), hypoxia-ischemia (Akhavan et al. 2012, Marcelino et al. 2015), and Alzheimer disease model (Herring et al. 2012, Klein et al. 2019).

Although maternal exercise did not prevent the body fat and weight gain induced by HFD in adult offspring, the diet-induced effects on glycemia and hippocampal redox homeostasis were partially prevented and the practice of swimming exercise without overload seems to be more beneficial, considering our data. Also, whether preclinical studies of maternal physical exercise during pregnancy are a promising strategy in the prevention and treatment of chronic non-transmissible diseases, further studies are still needed in order to define the type and

intensity of maternal exercise necessary to induce only beneficial effects to mother and offspring.

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Conflict of interest

The authors declare no conflicts of interest or competing interest.

References

Aebi, H. (1984). "Catalase in vitro." Methods Enzymol **105**: 121-126.

Akana, S. F., J. Shinsako and M. F. Dallman (1983). "Relationships among adrenal weight, corticosterone, and stimulated adrenocorticotropin levels in rats." Endocrinology **113**(6): 2226-2231.

Akhavan, M. M., M. Emami-Abarghoie, M. Safari, B. Sadighi-Moghaddam, A. A. Vafaei, A. R. Bandegi and A. Rashidy-Pour (2008). "Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups." Neuroscience **151**(4): 1173-1183.

Akhavan, M. M., T. Foroutan, M. Safari, B. Sadighi-Moghaddam, M. Emami-Abarghoie and A. Rashidy-Pour (2012). "Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring." Pak J Pharm Sci **25**(1): 233-238.

Alkadhi, K. A. (2018). "Exercise as a Positive Modulator of Brain Function." Mol Neurobiol **55**(4): 3112-3130.

Andrich, D. E., L. Melbouci, Y. Ou, J. P. Leduc-Gaudet, F. Chabot, F. Lalonde, F. S. Lira, B. D. Gaylinn, et al. (2018). "Altered Feeding Behaviors and Adiposity Precede Observable Weight Gain in Young Rats Submitted to a Short-Term High-Fat Diet." J Nutr Metab **2018**: 1498150.

August, P. M., R. M. Maurmann, A. B. Saccomori, M. C. Scortegagna, E. B. Flores, C. P. Klein, B. G. Dos Santos, V. Stone, et al. (2018). "Effect of maternal antioxidant supplementation and/or exercise practice during pregnancy on postnatal overnutrition induced by litter size reduction: Brain redox homeostasis at weaning." Int J Dev Neurosci **71**: 146-155.

Barella, L. F., J. C. de Oliveira, R. C. Branco, R. L. Camargo, R. M. Gomes, F. C. Mendes, R. A. Miranda, C. Gravena, et al. (2012). "Early exposure to a high-fat diet has more drastic consequences on metabolism compared with exposure during adulthood in rats." Horm Metab Res **44**(6): 458-464.

Barha, C. K., L. A. Galea, L. S. Nagamatsu, K. I. Erickson and T. Liu-Ambrose (2017). "Personalising exercise recommendations for brain health: considerations and future directions." Br J Sports Med **51**(8): 636-639.

Boitard, C., N. Etchamendy, J. Sauvant, A. Aubert, S. Tronel, A. Marighetto, S. Laye and G. Ferreira (2012). "Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice." Hippocampus **22**(11): 2095-2100.

Browne, R. W. and D. Armstrong (1998). "Reduced glutathione and glutathione disulfide." Methods Mol Biol **108**: 347-352.

Bustamante, C., R. Henriquez, F. Medina, C. Reinoso, R. Vargas and R. Pascual (2013). "Maternal exercise during pregnancy ameliorates the postnatal neuronal impairments induced by prenatal restraint stress in mice." Int J Dev Neurosci **31**(4): 267-273.

Carter, L. G., K. N. Lewis, D. C. Wilkerson, C. M. Tobia, S. Y. Ngo Tenlep, P. Shridas, M. L. Garcia-Cazarin, G. Wolff, et al. (2012). "Perinatal exercise improves glucose homeostasis in adult offspring." Am J Physiol Endocrinol Metab **303**(8): E1061-1068.

Chinkin, A. S. (2013). "THE EFFECTS OF VARIOUS SWIMMING TRAINING PROTOCOLS ON CARDIAC CAPACITY AND VENTRICULAR FIBRILLATION THRESHOLD IN RATS." Central European Journal of Sport Sciences and Medicine **2**: 9-14.

Clapp, J. F., 3rd (1996). "Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy." J Pediatr **129**(6): 856-863.

Clapp, J. F., 3rd, B. Lopez and R. Harcar-Sevcik (1999). "Neonatal behavioral profile of the offspring of women who continued to exercise regularly throughout pregnancy." Am J Obstet Gynecol **180**(1 Pt 1): 91-94.

Collaborators, G. B. D. C. o. D. (2017). "Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016." Lancet **390**(10100): 1151-1210.
Crimmins, E. M. (2015). "Lifespan and Healthspan: Past, Present, and Promise." Gerontologist **55**(6): 901-911.
Dahly, D. L., X. Li, H. A. Smith, A. S. Khashan, D. M. Murray, M. E. Kiely, O. B. H. J, F. P. McCarthy, et al. (2018). "Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study." Int J Epidemiol **47**(1): 131-145.
Dayi, A., S. Agilkaya, S. Ozbal, F. Cetin, I. Aksu, C. Gencoglu, S. Cingoz, C. Pekcetin, et al. (2012). "Maternal aerobic exercise during pregnancy can increase spatial learning by affecting leptin expression on offspring's early and late period in life depending on gender." ScientificWorldJournal **2012**: 429803.
De Lorenzo, A., S. Gratteri, P. Gualtieri, A. Cammarano, P. Bertucci and L. Di Renzo (2019). "Why primary obesity is a disease?" J Transl Med **17**(1): 169.
de Mello, A. H., A. B. Costa, J. D. G. Engel and G. T. Rezin (2018). "Mitochondrial dysfunction in obesity." Life Sci **192**: 26-32.
Della Vedova, M. C., M. D. Munoz, L. D. Santillan, M. G. Plateo-Pignatari, M. J. Germano, M. E. Rinaldi Tosi, S. Garcia, N. N. Gomez, et al. (2016). "A Mouse Model of Diet-Induced Obesity Resembling Most Features of Human Metabolic Syndrome." Nutr Metab Insights **9**: 93-102.
Gaesser, G. A. (2007). "Exercise for prevention and treatment of cardiovascular disease, type 2 diabetes, and metabolic syndrome." Curr Diab Rep **7**(1): 14-19.
Garcia-Carrizo, F., T. Priego, N. Szostaczuk, A. Palou and C. Picó (2017). "Sexual Dimorphism in the Age-Induced Insulin Resistance, Liver Steatosis, and Adipose Tissue Function in Rats." Front Physiol **8**: 445.
Garnaes, K. K., S. Morkved, O. Salvesen and T. Moholdt (2016). "Exercise Training and Weight Gain in Obese Pregnant Women: A Randomized Controlled Trial (ETIP Trial)." PLoS Med **13**(7): e1002079.
Gomes da Silva, S., A. A. de Almeida, J. Fernandes, G. M. Lopim, F. R. Cabral, D. A. Scerni, A. V. de Oliveira-Pinto, R. Lent, et al. (2016). "Maternal Exercise during Pregnancy Increases BDNF Levels and Cell Numbers in the Hippocampal Formation but Not in the Cerebral Cortex of Adult Rat Offspring." PLoS One **11**(1): e0147200.
Hajiluian, G., M. Abbasalizad Farhangi, G. Nameni, P. Shahabi and M. Megari-Abbasi (2018). "Oxidative stress-induced cognitive impairment in obesity can be reversed by vitamin D administration in rats." Nutr Neurosci **21**(10): 744-752.
Hamer, M. and Y. Chida (2009). "Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence." Psychol Med **39**(1): 3-11.
Hanson, M. A. (2016). "Background to the Cape Town Manifesto: harnessing the power of the normal." J Dev Orig Health Dis **7**(5): 498-500.
Hariri, N. and L. Thibault (2010). "High-fat diet-induced obesity in animal models." Nutr Res Rev **23**(2): 270-299.
Herring, A., A. Donath, M. Yarmolenko, E. Uslar, C. Conzen, D. Kanakis, C. Bosma, K. Worm, et al. (2012). "Exercise during pregnancy mitigates Alzheimer-like pathology in mouse offspring." FASEB J **26**(1): 117-128.
Kanoski, S. E. and H. J. Grill (2017). "Hippocampus Contributions to Food Intake Control: Mnemonic, Neuroanatomical, and Endocrine Mechanisms." Biol Psychiatry **81**(9): 748-756.
Kaur, J., R. K. Sodhi, J. Madan, S. K. Chahal and R. Kumar (2018). "Forskolin convalesces memory in high fat diet-induced dementia in wistar rats-Plausible role of pregnane x receptors." Pharmacol Rep **70**(1): 161-171.

Kawanishi, N., H. Yano, Y. Yokogawa and K. Suzuki (2010). "Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice." Exerc Immunol Rev **16**: 105-118.
Kim, H., S. H. Lee, S. S. Kim, J. H. Yoo and C. J. Kim (2007). "The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups." Int J Dev Neurosci **25**(4): 243-249.
Klein, C. P., K. Dos Santos Rodrigues, R. M. Hozer, N. de Sa Couto-Pereira, A. B. Saccomori, B. M. Dal Magro, M. S. Crestani, J. B. Hoppe, et al. (2018). "Swimming exercise before and during pregnancy: Promising preventive approach to impact offspring s health." Int J Dev Neurosci **71**: 83-93.
Klein, C. P., J. B. Hoppe, A. B. Saccomori, B. G. Dos Santos, J. P. Sagini, M. S. Crestani, P. M. August, R. M. Hozer, et al. (2019). "Physical Exercise During Pregnancy Prevents Cognitive Impairment Induced by Amyloid-beta in Adult Offspring Rats." Mol Neurobiol **56**(3): 2022-2038.
Kolahdouzi, S., E. Talebi-Garakani, G. Hamidian and A. Safarzade (2019). "Exercise training prevents high-fat diet-induced adipose tissue remodeling by promoting capillary density and macrophage polarization." Life Sci **220**: 32-43.
Kruk, J. (2007). "Physical activity in the prevention of the most frequent chronic diseases: an analysis of the recent evidence." Asian Pac J Cancer Prev **8**(3): 325-338.
Labonte-Lemoyne, E., D. Curnier and D. Ellemberg (2017). "Exercise during pregnancy enhances cerebral maturation in the newborn: A randomized controlled trial." J Clin Exp Neuropsychol **39**(4): 347-354.
Lee, H. H., H. Kim, J. W. Lee, Y. S. Kim, H. Y. Yang, H. K. Chang, T. H. Lee, M. C. Shin, et al. (2006). "Maternal swimming during pregnancy enhances short-term memory and neurogenesis in the hippocampus of rat pups." Brain Dev **28**(3): 147-154.
Liu, Y., X. Fu, N. Lan, S. Li, J. Zhang, S. Wang, C. Li, Y. Shang, et al. (2014). "Luteolin protects against high fat diet-induced cognitive deficits in obesity mice." Behav Brain Res **267**: 178-188.
Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). "Protein measurement with the Folin phenol reagent." J Biol Chem **193**(1): 265-275.
Lushchak, V. I. (2014). "Classification of oxidative stress based on its intensity." EXCLI J **13**: 922-937.
Maciejczyk, M., E. Zebrowska, A. Zalewska and A. Chabowski (2018). "Redox Balance, Antioxidant Defense, and Oxidative Damage in the Hypothalamus and Cerebral Cortex of Rats with High Fat Diet-Induced Insulin Resistance." Oxid Med Cell Longev **2018**: 6940515.
Magro-Malosso, E. R., G. Saccone, D. Di Mascio, M. Di Tommaso and V. Berghella (2017). "Exercise during pregnancy and risk of preterm birth in overweight and obese women: a systematic review and meta-analysis of randomized controlled trials." Acta Obstet Gynecol Scand **96**(3): 263-273.
Marcelino, T. B., P. I. de Lemos Rodrigues, P. M. Miguel, C. A. Netto, L. O. Pereira Silva and C. Matte (2015). "Effect of maternal exercise on biochemical parameters in rats submitted to neonatal hypoxia-ischemia." Brain Res **1622**: 91-101.
Marcelino, T. B., A. Longoni, K. Y. Kudo, V. Stone, A. Rech, A. M. de Assis, E. B. Scherer, M. J. da Cunha, et al. (2013). "Evidences that maternal swimming exercise improves antioxidant defenses and induces mitochondrial biogenesis in the brain of young Wistar rats." Neuroscience **246**: 28-39.
Misra, H. P. and I. Fridovich (1972). "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase." J Biol Chem **247**(10): 3170-3175.
Morrison, C. D., P. J. Pistell, D. K. Ingram, W. D. Johnson, Y. Liu, S. O. Fernandez-Kim, C. L. White, M. N. Purpera, et al. (2010). "High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling." J Neurochem **114**(6): 1581-1589.

Mudd, L. M., J. Pivarnik, C. B. Holzman, N. Paneth, K. Pfeiffer and H. Chung (2012). "Leisure-time physical activity in pregnancy and the birth weight distribution: where is the effect?" J Phys Act Health **9**(8): 1168-1177.
Nakandakari, S., V. R. Munoz, G. K. Kuga, R. C. Gaspar, M. R. Sant'Ana, I. C. B. Pavan, L. G. S. da Silva, A. P. Morelli, et al. (2019). "Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice." Brain Behav Immun **79**: 284-293.
Ng, M., T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, E. C. Mullany, S. Biryukov, et al. (2014). "Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013." Lancet **384**(9945): 766-781.
O'Brien, P. D., L. M. Hinder, B. C. Callaghan and E. L. Feldman (2017). "Neurological consequences of obesity." Lancet Neurol **16**(6): 465-477.
Opitz, B. (2014). "Memory function and the hippocampus." Front Neurol Neurosci **34**: 51-59.
Organization, W. H. (2018). Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016.
Park, H. S., S. S. Park, C. J. Kim, M. S. Shin and T. W. Kim (2019). "Exercise Alleviates Cognitive Functions by Enhancing Hippocampal Insulin Signaling and Neuroplasticity in High-Fat Diet-Induced Obesity." Nutrients **11**(7).
Popkin, B. M., L. S. Adair and S. W. Ng (2012). "Global nutrition transition and the pandemic of obesity in developing countries." Nutr Rev **70**(1): 3-21.
Quiclet, C., H. Dubouchaud, P. Berthon, H. Sanchez, G. Vial, F. Siti, E. Fontaine, C. Batandier, et al. (2017). "Maternal exercise modifies body composition and energy substrates handling in male offspring fed a high-fat/high-sucrose diet." J Physiol **595**(23): 7049-7062.
Quiclet, C., F. Siti, H. Dubouchaud, G. Vial, P. Berthon, E. Fontaine, C. Batandier and K. Couturier (2016). "Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function." Am J Physiol Endocrinol Metab **311**(2): E508-518.
Raipuria, M., H. Bahari and M. J. Morris (2015). "Effects of maternal diet and exercise during pregnancy on glucose metabolism in skeletal muscle and fat of weanling rats." PLoS One **10**(4): e0120980.
Reznick, A. Z. and L. Packer (1994). "Oxidative damage to proteins: spectrophotometric method for carbonyl assay." Methods Enzymol **233**: 357-363.
Ribeiro, T. A., L. P. Tofolo, I. P. Martins, A. Pavanello, J. C. de Oliveira, K. V. Prates, R. A. Miranda, C. C. da Silva Franco, et al. (2017). "Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition." Sci Rep **7**(1): 7634.
Robinson, A. M. and D. J. Bucci (2014). "Physical exercise during pregnancy improves object recognition memory in adult offspring." Neuroscience **256**: 53-60.
Salim, S. (2017). "Oxidative Stress and the Central Nervous System." J Pharmacol Exp Ther **360**(1): 201-205.
Sheldon, R. D., A. Nicole Blaize, J. A. Fletcher, K. J. Pearson, S. S. Donkin, S. C. Newcomer and R. S. Rector (2016). "Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis." J Hepatol **64**(1): 171-178.
Si, X., Y. Li, Y. Jiang, W. Shang, G. Shui, S. M. Lam, C. Blanchard, P. Strappe, et al. (2019). "gamma-Aminobutyric Acid Attenuates High-Fat Diet-Induced Cerebral Oxidative Impairment via Enhanced Synthesis of Hippocampal Sulfatides." J Agric Food Chem **67**(4): 1081-1091.
Singh, A., R. Kukreti, L. Saso and S. Kukreti (2019). "Oxidative Stress: A Key Modulator in Neurodegenerative Diseases." Molecules **24**(8).

Stanford, K. I., M. Y. Lee, K. M. Getchell, K. So, M. F. Hirshman and L. J. Goodyear (2015). "Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring." Diabetes **64**(2): 427-433.
Tan, B. L. and M. E. Norhaizan (2019). "Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function." Nutrients **11**(11).
Thaker, V. V. (2017). "Genetic and Epigenetic Causes of Obesity." Adolesc Med State Art Rev **28**(2): 379-405.
Tomiga, Y., S. Yoshimura, A. Ito, S. Nakashima, K. Kawanaka, Y. Uehara, H. Tanaka and Y. Higaki (2017). "Exercise training rescues high fat diet-induced neuronal nitric oxide synthase expression in the hippocampus and cerebral cortex of mice." Nitric Oxide **66**: 71-77.
Ulrich-Lai, Y. M., H. F. Figueiredo, M. M. Ostrander, D. C. Choi, W. C. Engeland and J. P. Herman (2006). "Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner." Am J Physiol Endocrinol Metab **291**(5): E965-973.
Venezia, A. C., L. M. Guth, E. E. Spangenburg and S. M. Roth (2015). "Lifelong parental voluntary wheel running increases offspring hippocampal Pgc-1alpha mRNA expression but not mitochondrial content or Bdnf expression." Neuroreport **26**(8): 467-472.
Vina, J., F. Sanchis-Gomar, V. Martinez-Bello and M. C. Gomez-Cabrera (2012). "Exercise acts as a drug; the pharmacological benefits of exercise." Br J Pharmacol **167**(1): 1-12.
Voss, M. W., C. Vivar, A. F. Kramer and H. van Praag (2013). "Bridging animal and human models of exercise-induced brain plasticity." Trends Cogn Sci **17**(10): 525-544.
Wang, C., Y. Wei, X. Zhang, Y. Zhang, Q. Xu, Y. Sun, S. Su, L. Zhang, et al. (2017). "A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women." Am J Obstet Gynecol **216**(4): 340-351.
Warburton, D. E., C. W. Nicol and S. S. Bredin (2006). "Health benefits of physical activity: the evidence." CMAJ **174**(6): 801-809.
Wasinski, F., R. F. Bacurau, G. R. Estrela, F. Klempin, A. M. Arakaki, R. O. Batista, F. F. Mafra, L. F. do Nascimento, et al. (2015). "Exercise during pregnancy protects adult mouse offspring from diet-induced obesity." Nutr Metab (Lond) **12**: 56.
Wasinski, F., G. R. Estrela, A. M. Arakaki, M. Bader, N. Alenina, F. Klempin and R. C. Araujo (2016). "Maternal Forced Swimming Reduces Cell Proliferation in the Postnatal Dentate Gyrus of Mouse Offspring." Front Neurosci **10**: 402.
Wendel, A. (1981). "Glutathione peroxidase." Methods Enzymol **77**: 325-333.
Wu, H., Q. Liu, P. K. Kalavagunta, Q. Huang, W. Lv, X. An, H. Chen, T. Wang, et al. (2018). "Normal diet Vs High fat diet - A comparative study: Behavioral and neuroimmunological changes in adolescent male mice." Metab Brain Dis **33**(1): 177-190.
Yau, S. Y., T. H. Lee, D. A. Formolo, W. L. Lee, L. C. Li, P. M. Siu and C. C. H. Chan (2019). "Effects of Maternal Voluntary Wheel Running During Pregnancy on Adult Hippocampal Neurogenesis, Temporal Order Memory, and Depression-Like Behavior in Adult Female and Male Offspring." Front Neurosci **13**: 470.

Figure captions

Figure 1. Maternal experimental design

Figure 2. Offspring weight gain from weaning to 60 days of age. Results are expressed as mean + SEM for n=7-21. Results were analyzed by two-way ANOVA followed by Tukey's post-test.

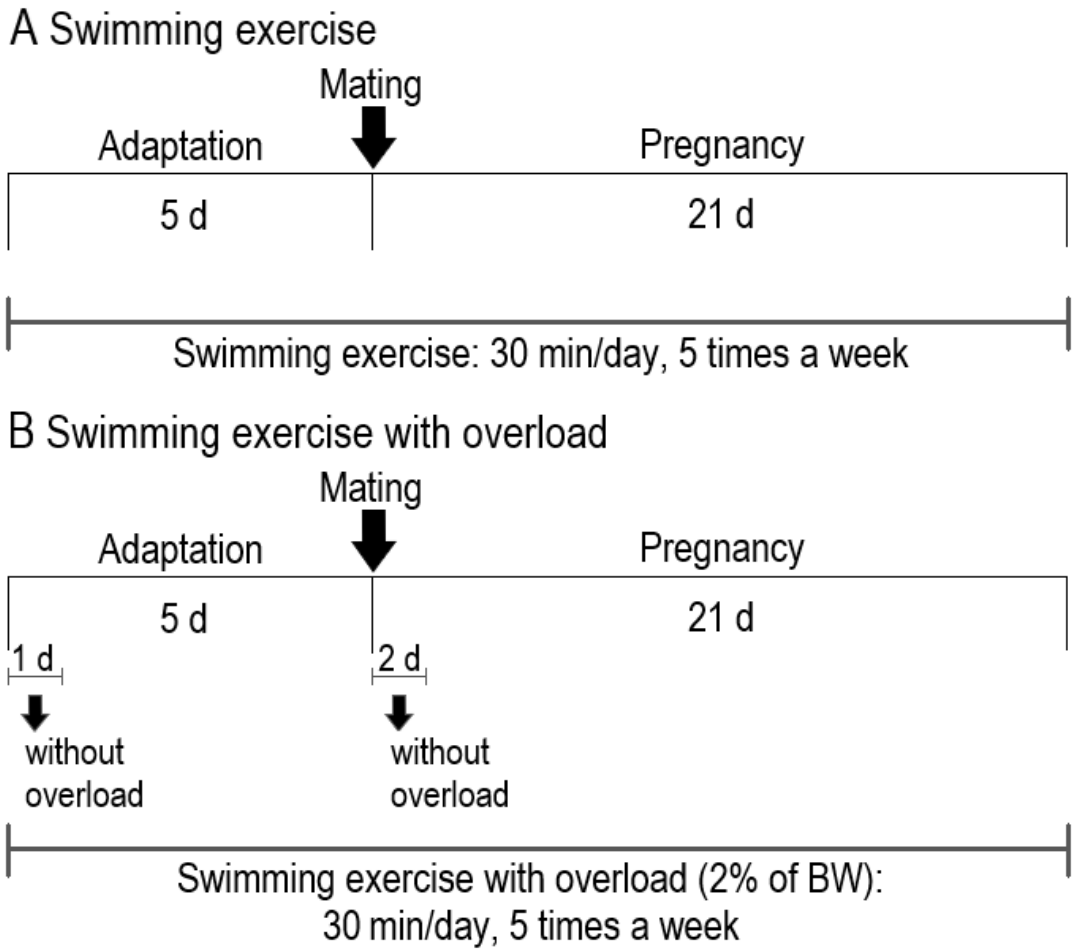
Figure 3. Offspring consumption profile for 30 days of high-fat diet. Results are expressed as mean for n=5-7. Results are expressed as mean + SEM and were analyzed by two-way ANOVA followed by Tukey's post-test. *p<0.05, **p<0.01, ***p<0.001 different from the control group (SN).

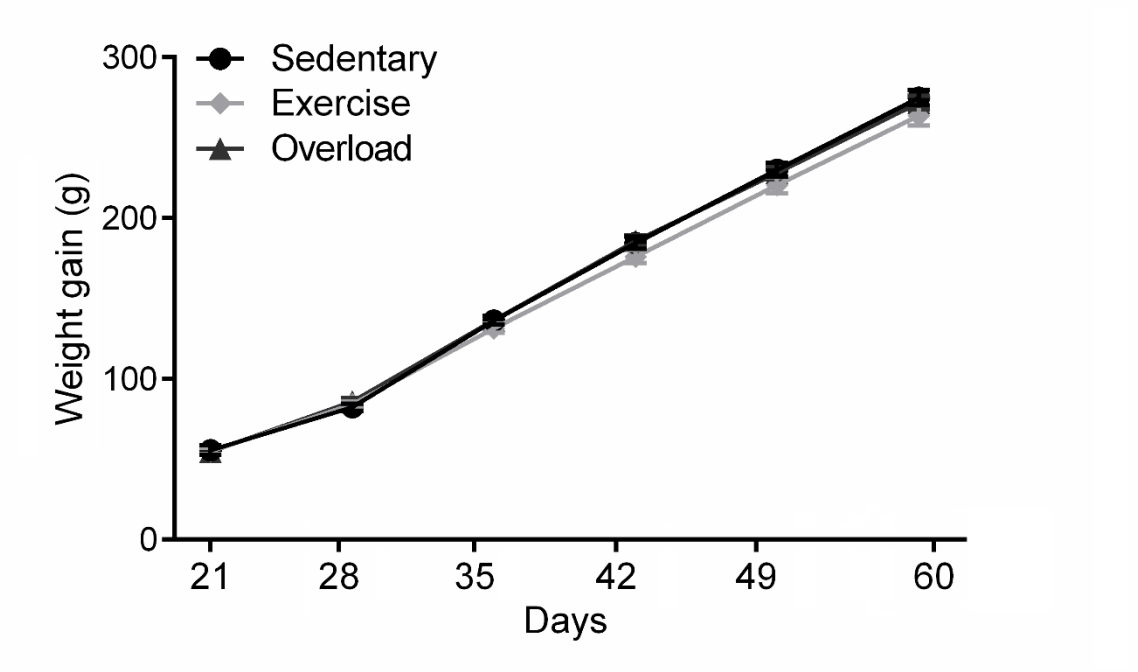
Figure 4. Offspring weight gain during 30 days of a high-fat diet. Results are expressed as mean for n=12-18. Results are expressed as mean + SEM and were analyzed by two-way ANOVA with Tukey post-test. *p<0.05, **p<0.01, different from the control group (SN).

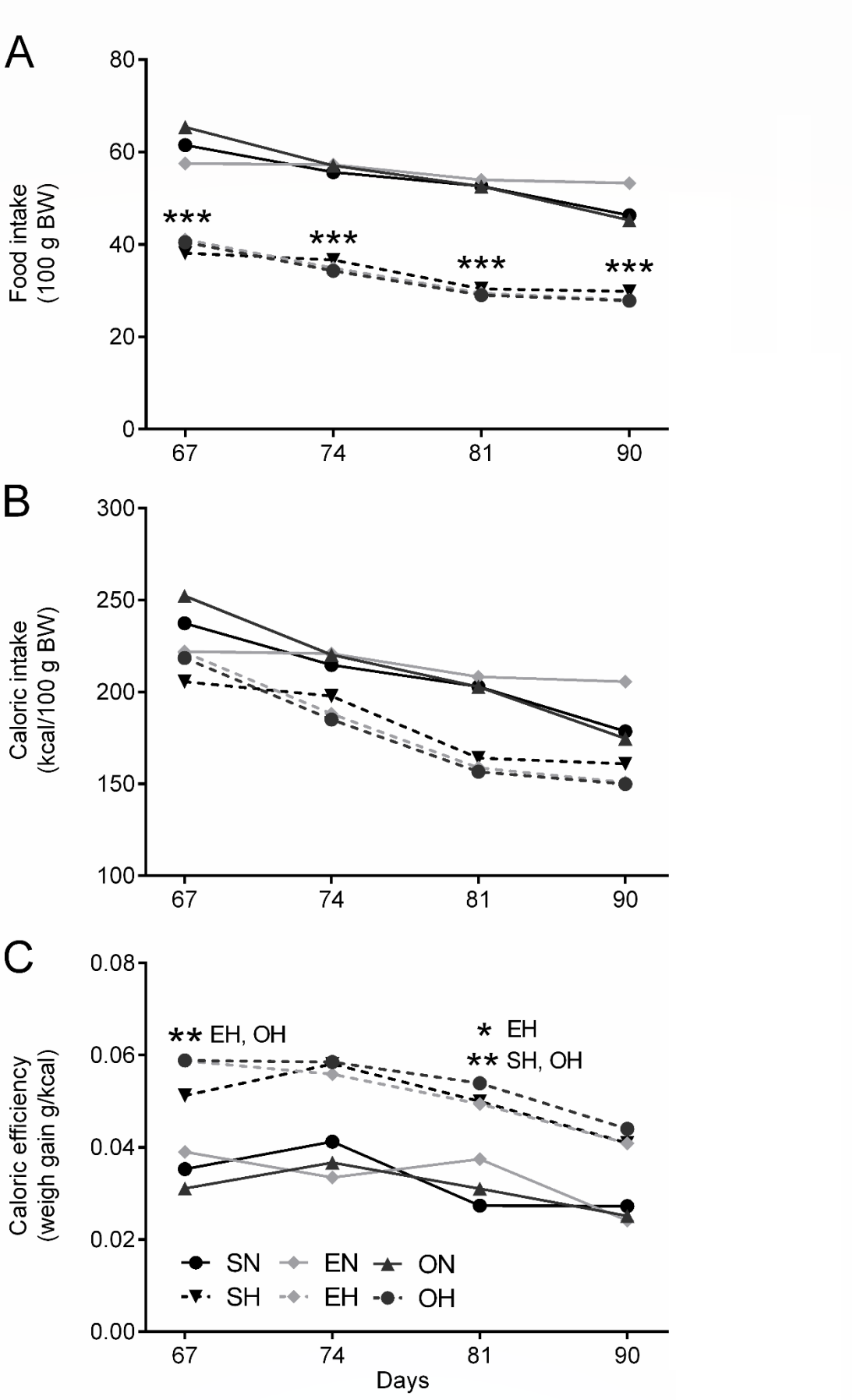
Figure 5. Offspring body fat after 30 days of a high-fat diet. Results are expressed as mean + SEM for n=12-18. Results were analyzed by two-way ANOVA with Tukey post-test. ***p<0.001, different from the control group.

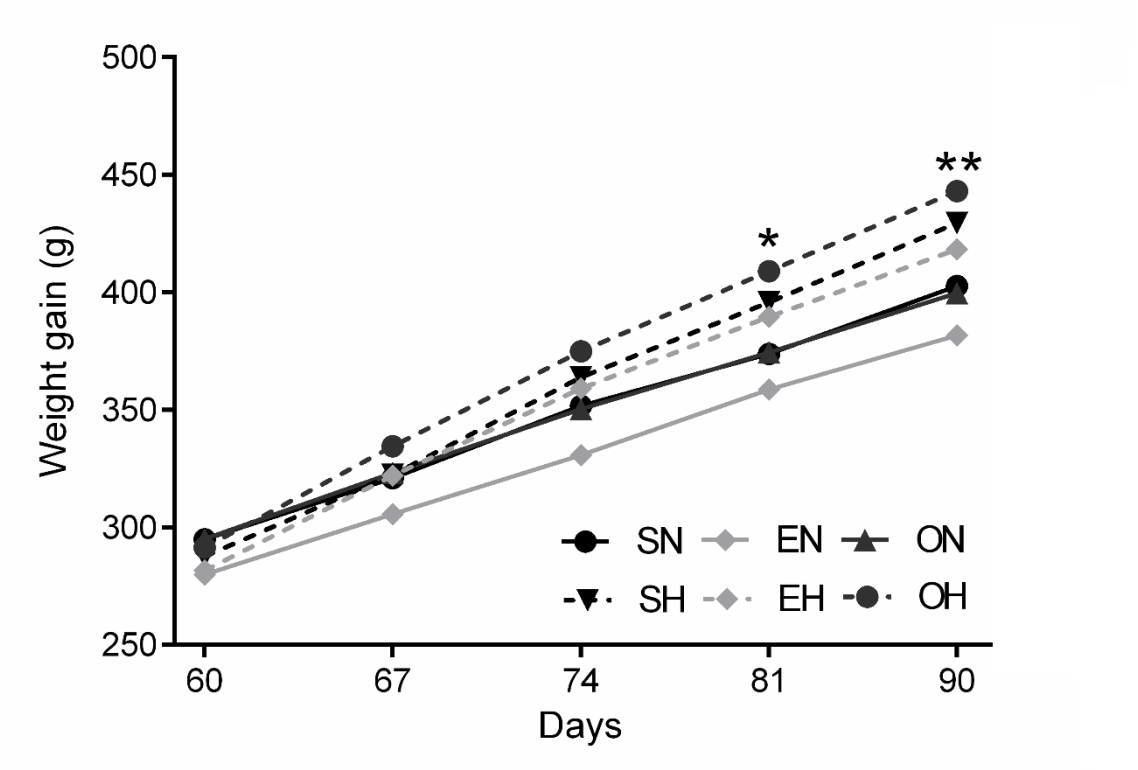
Figure 6. Effect of maternal exercise and high-fat diet in offspring hippocampus on 90 days of age. (A) DCF fluorescence, and (B) MitoSOX fluorescence. Results are expressed as mean + SEM for n=6-10. Results were analyzed by two-way ANOVA followed by Tukey's post-test or Kruskall-Wallis with Dunn’s post-test. *p<0.001, ***p<0.05, different from the control group.

Figure 7. Effect of maternal exercise and high-fat diet in antioxidant defense at offspring hippocampus. (A) Superoxide dismutase (SOD) activity, (B) Catalase (CAT) activity, (C) Glutathione peroxidase (GPx) activity, and (D) Reduced glutathione levels. Results are expressed as mean + SEM for n=6-10. Results were analyzed by two-way ANOVA followed by Tukey's post-test. *p<0.05, **p<0.01, different from the control group.


A Swimming exercise
Mating
Adaptation
Pregnancy
5 d
21 d
Swimming exercise: 30 min/day, 5 times a week
B Swimming exercise with overload
Mating
Adaptation
Pregnancy
5 d
21 d
1 d
2 d
without overload
without overload
Swimming exercise with overload (2% of BW):
30 min/day, 5 times a week


Sedentary
Exercise
Overload
Weight gain (g)
300
200
100
0
21
28
35
42
49
60
Days


A
Food intake
(100 g BW)
80
60
40
20
0
***
***
***
***
67
74
81
90
B
Caloric intake
(kcal/100 g BW)
300
250
200
150
100
67
74
81
90
C
Caloric efficiency
(weigh gain g/kcal)
0.08
0.06
0.04
0.02
0.00
** EH, OH
* EH
** SH, OH
SN
EN
ON
SH
EH
OH
67
74
81
90
Days


500
450
400
350
300
250
Weight gain (g)
60
67
74
81
90
Days
*
**
SN
EN
ON
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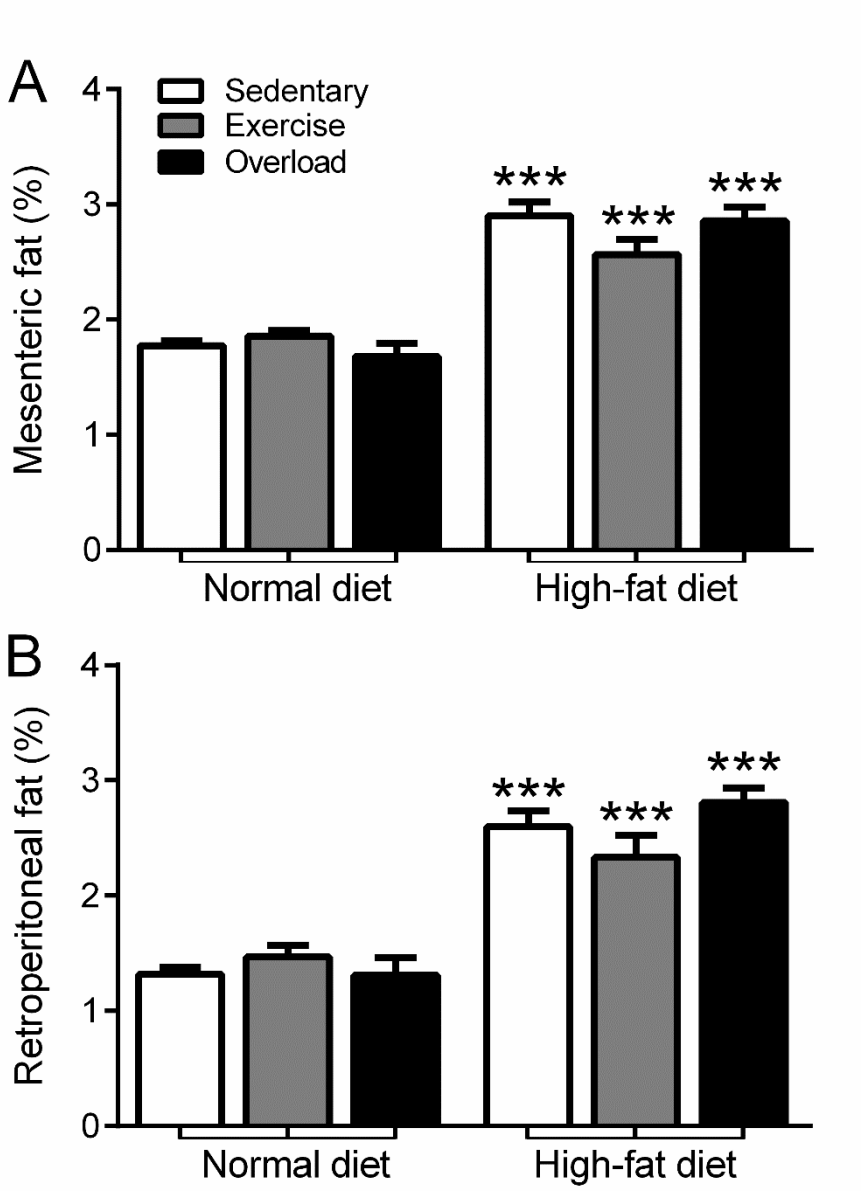
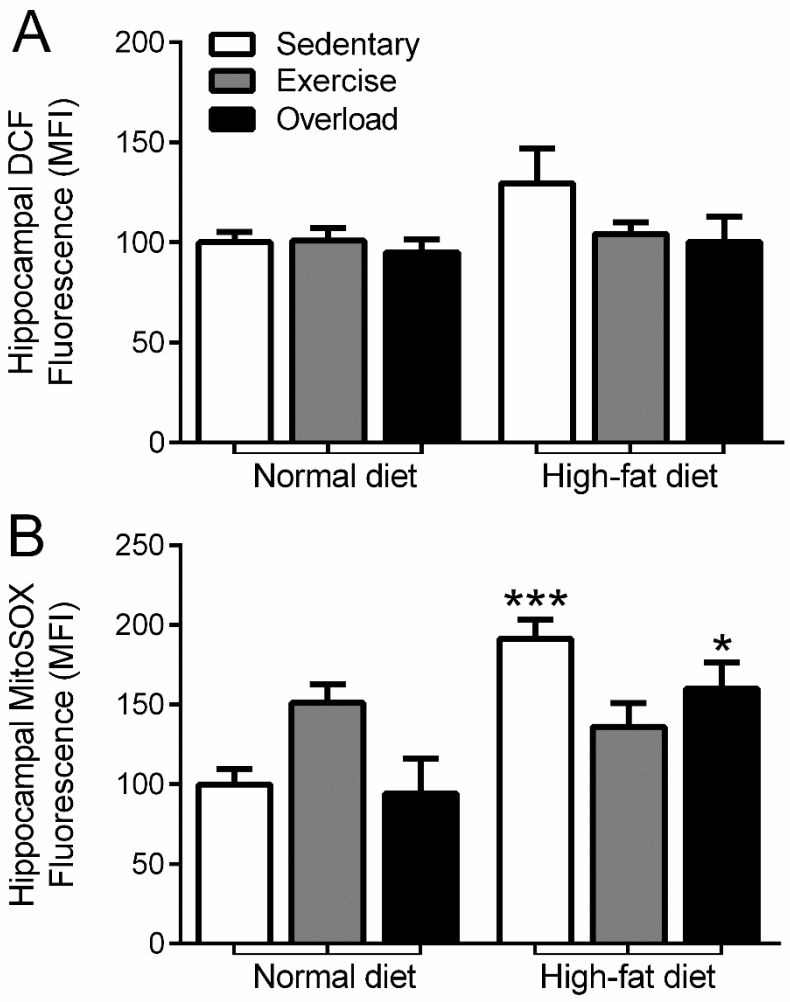
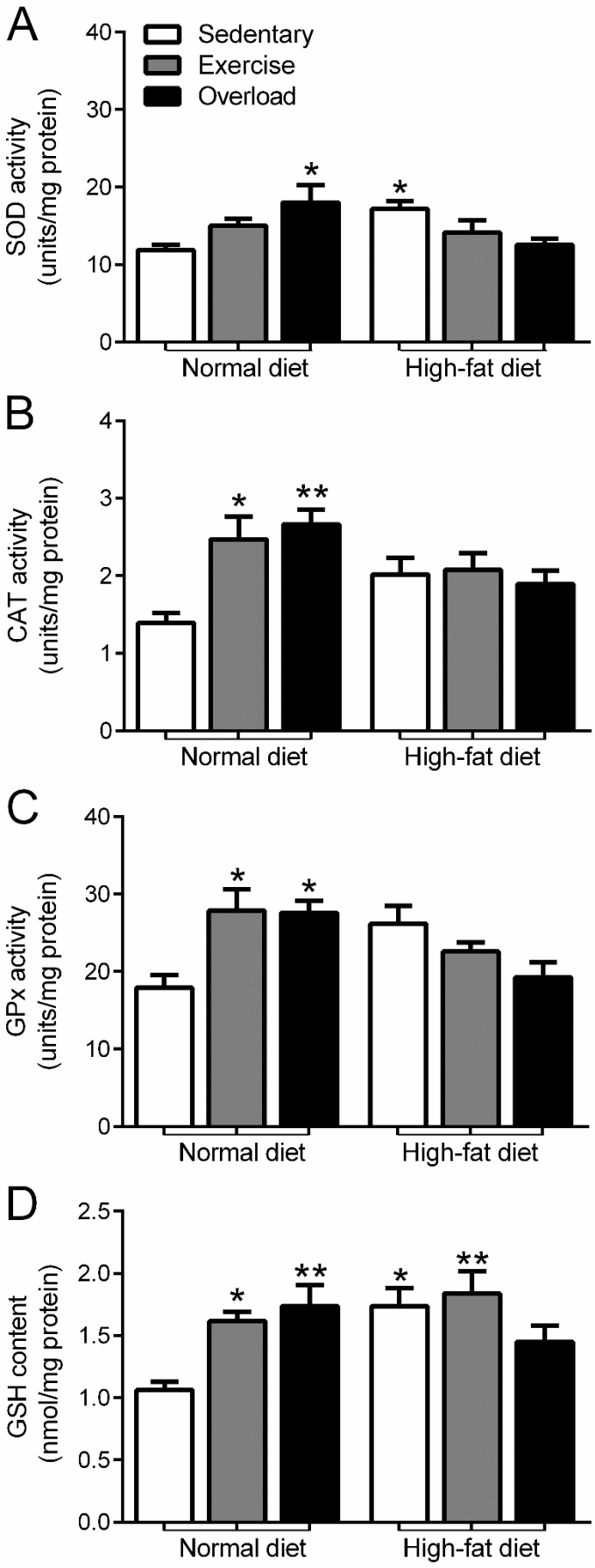
EH
OH


A
Sedentary
Exercise
Overload
Mesenteric fat (%)
4
3
2
1
0
***
***
***
Normal diet
High-fat diet
B
Retroperitoneal fat (%)
4
3
2
1
0
***
***
***
Normal diet
High-fat diet


A
Hippocampal DCF Fluorescence (MFI)
200
150
100
50
0
Sedentary
Exercise
Overload
Normal diet
High-fat diet
B
Hippocampal MitoSOX Fluorescence (MFI)
250
200
150
100
50
0
***
*
Normal diet
High-fat diet


A
Sedentary
Exercise
Overload
SOD activity
(units/mg protein)
40
30
20
10
0
*
*
Normal diet
High-fat diet
B
CAT activity
(units/mg protein)
4
3
2
1
0
*
**
Normal diet
High-fat diet
C
GPx activity
(units/mg protein)
40
30
20
10
0
*
*
Normal diet
High-fat diet
D
GSH content
(nmol/mg protein)
2.5
2.0
1.5
1.0
0.5
0.0
*
**
*
**
Normal diet
High-fat diet

IV. DISCUSSÃO

Neste trabalho avaliamos o efeito de intervenções gestacionais em resposta a dois modelos de indução de sobrepeso na prole: inicialmente, a prática de exercício materno e a suplementação com naringenina, aliados ou não, sobre o modelo de superalimentação durante a lactação. Posteriormente, o efeito de dois tipos de natação durante a gestação, com e sem sobrecarga, sobre a dieta rica em gordura na prole adulta, sempre utilizando apenas os filhotes machos. Os protocolos de exercício materno são de intensidade moderada.

As intervenções maternas ocorreram durante toda a gestação, no caso da suplementação com naringenina, ou também uma semana antes para adaptação, como ocorreu com o exercício de natação. Tanto a suplementação com naringenina quanto o exercício de natação, com ou sem sobrecarga, não causaram alterações na taxa de prenhez, ganho de peso gestacional, tamanho de ninhada ou peso dos filhotes ao primeiro dia de vida. No modelo de superalimentação na lactação não houve diferença no tempo para abertura dos olhos ou aparecimento dos dentes incisivos na prole, exposta ou não ao exercício materno.

Outros trabalhos já analisaram em modelo animal o consumo de polifenóis durante a gestação. No estudo de LI; ZHANG; SUN; ZHANG *et al.* (2019) a suplementação materna com naringenina foi capaz de prevenir o aumento do peso e a redução no tamanho de ninhada ao nascer, induzida pelo diabetes gestacional, entretanto não foi avaliado o efeito da naringenina *per se*. A suplementação materna com naringina, forma glicosilada da naringenina, não causou alteração no tamanho da ninhada e no peso dos filhotes aos 9 dias de vida (SACCO; SAINT; LEBLANC;

WARD, 2017). Quanto à utilização de outros polifenóis, já foi demonstrado em roedores a ausência de efeito após a suplementação materna com resveratrol na dose entre 2,0 e 2,5 mg/kg/dia (ROS; DIAZ; FREIRE-REGATILLO; ARGENTE-ARIZON *et al.*, 2018) e também redução no tamanho de ninhada com a ingestão de suco de romã na concentração de 3% na água dos animais disponibilizada durante a gestação (FINN-SELL; COTTRELL; GREENWOOD; DILWORTH *et al.*, 2018).

Quanto à prática de exercício gestacional, em humanos, não foi encontrado efeito negativo no peso ao nascer (CLAPP, 1996; MUDD; PIVARNIK; HOLZMAN; PANETH *et al.*, 2012), havendo prevenção contra o baixo peso (SIEBEL; CAREY; KINGWELL, 2012) ou a macrossomia (BARAKAT; PELAEZ; CORDERO; PERALES *et al.*, 2016). O efeito positivo do exercício materno no controle do peso ao nascer é relacionado à melhora no metabolismo da glicose (TOMIC; SPORIS; TOMIC; MILANOVIC *et al.*, 2013), visto que em mulheres praticantes de exercício físico antes e durante a gestação ocorre melhora na tolerância à glicose e menor risco para diabetes gestacional (OKEN; NING; RIFAS-SHIMAN; RADESKY *et al.*, 2006). Em modelo animal, a ausência de dano causado pelo exercício gestacional nestes parâmetros também já foi demonstrada (MARCELINO; LONGONI; KUDO; STONE *et al.*, 2013; RAIPURIA; BAHARI; MORRIS, 2015; RIBEIRO; TOFOLO; MARTINS; PAVANELLO *et al.*, 2017).

A prática de exercício de natação sem carga e a superalimentação durante a lactação não causaram alteração nos parâmetros de desenvolvimento da prole, avaliados pela aparência dos dentes incisivos e abertura dos olhos. No estudo de KLEIN; DOS SANTOS RODRIGUES; HOZER; DE SA COUTO-PEREIRA *et al.*

(2018) o exercício de natação materna em roedores também não afetou o desenvolvimento dos reflexos sensório-motores, mas trouxe um desenvolvimento motor mais maduro na prole. A redução de ninhada parece afetar positivamente o aparecimento desses fatores, no entanto, quando avaliadas em ninhadas nascidas em pequeno número de forma natural e não nas reduzidas após o nascimento (CHAHOUD; PAUMGARTTEN, 2009).

Nos filhotes machos a suplementação com naringenina, aliada ou não ao exercício gestacional, não causou alteração no peso ao nascer e no peso e gordura corporal aos 21 dias de vida. A prática de exercício de natação, com ou sem sobrecarga, não causou efeito no peso ao nascer e no peso e gordura corporal da prole aos 21, 60 e 90 dias de vida. Em humanos, a prática de exercício materno já demonstrou causar redução no percentual de gordura ao nascimento (DAHLY; LI; SMITH; KHASHAN *et al.*, 2018) e também em peso e percentual de gordura aos 5 anos de idade (CLAPP, 1996). Em roedores, a prática de exercício materno em roda de corrida ou esteira reduziu o peso e gordura corporal durante toda a vida do animal (CARTER; LEWIS; WILKERSON; TOBIA *et al.*, 2012; STANFORD; LEE; GETCHELL; SO *et al.*, 2015), entretanto trabalhos que avaliaram a prática de exercício de natação não encontraram o mesmo efeito (AUGUST; MAURMANN; SACCOMORI; SCORTEGAGNA *et al.*, 2018; KLEIN; DOS SANTOS RODRIGUES; HOZER; DE SA COUTO-PEREIRA *et al.*, 2018; WASINSKI; BACURAU; ESTRELA; KLEMPIN *et al.*, 2015; WASINSKI; ESTRELA; ARAKAKI; BADER *et al.*, 2016). O exercício gestacional em modelo animal parece trazer maiores efeitos sobre danos metabólicos na prole quando utilizados protocolos de corrida ou de roda de corrida.

No modelo de superalimentação induzida durante a lactação, houve aumento no peso e percentual de gordura dos filhotes aos 21 dias de vida, sem haver prevenção da suplementação com naringenina ou do exercício físico gestacional, aliados ou não, apenas o atraso no aumento do ganho de peso pelos tratamentos isolados. O aumento no peso e gordura corporal por meio da superalimentação induzida por redução de ninhada está bem descrito (CHEN; SIMAR; LAMBERT; MERCIER *et al.*, 2008; CONCEIÇÃO; KAEZER; PEIXOTO-SILVA; FELZENSZWALB *et al.*, 2016; RODRIGUES; DE MOURA; PASSOS; DUTRA *et al.*, 2009). A prática de exercício de corrida gestacional preveniu o ganho de peso e reduziu o aumento de gordura corporal na prole neste mesmo modelo, entretanto as ratas foram exercitadas durante toda a gestação e lactação, a 30% do VO_2Max, por 30 min/3 vezes/semana (RIBEIRO; TOFOLO; MARTINS; PAVANELLO *et al.*, 2017), sendo um exercício de baixa intensidade. Não foram encontrados outros trabalhos avaliando a suplementação com naringenina ou outros polifenóis durante a gestação em modelo de superalimentação pós-natal.

A redução de ninhada causa aumento no ganho de peso e gordura corporal dos filhotes por meio da menor competição na lactação, aumento na concentração lipídica do leite, imaturidade no controle do apetite e também maior cuidado materno (ENES-MARQUES; GIUSTI-PAIVA, 2018; KENNEDY, 1957; MOZES; SEFCIKOVA; RACEK, 2014; SEFCIKOVA; RACEK, 2015). Quando avaliamos o cuidado materno entre os dias 2 e 9 de vida dos filhotes, encontramos nas mães de ninhadas reduzidas uma menor frequência sem contato com a prole em diferentes horários do dia, sem efeito do exercício físico materno, concordando com o que já foi

demonstrado por Enes-Marques and Giusti-Paiva (ENES-MARQUES; GIUSTI-PAIVA, 2018). As ratas deste grupo também se mantiveram por mais períodos na posição de lactação arqueada (3 dos 5 avaliados), que é a considerada mais importante para a nutrição do filhote ao início da vida (STERN, 1997), chegando a dispender o dobro do tempo nesta posição quando comparada à ninhada normalizada. O exercício gestacional pôde prevenir parte deste comportamento, mas ainda mantendo a sua frequência aumentada em um dos períodos do dia, justificando o atraso no aumento de ganho de peso encontrado nos filhotes.

Estas alterações no comportamento materno auxiliam no entendimento de como ocorre o aumento no ganho de peso da prole quando a ninhada é reduzida, entretanto ainda não se sabe ao certo qual a motivação materna. Sabe-se que em diversos protocolos de estresse no período de lactação, incluindo a separação materna, ocorre aumento no cuidado materno para compensar possivelmente o estresse pelo tempo ausente (ORSO; CREUTZBERG; WEARICK-SILVA; WENDT VIOLA *et al.*, 2019), entretanto o modelo de redução de ninhada não demonstra aumentar os indicadores de estresse na rata mãe (CAPRIGLIONI CANCIAN; LEITE; MONTES; FISHER *et al.*, 2016; PLUMEL; STIER; THIERSE; VAN DORSSELAER *et al.*, 2014; VAN HAASTEREN; VAN TOOR; KLOOTWIJK; HANDLER *et al.*, 1996; XAVIER; SCOMPARIN; PONTES; RIBEIRO *et al.*, 2019), sendo possivelmente o aumento do cuidado materno não relacionado ao estresse. Uma hipótese é de que a rata mãe aumente seu cuidado em resposta à inabilidade dos filhotes em procurar pelo seu cuidado e também de realizar a sucção do leite, já demonstrado anteriormente em ninhadas com três filhotes (TEICHER; KENNY, 1978), apesar de

haver uma menor ativação neuronal na área pré-óptica medial das mães, que seria responsável pelo comportamento materno (FERREIRA; DUARTE; DINIZ; BITTENCOURT, 2017).

Quando expostos ao tamanho reduzido da ninhada, na idade adulta os filhotes geralmente apresentam risco aumentado para várias doenças, aumento de peso e gordura corporal, aumento de glicose, triglicerídeos, colesterol total e níveis de insulina, aliados à diminuição do HDL, resistência à leptina e efeito deletério em vários tecidos, como coração, fígado, rim e cérebro (ENES-MARQUES; GIUSTI-PAIVA, 2018; HABBOUT; LI; ROCHETTE; VERGELY, 2013).

Na segunda intervenção realizada a partir do modelo de dieta rica em gordura nos filhotes machos já adultos, independente da intervenção gestacional, houve uma redução no consumo sem alteração significativa na quantidade de calorias ingerida pelos grupos. Ainda houve aumento na eficiência calórica, que representa a quantidade de calorias ingeridas que é efetivamente convertida em peso corporal. Este efeito é esperado em dietas com maior densidade, pois os animais tendem a ajustar o seu consumo de acordo com a sua necessidade, consumindo assim menor quantidade em gramas de ração (BOITARD; ETCHAMENDY; SAUVANT; AUBERT *et al.*, 2012; HARIRI; THIBAULT, 2010; MACIEJCZYK; ZEBROWSKA; ZALEWSKA; CHABOWSKI, 2018; WU; LIU; KALAVAGUNTA; HUANG *et al.*, 2018).

A dieta causou aumento no percentual de gordura mesentérica e retroperitoneal, sem prevenção do exercício materno, não causando alteração no ganho de peso. A ausência de aumento no peso corporal pelo consumo de dieta HF por 4 semanas já foi demonstrada por DELLA VEDOVA; MUNOZ; SANTILLAN;

PLATEO-PIGNATARI *et al.* (2016), e acreditamos que se deve justamente ao curto tempo de exposição, visto que em protocolos com mais semanas de consumo os animais tendem a apresentar aumento no peso corporal (BOITARD; ETCHAMENDY; SAUVANT; AUBERT *et al.*, 2012; MACIEJCZYK; ZEBROWSKA; ZALEWSKA; CHABOWSKI, 2018). O aumento na gordura corporal em resposta ao consumo de dieta rica em gordura já foi demonstrada em diversos trabalhos (MORRISON; PISTELL; INGRAM; JOHNSON *et al.*, 2010; TOMIGA; YOSHIMURA; ITO; NAKASHIMA *et al.*, 2017), ocorrendo em protocolos de baixo tempo de exposição antes mesmo do aumento do peso corporal (ANDRICH; MELBOUCI; OU; LEDUC-GAUDET *et al.*, 2018).

Houve aumento do ganho de peso apenas nos filhotes que aliaram a dieta HF com o exercício materno com sobrecarga, aos 81 e 90 dias de vida. Em outro trabalho que utilizou sobrecarga de 3% no exercício de natação materno antes e durante a gestação (menos na última semana de prenhez), por um período de 60 min ao dia aliado a utilização de um sistema de bolhas para impossibilitar o descanso dos animais, ocorreu uma diminuição no ganho de peso da prole induzida por dieta HF [40]. Novamente, assim como no modelo de superalimentação na lactação, o exercício materno em roda de corrida (QUICLET; DUBOUCHAUD; BERTHON; SANCHEZ *et al.*, 2017) ou em esteira (SHELDON; NICOLE BLAIZE; FLETCHER; PEARSON *et al.*, 2016) causaram redução no ganho de peso e/ou percentual de gordura induzido por dieta na prole, sendo o exercício materno de natação incapaz de exercer o mesmo efeito.

Quanto aos parâmetros sorológicos, ao desmame os filhotes machos apresentaram menores níveis de glicose quando expostos ao exercício ou a suplementação com naringenina materna, mas o efeito desaparece quando as duas intervenções são aliadas. O modelo de redução de ninhada não causou alteração nos níveis de glicose no soro, como já demonstrado em outros trabalhos (BOULLU-CIOCCA; DUTOUR; GUILLAUME; ACHARD *et al.*, 2005; CHEN; SIMAR; LAMBERT; MERCIER *et al.*, 2008; PLAGEMANN; HARDER; RAKE; VOITS *et al.*, 1999). Quando expostos à dieta HF, os filhotes machos aos 90 dias de vida apresentam maiores níveis de glicose no soro, efeito prevenido pela prática materna de exercício de natação sem carga.

O efeito do exercício físico no aumento da expressão do transportador de glicose 4 (GLUT4), responsável pela captação de glicose muscular, já é bem demonstrado em humanos e roedores (GOODYEAR; KAHN, 1998; RICHTER; HARGREAVES, 2013). O exercício materno também melhora o metabolismo da glicose na prole, do desmame até a vida adulta (QUICLET; SITI; DUBOUCHAUD; VIAL *et al.*, 2016; STANFORD; LEE; GETCHELL; SO *et al.*, 2015). O mecanismo pode estar relacionado à expressão do GLUT4 via ativação de PGC-1α (WENDE; SCHAEFFER; PARKER; ZECHNER *et al.*, 2007), sendo o exercício materno capaz de aumentar na prole aos 19 dias de vida a expressão de GLUT4 e PGC-1α no tecido adiposo branco e de PGC-1α no músculo, também prevenido a redução na expressão do GLUT4 induzida por dieta HF no mesmo tecido (RAIPURIA; BAHARI; MORRIS, 2015). A natação com sobrecarga parece impedir o efeito benéfico do exercício sobre os níveis de glicose, como também demonstrado no trabalho de

WASINSKI; BACURAU; ESTRELA; KLEMPIN *et al.* (2015) a ausência de efeito da natação materna com sobrecarga sobre os níveis de glicose induzidos por dieta HF na prole.

O consumo de polifenóis também é altamente relacionado à melhora no metabolismo da glicose a partir de diversos mecanismos, incluindo a menor absorção de glicose e melhora na sensibilidade à insulina (KIM; KEOGH; CLIFTON, 2016). Em relação ao efeito durante a gestação, o consumo de polifenóis também traz benefícios. No estudo de BRAWERMAN; KERELIUK; BRAR; COLE *et al.* (2019), ratas foram expostas a uma dieta rica em gordura e sacarose para indução de GDM e também suplementadas com resveratrol antes e durante a gestação. Após o desmame, os filhotes machos receberam a dieta para indução de síndrome metabólica, causando após 12 semanas resistência à insulina, intolerância à glicose e desregulação na gliconeogênese, sendo todos os parâmetros atenuados pela suplementação materna com resveratrol.

No modelo de superalimentação durante a lactação também foram avaliados parâmetros de homeostase redox encefálicos. Na redução de ninhada houve um aumento na atividade de enzimas antioxidantes SOD, GPx, e GLO 1 no hipocampo da prole ao desmame, talvez em resposta ao ambiente pró-inflamatório observado nesse modelo (DE LUCA; ZIKO; DHUNA; SOMINSKY *et al.*, 2017; DE LUCA; ZIKO; SOMINSKY; NGUYEN *et al.*, 2016). O exercício materno, aliado ou não à redução de ninhada, causou aumento no dano oxidativo a proteínas. Considerando que já foi demonstrado que a prática de exercício contínuo leva à redução no dano oxidativo a proteínas, enquanto o exercício agudo pode levar ao aumento deste marcador no

plasma (WADLEY; TURNER; ALDRED, 2016), acreditamos que o hipocampo responde de forma diferente ao exercício materno.

Já no cerebelo, a prática de exercício físico gestacional causou redução no dano oxidativo a proteínas, efeito já demonstrado na mesma estrutura quando o exercício foi praticado por animais adultos (CASUSO; MARTINEZ-AMAT; HITA-CONTRERAS; CAMILETTI-MOIRON *et al.*, 2015). A superalimentação durante a lactação causou redução na atividade da enzima TrxR, e a suplementação materna com naringenina pôde prevenir completamente este efeito. Em animais adultos, a suplementação com polifenóis já demonstrou aumentar a expressão da enzima (JI; JIANG; LU; SHENG *et al.*, 2013).

No hipotálamo o exercício materno causou um aumento nas espécies reativas. Ocorreu também redução na atividade da GPx em quatro grupos: ninhada superalimentada, na prática de exercício materno e também com o exercício materno aliado a suplementação com naringenina, tanto na ninhada controle quanto na redução de ninhada. A redução na atividade da GPx em cérebro total de ratos adultos já foi demonstrada após 12 semanas de dieta HF, sendo relacionada ao efeito negativo da superalimentação (AMRI; GHORBEL; TURKI; AKROUT *et al.*, 2017). A atividade da SOD e GLO1 foi aumentada no hipotálamo em resposta ao exercício materno quando aliado à superalimentação de ninhada, demonstrando também uma possível proteção contra o ambiente pró-inflamatório causado pelo modelo (ZIKO; DE LUCA; DINAN; BARWOOD *et al.*, 2014).

As diferenças na resposta das intervenções maternas e neonatais podem se dar pelos estágios de desenvolvimento das estruturas encefálicas. O

desenvolvimento do cerebelo em ratos Wistar inicia na gestação, estando no estágio 2/5 de maturação ao nascimento, e tem seu completo desenvolvimento relacionado a abertura dos olhos dos animais, que ocorre entre os dias 14 e 15 (SANCHEZ-VILLAGRA; SULTAN, 2002). Com o hipocampo é semelhante, havendo já percepção da estrutura no dia embrionário 14.5 e sua formação praticamente completa ao dia pós-natal 14 (URBAN; GUILLEMOT, 2014).

O hipotálamo também inicia sua formação entre os dias embrionários 12 e 17, mas apresenta maior maturação apenas ao final da terceira semana de vida em roedores (BOURET, 2010). Essa estrutura é pouco avaliada em resposta ao exercício materno, entretanto no estudo de SEO; KIM; KIM; SUNG *et al.* (2013) foi vista uma proteção do exercício de corrida materno contra o estresse pré-natal. O hipotálamo também é fortemente influenciado pela nutrição materna, entretanto por ter seu maior desenvolvimento pós-natal dificulta a comparação com humanos, que tem seu hipotálamo desenvolvido em grande parte ainda no período pré-natal (BOURET, 2010; GALI RAMAMOORTHY; BEGUM; HARNO; WHITE, 2015). A redução de ninhada já demonstrou causar alterações no controle da fome, por meio do aumento nos neurônios anorexígenos NPY e outras alterações importantes no hipotálamo, entretanto o aumento no estresse oxidativo neste modelo foi demonstrado apenas em plasma, coração e fígado (HABBOUT; LI; ROCHETTE; VERGELY, 2013; PLAGEMANN; HARDER; RAKE; WAAS *et al.*, 1999).

Apesar da superalimentação trazer na vida adulta dos animais diversas alterações metabólicas negativas, causou no encéfalo modificações dependentes da estrutura e algumas delas podem ser positivas, como o aumento de capacidade

antioxidante no hipocampo que pode estar relacionado ao aumento no comportamento materno. Ninhadas reduzidas parecem trazem um efeito protetor ao cérebro, visto o menor peso cerebral e desenvolvimento comportamental em ninhadas maiores em relação às menores (WAINWRIGHT; PELKMAN; WAHLSTEN, 1989). Quando avaliados na idade adulta, filhotes expostos à redução de ninhada durante a lactação apresentaram menor comportamento do tipo ansioso aos 60 e 90 dias de vida (ENES-MARQUES; GIUSTI-PAIVA, 2018; SALARI; SAMADI; HOMBERG; KOSARI-NASAB, 2018), menores níveis plasmáticos de corticosterona e hormônio adrenocortitrófico e também menor resposta do eixo hipotálamo-pituitária-adrenal em resposta ao estresse, que foi relacionada ao maior comportamento materno (LIU; DIORIO; TANNENBAUM; CALDJI *et al.*, 1997). Considerando esses estudos, a redução de ninhada levaria a uma vida adulta com comportamento tipo ansiedade reduzido nos animais. Entretanto, em outra espécie de roedor foi encontrado aumento do comportamento do tipo ansioso e do estresse induzido aos 90 dias de vida, aliado à piora na memória espacial (SALARI; SAMADI; HOMBERG; KOSARI-NASAB, 2018).

Também vale a pena ressaltar que os efeitos encontrados tanto pelo exercício materno quanto pela suplementação com naringenina no ganho de peso, níveis de glicose e alterações na homeostase redox foram abolidos quando os dois tratamentos maternos foram aliados. A utilização de antioxidantes impede a adaptação causada pela prática de exercício físico em animais adultos (CASUSO; MARTINEZ-LOPEZ; HITA-CONTRERAS; CAMILETTI-MOIRON *et al.*, 2014; CASUSO; MARTINEZ-LOPEZ; NORDSBORG; HITA-CONTRERAS *et al.*, 2013;

GOMEZ-CABRERA; DOMENECH; VINA, 2008), sendo agora demonstrado que este bloqueio pode ocorrer inclusive para a próxima geração.

Em relação a dieta HF, à qual a prole foi exposta na vida adulta, também foi avaliada a homeostase redox encefálica. Apesar de não haver alterações significativas em cerebelo e hipocampo da prole (ver material suplementar), o hipocampo foi altamente afetado pela dieta HF, causando níveis aumentados de superóxido e de outras espécies reativas (apesar de não significativos), aumento na atividade SOD e conteúdo de GSH, sem alterações no dano oxidativo à proteína ou na função mitocondrial. Como a dieta foi mantida por um curto período, acreditamos que houve um desequilíbrio na homeostase redox, com aumento dos níveis de oxidantes que promovem a ativação da resposta antioxidante, entretanto sem causar danos oxidativos significativos ao tecido (LUSHCHAK, 2014). Alterações nos parâmetros redox do hipocampo induzidos por HFD já foram descritas em diferentes modelos de roedores e variados períodos de consumo, geralmente apresentando um aumento nos parâmetros oxidantes e defesas antioxidantes reduzidas quando consumidas por períodos mais longos, entre 7 e 16 semanas (HAJILUIAN; ABBASALIZAD FARHANGI; NAMENI; SHAHABI *et al.*, 2018; KAUR; SODHI; MADAN; CHAHAL *et al.*, 2018; MORRISON; PISTELL; INGRAM; JOHNSON *et al.*, 2010; SI; LI; JIANG; SHANG *et al.*, 2019).

O exercício materno causou alterações na homeostase redox hipocampal aliada ou não ao consumo de HFD. Em nossas condições experimentais, a natação durante a gestação trouxe melhora na capacidade antioxidante, com aumento das atividades de CAT e GPx, além do aumento no conteúdo de GSH nos dois tipos de

exercícios. Também observamos aumento da atividade da SOD como resultado do exercício com sobrecarga materno, sem alteração no dano oxidativo a proteínas, bem como na função mitocondrial.

Em resposta à HFD, o exercício materno também causou efeitos dependentes da modalidade no hipocampo da prole. A natação sem sobrecarga durante a gravidez preveniu completamente o aumento do superóxido induzido pela dieta, enquanto o exercício com sobrecarga reduziu parcialmente o aumento dos níveis. A prática de exercício físico afeta positivamente a função cerebral (ALKADHI, 2018; VOSS; VIVAR; KRAMER; VAN PRAAG, 2013) e seu efeito durante a gravidez na programação metabólica cerebral da prole parece agir de maneira semelhante. O exercício de natação materna influencia a homeostase redox cerebral dos filhos do período neonatal (MARCELINO; LONGONI; KUDO; STONE *et al.*, 2013) até o desmame (MARCELINO; DE LEMOS RODRIGUES; MIGUEL; NETTO *et al.*, 2015), e agora nós confirmamos o efeito também no desmame e na vida adulta. Quando avaliada no mesmo modelo de natação materna sem carga, a função mitocondrial se mostra aumentada no hipocampo da prole aos 74 dias de vida (KLEIN; HOPPE; SACCOMORI; DOS SANTOS *et al.*, 2019), no entanto, não encontramos o mesmo efeito em animais com 3 meses de idade.

O efeito positivo do exercício materno na homeostase redox cerebral da prole não é bem compreendido, mas podemos especular que possa ocorrer por meio da ativação de reguladores importantes, como PGC1α e NRF1, que estão aumentados no hipocampo da prole com 3 dias de vida expostos ao exercício materno [75], e também BDNF, que apresenta níveis aumentados no hipocampo de animais com 2

meses de idade (GOMES DA SILVA; DE ALMEIDA; FERNANDES; LOPIM *et al.*, 2016). Esses efeitos positivos na homeostase redox, associados a uma maior neurogênese (DAYI; AGILKAYA; OZBAL; CETIN *et al.*, 2012; GOMES DA SILVA; DE ALMEIDA; FERNANDES; LOPIM *et al.*, 2016) e ativação neuronal (ROBINSON; BUCCI, 2014) pode trazer benefícios para a função cognitiva da prole, uma vez que uma gravidez ativa em um modelo animal já demonstrou melhorar o aprendizado e a memória (AKHAVAN; EMAMI-ABARGHOIE; SAFARI; SADIGHI-MOGHADDAM *et al.*, 2008; DAYI; AGILKAYA; OZBAL; CETIN *et al.*, 2012; GOMES DA SILVA; DE ALMEIDA; FERNANDES; LOPIM *et al.*, 2016; KIM; LEE; KIM; YOO *et al.*, 2007; ROBINSON; BUCCI, 2014) e proteger alguns parâmetros no cérebro de ratos quando expostos ao estresse pré-natal (BUSTAMANTE; HENRIQUEZ; MEDINA; REINOSO *et al.*, 2013), hipóxia isquemia (AKHAVAN; FOROUTAN; SAFARI; SADIGHI-MOGHADDAM *et al.*, 2012; MARCELINO; DE LEMOS RODRIGUES; MIGUEL; NETTO *et al.*, 2015), e modelo de doença de Alzheimer (HERRING; DONATH; YARMOLENKO; USLAR *et al.*, 2012; KLEIN; HOPPE; SACCOMORI; DOS SANTOS *et al.*, 2019).

A programação metabólica da prole por meio das intervenções maternas e neonatais pode ocorrer por modificações epigenéticas, que são herdáveis, mas não alteram a sequência de DNA (BALE, 2015). Estudos em humanos relacionam os benefícios do exercício no tratamento e prevenção a alterações epigenéticas, que ocorre pela acetilação de histonas, em especial a H3 e H4, em diversos tecidos. Estas alterações podem estimular vias benéficas ou inibir a progressão de doenças, como demonstrado com a modulação específica de genes envolvidos na obesidade,

diabetes e doenças cardiovasculares, como a hipometilação do gene PGC-1α no músculo induzida pelo exercício (GRAZIOLI; DIMAURO; MERCATELLI; WANG *et al.*, 2017)..

Em relação ao exercício pré-natal, a prática paterna de exercício em roda de corrida semanas antes do acasalamento causou nos filhotes adultos um menor nível de metilação no hipocampo, aliado à melhora na memória espacial (MEGA; DE MEIRELES; PIAZZA; SPINDLER *et al.*, 2018; SPINDLER; SEGABINAZI; MEIRELES; PIAZZA *et al.*, 2019). Uma das hipóteses para esclarecer esse efeito é por meio das alterações epigenéticas ocorridas no esperma, como já demonstrado tanto em humanos quanto em modelo animal com a prática de exercício físico (DENHAM; O'BRIEN; HARVEY; CHARCHAR, 2015). O exercício materno também causa alterações epigenéticas na prole já adulta, prevenindo a hipermetilação do gene responsável pela transcrição de PGC-1□□induzido por dieta HF materna (JORNAYVAZ; SHULMAN, 2010).

O consumo de polifenóis também causa alterações epigenéticas, trazendo melhora na prevenção e tratamento de doenças em modelos *in vitro* e *in vivo* (ARORA; SHARMA; TOLLEFSBOL, 2019; BAG; BAG, 2018), ocorrendo pela acetilação de histonas, metilação do DNA e também inibição da família de enzimas DNA metil transferases (DNMTs) (LI, 2018). Em modelo de diabetes gestacional, a suplementação materna com galato de epigalocatequina impediu a hipermetilação global do DNA por meio do bloqueio da atividade e expressão das enzimas DNMT 3a e 3b no tubo neural dos embriões com 10 dias (ZHONG; XU; REECE; YANG, 2016). O consumo de chá verde durante a gestação também foi capaz de atenuar o

dano ao fígado induzido por dieta hipoprotéica materna e HF na prole, com alterações na DNMT1 e outros marcadores (KATAOKA; NORIKURA; SATO, 2018).

Também foram encontradas diferenças na resposta da prole aos dois protocolos de natação utilizados. PARK; KIM; EO; LEE *et al.* (2013) observaram diferentes respostas no cérebro da prole em relação a diferentes durações de exercício materno: quando realizada corrida em esteira por 20 min/dia, houve diminuição na atividade da STEM; quando realizada por 30 min/dia não houve efeito e por 40/min, aumento na atividade. Fica clara a necessidade de maiores estudos para definir o melhor tipo de exercício e duração para trazer benefícios para mãe e prole. Enquanto o exercício sem sobrecarga trouxe benefícios à prole e também proteção contra danos causados pela dieta HF, a sobrecarga causou aumento do peso corporal quando aliada a dieta HF na prole, e também não causou proteção aos níveis aumentados de glicose pela dieta, efeito encontrado sem a sobrecarga.

A suplementação com naringenina trouxe atraso no ganho de peso induzido por superalimentação durante a lactação, reduziu os níveis de glicose no soro e preveniu a redução na atividade da enzima TrxR no cerebelo. A suplementação materna com 50 mg/dia parece ser benéfica nos parâmetros avaliados, entretanto é necessária cautela na administração de polifenóis durante a gestação, visto a série de trabalhos realizados por Zielinsky e colaboradores (ZIELINSKY; BUSATO, 2013; ZIELINSKY; PICCOLI; MANICA; NICOLOSO *et al.*, 2010; ZIELINSKY; PICCOLI; MANICA; NICOLOSO *et al.*, 2012) demonstrando, em humanos, que o alto consumo de polifenóis ao final da gestação causa a constrição do canal arterial coronário fetal.

Apesar da maior atenção recebida ultimamente, a área em DOHaD ainda é extremamente recente. Grande parte dos trabalhos são realizados em modelo animal e os realizados em humanos são muitas vezes inconclusivos ou com resultados contrastantes, havendo dificuldade em isolar o efeito real com tantas variáveis existentes na vida humana. Estudos mais aprofundados são necessários para avaliar os mecanismos exatos dos efeitos de intervenções pré- e pós-natais, a fim de obter um impacto positivo na saúde na próxima geração sem trazer efeitos colaterais.

V. CONCLUSÃO

A partir dos resultados encontrados é possível afirmar que intervenções benéficas no estilo de vida realizadas no período pré-natal podem trazer melhora contra modelo de obesidade no período pós-natal. A prática de exercício físico gestacional trouxe melhora importante na redução dos níveis de glicose plasmáticos ao desmame e também em resposta a dieta rica em gordura na vida adulta. Os benefícios também se aplicaram ao cérebro, onde além de prevenir o aumento de espécies reativas induzido pela dieta, trouxe aumento na capacidade antioxidante hipocampal.

Em modelo de superalimentação na lactação, a prática de exercício materno e a suplementação com naringenina atrasaram o aumento no ganho de peso, no caso do exercício, por alterações no comportamento materno, entretanto sem causar efeito preventivo contra o aumento no percentual de gordura e no peso final dos animais. No encéfalo as alterações foram dependentes da estrutura, havendo um aumento importante na capacidade antioxidante do hipocampo. Também é

importante destacar a anulação dos efeitos quando são aliadas a suplementação com naringenina e a prática de exercício materno, reforçando dados da literatura de que antioxidantes bloqueiam a adaptação induzida pela prática de exercício físico.

VI. PERSPECTIVAS

Avaliar, na prole superalimentada no período pós-natal, o efeito do exercício físico materno sobre os seguintes parâmetros:

Avaliar, no plasma da prole:

1. níveis de insulina,
2. níveis de corticosterona, e
3. níveis de leptina e adiponectina.

Avaliar, no hipocampo:

1. atividade e imunoconteúdo das histonas desacetilases 3 e 11,
2. massa e potencial de membrana mitocondrial, e
3. expressão gênica de PGC1α, MFN 1 e 2 e DRP.

Avaliar, no tecido adiposo mesentérico e retroperitoneal:

1. atividade e imunoconteúdo das histonas desacetilases 3 e 11,
2. níveis de espécies reativas,
3. massa e potencial de membrana mitocondrial, e
4. expressão gênica de TNF, leptina, adiponectina e IL6.

VII. Referências

ACHARD, V.; SANCHEZ, C.; TASSISTRO, V.; VERDIER, M. *et al.* Immediate Postnatal Overfeeding in Rats Programs Aortic Wall Structure Alterations and Metalloproteinases Dysregulation in Adulthood. **Am J Hypertens**, Nov 6 2015.

ACOG. ACOG committee opinion. Exercise during pregnancy and the postpartum period. Number 267, January 2002. American College of Obstetricians and Gynecologists. **Int J Gynaecol Obstet**, 77, n. 1, p. 79-81, Apr 2002.

ACOG. ACOG Committee Opinion No. 650: Physical Activity and Exercise During Pregnancy and the Postpartum Period. **Obstet Gynecol**, 126, n. 6, p. e135-142, Dec 2015a.

ACOG. Committee Opinion No. 650: Physical Activity and Exercise During Pregnancy and the Postpartum Period. **Obstet Gynecol**, 126, n. 6, p. e135-142, Dec 2015b.

AKHAVAN, M. M.; EMAMI-ABARGHOIE, M.; SAFARI, M.; SADIGHI-MOGHADDAM, B. *et al.* Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups. **Neuroscience**, 151, n. 4, p. 1173-1183, Feb 19 2008.

AKHAVAN, M. M.; FOROUTAN, T.; SAFARI, M.; SADIGHI-MOGHADDAM, B. *et al.* Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring. **Pak J Pharm Sci**, 25, n. 1, p. 233-238, Jan 2012.

ALCOLEA, M. P.; COLOM, B.; LLADO, I.; GIANOTTI, M. *et al.* Mitochondrial transcription factor A (TFAM) is increased in rat embryo during placentation and associated with mitochondrial differentiation. **Cell Physiol Biochem**, 17, n. 1-2, p. 79-88, 2006.

ALKADHI, K. A. Exercise as a Positive Modulator of Brain Function. **Mol Neurobiol**, 55, n. 4, p. 3112-3130, Apr 2018.

ALVAREZ-BUENO, C.; CAVERO-REDONDO, I.; SANCHEZ-LOPEZ, M.; GARRIDO-MIGUEL, M. *et al.* Pregnancy leisure physical activity and children's neurodevelopment: a narrative review. **BJOG**, 125, n. 10, p. 1235-1242, Sep 2018.

AMATI, F.; HASSOUNAH, S.; SWAKA, A. The Impact of Mediterranean Dietary Patterns During Pregnancy on Maternal and Offspring Health. **Nutrients**, 11, n. 5, May 17 2019.

AMRI, Z.; GHORBEL, A.; TURKI, M.; AKROUT, F. M. *et al.* Effect of pomegranate extracts on brain antioxidant markers and cholinesterase activity in high fat-high fructose diet induced obesity in rat model. **BMC Complement Altern Med**, 17, n. 1, p. 339, Jun 27 2017.

ANDRICH, D. E.; MELBOUCI, L.; OU, Y.; LEDUC-GAUDET, J. P. *et al.* Altered Feeding Behaviors and Adiposity Precede Observable Weight Gain in Young Rats Submitted to a Short-Term High-Fat Diet. **J Nutr Metab**, 2018, p. 1498150, 2018.

ANNADURAI, T.; MURALIDHARAN, A. R.; JOSEPH, T.; HSU, M. J. *et al.* Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats. **J Physiol Biochem**, 68, n. 3, p. 307-318, Sep 2012.

ARORA, I.; SHARMA, M.; TOLLEFSBOL, T. O. Combinatorial Epigenetics Impact of Polyphenols and Phytochemicals in Cancer Prevention and Therapy. **Int J Mol Sci**, 20, n. 18, Sep 14 2019.

AUGUST, P. M.; MAURMANN, R. M.; SACCOMORI, A. B.; SCORTEGAGNA, M. C. *et al.* Effect of maternal antioxidant supplementation and/or exercise practice during pregnancy on postnatal overnutrition induced by litter size reduction: Brain redox homeostasis at weaning. **Int J Dev Neurosci**, 71, p. 146-155, Dec 2018.

AURELI, M.; GRASSI, S.; PRIONI, S.; SONNINO, S. *et al.* Lipid membrane domains in the brain. **Biochim Biophys Acta**, 1851, n. 8, p. 1006-1016, Aug 2015.

BAG, A.; BAG, N. Tea Polyphenols and Prevention of Epigenetic Aberrations in Cancer. **J Nat Sci Biol Med**, 9, n. 1, p. 2-5, Jan-Jun 2018.

BAHLS, M.; SHELDON, R. D.; TAHERIPOUR, P.; CLIFFORD, K. A. *et al.* Mother's exercise during pregnancy programmes vasomotor function in adult offspring. **Exp Physiol**, 99, n. 1, p. 205-219, Jan 2014.

BALE, T. L. Epigenetic and transgenerational reprogramming of brain development. **Nat Rev Neurosci**, 16, n. 6, p. 332-344, Jun 2015.

BALE, T. L.; BARAM, T. Z.; BROWN, A. S.; GOLDSTEIN, J. M. *et al.* Early life programming and neurodevelopmental disorders. **Biol Psychiatry**, 68, n. 4, p. 314-319, Aug 15 2010.

BARAKAT, R.; PELAEZ, M.; CORDERO, Y.; PERALES, M. *et al.* Exercise during pregnancy protects against hypertension and macrosomia: randomized clinical trial. **Am J Obstet Gynecol**, 214, n. 5, p. 649 e641-648, May 2016.

BARKER, D. J. The fetal and infant origins of adult disease. **BMJ**, 301, n. 6761, p. 1111, Nov 17 1990.

BARKER, D. J.; HALES, C. N.; FALL, C. H.; OSMOND, C. *et al.* Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. **Diabetologia**, 36, n. 1, p. 62-67, Jan 1993.

BARKER, D. J.; WINTER, P. D.; OSMOND, C.; MARGETTS, B. *et al.* Weight in infancy and death from ischaemic heart disease. **Lancet**, 2, n. 8663, p. 577-580, Sep 9 1989.

BARTON, M. Childhood obesity: a life-long health risk. **Acta Pharmacol Sin**, 33, n. 2, p. 189-193, Feb 2012.

BAUDRAND, R.; VAIDYA, A. Cortisol dysregulation in obesity-related metabolic disorders. **Curr Opin Endocrinol Diabetes Obes**, 22, n. 3, p. 143-149, Jun 2015.

BECKMAN, J. S.; KOPPENOL, W. H. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. **Am J Physiol**, 271, n. 5 Pt 1, p. C1424-1437, Nov 1996.

BENKHALIFA, M.; FERREIRA, Y. J.; CHAHINE, H.; LOUANJLI, N. *et al.* Mitochondria: participation to infertility as source of energy and cause of senescence. **Int J Biochem Cell Biol**, 55, p. 60-64, Oct 2014.

BIRO, F. M.; WIEN, M. Childhood obesity and adult morbidities. **Am J Clin Nutr**, 91, n. 5, p. 1499S-1505S, May 2010.

BLACK, R. E.; VICTORA, C. G.; WALKER, S. P.; BHUTTA, Z. A. *et al.* Maternal and child undernutrition and overweight in low-income and middle-income countries. **Lancet**, 382, n. 9890, p. 427-451, Aug 3 2013.

BOITARD, C.; ETCHAMENDY, N.; SAUVANT, J.; AUBERT, A. *et al.* Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. **Hippocampus**, 22, n. 11, p. 2095-2100, Nov 2012.

BOULLU-CIOCCA, S.; DUTOUR, A.; GUILLAUME, V.; ACHARD, V. *et al.* Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. **Diabetes**, 54, n. 1, p. 197-203, Jan 2005.

BOURET, S. G. Role of early hormonal and nutritional experiences in shaping feeding behavior and hypothalamic development. **J Nutr**, 140, n. 3, p. 653-657, Mar 2010.

BRAWERMAN, G. M.; KERELIUK, S. M.; BRAR, N.; COLE, L. K. *et al.* Maternal resveratrol administration protects against gestational diabetes-induced glucose intolerance and islet dysfunction in the rat offspring. **J Physiol**, 597, n. 16, p. 4175-4192, Aug 2019.

BRIGELIUS-FLOHE, R.; MAIORINO, M. Glutathione peroxidases. **Biochim Biophys Acta**, 1830, n. 5, p. 3289-3303, May 2013.

BUETTNER, G. R. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. **Arch Biochem Biophys**, 300, n. 2, p. 535-543, Feb 1 1993.

BUSTAMANTE, C.; HENRIQUEZ, R.; MEDINA, F.; REINOSO, C. *et al.* Maternal exercise during pregnancy ameliorates the postnatal neuronal impairments induced by prenatal restraint stress in mice. **Int J Dev Neurosci**, 31, n. 4, p. 267-273, Jun 2013.

CADENAS, E.; DAVIES, K. Mitochondrial free radical generation, oxidative stress, and aging. **Free Radic Biol Med**, 29, n. 3-4, p. 222-230, Aug 2000.

CANELLA, D. S.; LEVY, R. B.; MARTINS, A. P.; CLARO, R. M. *et al.* Ultra-processed food products and obesity in Brazilian households (2008-2009). **PLoS One**, 9, n. 3, p. e92752, 2014.

CAPRIGLIONI CANCIAN, C. R.; LEITE, N. C.; MONTES, E. G.; FISHER, S. V. *et al.* Histological and Metabolic State of Dams Suckling Small Litter or MSG-Treated Pups. **ScientificWorldJournal**, 2016, p. 1678541, 2016.

CARTER, L. G.; LEWIS, K. N.; WILKERSON, D. C.; TOBIA, C. M. *et al.* Perinatal exercise improves glucose homeostasis in adult offspring. **Am J Physiol Endocrinol Metab**, 303, n. 8, p. E1061-1068, Oct 15 2012.

CARTER, L. G.; QI, N. R.; DE CABO, R.; PEARSON, K. J. Maternal exercise improves insulin sensitivity in mature rat offspring. **Med Sci Sports Exerc**, 45, n. 5, p. 832-840, May 2013.

CASUSO, R. A.; MARTINEZ-AMAT, A.; HITA-CONTRERAS, F.; CAMILETTI-MOIRON, D. *et al.* Quercetin supplementation does not enhance cerebellar mitochondrial biogenesis and oxidative status in exercised rats. **Nutr Res**, 35, n. 7, p. 585-591, Jul 2015.

CASUSO, R. A.; MARTINEZ-LOPEZ, E. J.; HITA-CONTRERAS, F.; CAMILETTI-MOIRON, D. *et al.* The combination of oral quercetin supplementation and exercise prevents brain mitochondrial biogenesis. **Genes Nutr**, 9, n. 5, p. 420, Sep 2014.

CASUSO, R. A.; MARTINEZ-LOPEZ, E. J.; NORDSBORG, N. B.; HITA-CONTRERAS, F. *et al.* Oral quercetin supplementation hampers skeletal muscle adaptations in response to exercise training. **Scand J Med Sci Sports**, Oct 14 2013.

CHAHOUD, I.; PAUMGARTTEN, F. J. Influence of litter size on the postnatal growth of rat pups: is there a rationale for litter-size standardization in toxicity studies? **Environ Res**, 109, n. 8, p. 1021-1027, Nov 2009.

CHEN, H.; SIMAR, D.; LAMBERT, K.; MERCIER, J. *et al.* Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. **Endocrinology**, 149, n. 11, p. 5348-5356, Nov 2008.

CHENG, Z.; SCHMELZ, E. M.; LIU, D.; HULVER, M. W. Targeting mitochondrial alterations to prevent type 2 diabetes--evidence from studies of dietary redox-active compounds. **Mol Nutr Food Res**, 58, n. 8, p. 1739-1749, Aug 2014.

CHUGANI, H. T. A critical period of brain development: studies of cerebral glucose utilization with PET. **Prev Med**, 27, n. 2, p. 184-188, Mar-Apr 1998.

CLAPP, J. F., 3rd. Morphometric and neurodevelopmental outcome at age five years of the offspring of women who continued to exercise regularly throughout pregnancy. **J Pediatr**, 129, n. 6, p. 856-863, Dec 1996.

CLAPP, J. F., 3rd; LITTLE, K. D. Effect of recreational exercise on pregnancy weight gain and subcutaneous fat deposition. **Med Sci Sports Exerc**, 27, n. 2, p. 170-177, Feb 1995.

CLAPP, J. F., 3rd; LOPEZ, B.; HARCAR-SEVCIK, R. Neonatal behavioral profile of the offspring of women who continued to exercise regularly throughout pregnancy. **Am J Obstet Gynecol**, 180, n. 1 Pt 1, p. 91-94, Jan 1999.

CLAPP, J. F., 3rd; SIMONIAN, S.; LOPEZ, B.; APPLEBY-WINEBERG, S. *et al.* The one-year morphometric and neurodevelopmental outcome of the offspring of women who continued to exercise regularly throughout pregnancy. **Am J Obstet Gynecol**, 178, n. 3, p. 594-599, Mar 1998.

COLLABORATION, N. R. F. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. **Lancet**, 390, n. 10113, p. 2627-2642, Dec 16 2017.

CONCEICAO, E. P.; MOURA, E. G.; CARVALHO, J. C.; OLIVEIRA, E. *et al.* Early redox imbalance is associated with liver dysfunction at weaning in overfed rats. **J Physiol**, 593, n. 21, p. 4799-4811, Nov 1 2015.

CONCEIÇÃO, E. P. S.; KAEZER, A. R.; PEIXOTO-SILVA, N.; FELZENSZWALB, I. *et al.* Effects of Ilex paraguariensis (yerba mate) on the hypothalamic signalling of insulin and leptin and liver dysfunction in adult rats overfed during lactation. **Journal of Developmental Origins of Health and Disease**, 8, n. 01, p. 123-132, Feb 2016.

CORY, H.; PASSARELLI, S.; SZETO, J.; TAMEZ, M. *et al.* The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. **Front Nutr**, 5, p. 87, 2018.

CRUNKHORN, S.; DEARIE, F.; MANTZOROS, C.; GAMI, H. *et al.* Peroxisome proliferator activator receptor gamma coactivator-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 mitogen-activated protein kinase activation. **J Biol Chem**, 282, n. 21, p. 15439-15450, May 25 2007.

CURRIE, L. M.; WOOLCOTT, C. G.; FELL, D. B.; ARMSON, B. A. *et al.* The association between physical activity and maternal and neonatal outcomes: a prospective cohort. **Matern Child Health J**, 18, n. 8, p. 1823-1830, Oct 2014.

DAHLY, D. L.; LI, X.; SMITH, H. A.; KHASHAN, A. S. *et al.* Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study. **Int J Epidemiol**, 47, n. 1, p. 131-145, Feb 1 2018.

DAN DUNN, J.; ALVAREZ, L. A.; ZHANG, X.; SOLDATI, T. Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. **Redox Biol**, 6, p. 472-485, Dec 2015.

DAS, U. N. Nutritional factors in the prevention and management of coronary artery disease and heart failure. **Nutrition**, 31, n. 2, p. 283-291, Feb 2015.

DAYI, A.; AGILKAYA, S.; OZBAL, S.; CETIN, F. *et al.* Maternal aerobic exercise during pregnancy can increase spatial learning by affecting leptin expression on offspring's early and late period in life depending on gender. **ScientificWorldJournal**, 2012, p. 429803, 2012.

DE LUCA, S. N.; ZIKO, I.; DHUNA, K.; SOMINSKY, L. *et al.* Neonatal overfeeding by small-litter rearing sensitises hippocampal microglial responses to immune challenge: Reversal with neonatal repeated injections of saline or minocycline. **J Neuroendocrinol**, 29, n. 11, Nov 2017.

DE LUCA, S. N.; ZIKO, I.; SOMINSKY, L.; NGUYEN, J. C. *et al.* Early life overfeeding impairs spatial memory performance by reducing microglial sensitivity to learning. **J Neuroinflammation**, 13, n. 1, p. 112, May 18 2016.

DE TULLIO, M. C.; ASARD, H. Molecules tell stories: Reactive Oxygen, Nitrogen, Carbonyl and Sulfur Species take center stage. **Plant Physiol Biochem**, 59, p. 1-2, Oct 2012.

DELLA VEDOVA, M. C.; MUNOZ, M. D.; SANTILLAN, L. D.; PLATEO-PIGNATARI, M. G. *et al.* A Mouse Model of Diet-Induced Obesity Resembling Most Features of Human Metabolic Syndrome. **Nutr Metab Insights**, 9, p. 93-102, 2016.

DENHAM, J.; O'BRIEN, B. J.; HARVEY, J. T.; CHARCHAR, F. J. Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. **Epigenomics**, 7, n. 5, p. 717-731, Aug 2015.

DI MASCIO, D.; MAGRO-MALOSSO, E. R.; SACCONE, G.; MARHEFKA, G. D. *et al.* Exercise during pregnancy in normal-weight women and risk of preterm birth: a systematic review and meta-analysis of randomized controlled trials. **Am J Obstet Gynecol**, 215, n. 5, p. 561-571, Nov 2016.

DIENEL, G. A. Brain Glucose Metabolism: Integration of Energetics with Function. **Physiol Rev**, 99, n. 1, p. 949-1045, Jan 1 2019.

DISTLER, M. G.; PALMER, A. A. Role of Glyoxalase 1 (Glo1) and methylglyoxal (MG) in behavior: recent advances and mechanistic insights. **Front Genet**, 3, p. 250, 2012.

DORSAM, A. F.; PREISSL, H.; MICALI, N.; LORCHER, S. B. *et al.* The Impact of Maternal Eating Disorders on Dietary Intake and Eating Patterns during Pregnancy: A Systematic Review. **Nutrients**, 11, n. 4, Apr 13 2019.

DU, Q.; HOSODA, H.; UMEKAWA, T.; KINOUCHI, T. *et al.* Postnatal weight gain induced by overfeeding pups and maternal high-fat diet during the lactation period modulates glucose metabolism and the production of pancreatic and gastrointestinal peptides. **Peptides**, 70, p. 23-31, Aug 2015.

ELIAS, S. G.; VAN NOORD, P. A. H.; PEETERS, P. H. M.; TONKELAAR, I. D. *et al.* Caloric restriction reduces age at menopause: the effect of the 1944-1945 Dutch famine. **Menopause**, 25, n. 11, p. 1232-1237, Nov 2018.

ENES-MARQUES, S.; GIUSTI-PAIVA, A. Litter size reduction accentuates maternal care and alters behavioral and physiological phenotypes in rat adult offspring. **J Physiol Sci**, 68, n. 6, p. 789-798, Nov 2018.

ERIKSSON, J. G.; FORSEN, T.; TUOMILEHTO, J.; JADDOE, V. W. *et al.* Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. **Diabetologia**, 45, n. 3, p. 342-348, Mar 2002.

FARR, O. M.; LI, C. R.; MANTZOROS, C. S. Central nervous system regulation of eating: Insights from human brain imaging. **Metabolism**, 65, n. 5, p. 699-713, May 2016.

FERNANDEZ-TWINN, D. S.; OZANNE, S. E. Early life nutrition and metabolic programming. **Ann N Y Acad Sci**, 1212, p. 78-96, Nov 2010.

FERRARI, N.; BAE-GARTZ, I.; BAUER, C.; JANOSCHEK, R. *et al.* Exercise during pregnancy and its impact on mothers and offspring in humans and mice. **J Dev Orig Health Dis**, 9, n. 1, p. 63-76, Feb 2018.

FERREIRA, J. G. P.; DUARTE, J. C. G.; DINIZ, G. B.; BITTENCOURT, J. C. Litter size determines the number of melanin-concentrating hormone neurons in the medial preoptic area of Sprague Dawley lactating dams. **Physiol Behav**, 181, p. 75-79, Nov 1 2017.

FINN-SELL, S. L.; COTTRELL, E. C.; GREENWOOD, S. L.; DILWORTH, M. R. *et al.* Pomegranate Juice Supplementation Alters Utero-Placental Vascular Function and Fetal Growth in the eNOS(-/-) Mouse Model of Fetal Growth Restriction. **Front Physiol**, 9, p. 1145, 2018.

FORMAN, H. J.; URSINI, F.; MAIORINO, M. An overview of mechanisms of redox signaling. **J Mol Cell Cardiol**, 73, p. 2-9, Aug 2014.

FRANKLIN, T. B.; MANSUY, I. M. Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects. **Neurobiol Dis**, 39, n. 1, p. 61-65, Jul 2010.

FRUHBECK, G.; CATALAN, V.; RODRIGUEZ, A.; RAMIREZ, B. *et al.* Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. **Sci Rep**, 7, n. 1, p. 6619, Jul 26 2017.

GALI RAMAMOORTHY, T.; BEGUM, G.; HARNO, E.; WHITE, A. Developmental programming of hypothalamic neuronal circuits: impact on energy balance control. **Front Neurosci**, 9, p. 126, 2015.

GARNAES, K. K.; MORKVED, S.; SALVESEN, O.; MOHOLDT, T. Exercise Training and Weight Gain in Obese Pregnant Women: A Randomized Controlled Trial (ETIP Trial). **PLoS Med**, 13, n. 7, p. e1002079, Jul 2016.

GERLACH, M.; BEN-SHACHAR, D.; RIEDERER, P.; YOUDIM, M. B. Altered brain metabolism of iron as a cause of neurodegenerative diseases? **J Neurochem**, 63, n. 3, p. 793-807, Sep 1994.

GOMES DA SILVA, S.; DE ALMEIDA, A. A.; FERNANDES, J.; LOPIM, G. M. *et al.* Maternal Exercise during Pregnancy Increases BDNF Levels and Cell Numbers in the Hippocampal Formation but Not in the Cerebral Cortex of Adult Rat Offspring. **PLoS One**, 11, n. 1, p. e0147200, 2016.

GOMEZ-CABRERA, M. C.; DOMENECH, E.; VINA, J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. **Free Radic Biol Med**, 44, n. 2, p. 126-131, Jan 15 2008.

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

GOYAL, M. S.; HAWRYLYCZ, M.; MILLER, J. A.; SNYDER, A. Z. *et al.* Aerobic glycolysis in the human brain is associated with development and neotenous gene expression. **Cell Metab**, 19, n. 1, p. 49-57, Jan 7 2014.

GRAZIOLI, E.; DIMAURO, I.; MERCATELLI, N.; WANG, G. *et al.* Physical activity in the prevention of human diseases: role of epigenetic modifications. **BMC Genomics**, 18, n. Suppl 8, p. 802, Nov 14 2017.

GRUNDY, S. M. Adipose tissue and metabolic syndrome: too much, too little or neither. **Eur J Clin Invest**, 45, n. 11, p. 1209-1217, Nov 2015.

GUTOWSKI, M.; KOWALCZYK, S. A study of free radical chemistry: their role and pathophysiological significance. **Acta Biochim Pol**, 60, n. 1, p. 1-16, 2013.

GUTTERIDGE, J. M.; HALLIWELL, B. Free radicals and antioxidants in the year 2000. A historical look to the future. **Ann N Y Acad Sci**, 899, p. 136-147, 2000.

HABBOUT, A.; GUENANCIA, C.; LORIN, J.; RIGAL, E. *et al.* Postnatal overfeeding causes early shifts in gene expression in the heart and long-term alterations in cardiometabolic and oxidative parameters. **PLoS One**, 8, n. 2, p. e56981, 2013.

HABBOUT, A.; LI, N.; ROCHETTE, L.; VERGELY, C. Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences. **J Nutr**, 143, n. 5, p. 553-562, May 2013.

HAGBERG, H.; MALLARD, C.; ROUSSET, C. I.; THORNTON, C. Mitochondria: hub of injury responses in the developing brain. **Lancet Neurol**, 13, n. 2, p. 217-232, Feb 2014.

HAJILUIAN, G.; ABBASALIZAD FARHANGI, M.; NAMENI, G.; SHAHABI, P. *et al.* Oxidative stress-induced cognitive impairment in obesity can be reversed by vitamin D administration in rats. **Nutr Neurosci**, 21, n. 10, p. 744-752, Dec 2018.

HALES, C. N.; BARKER, D. J. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. **Diabetologia**, 35, n. 7, p. 595-601, Jul 1992.

HALLIWELL, B. Vitamin C: poison, prophylactic or panacea? **Trends Biochem Sci**, 24, n. 7, p. 255-259, Jul 1999.

HALLIWELL, B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. **Drugs Aging**, 18, n. 9, p. 685-716, 2001.

HALLIWELL, B. Oxidative stress and neurodegeneration: where are we now? **J Neurochem**, 97, n. 6, p. 1634-1658, Jun 2006a.

HALLIWELL, B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. **Plant Physiol**, 141, n. 2, p. 312-322, Jun 2006b.

HALLIWELL, B. Free radicals and antioxidants - quo vadis? **Trends Pharmacol Sci**, 32, n. 3, p. 125-130, Mar 2011.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radicals in Biology and Medicine**. Fourth ed. USA: Oxford University Press, 2007. 851 p. 978-0-19-856868-1.

HANSCHMANN, E. M.; GODOY, J. R.; BERNDT, C.; HUDEMANN, C. *et al.* Thioredoxins, glutaredoxins, and peroxiredoxins--molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. **Antioxid Redox Signal**, 19, n. 13, p. 1539-1605, Nov 1 2013.

HANSON, M. A. Background to the Cape Town Manifesto: harnessing the power of the normal. **J Dev Orig Health Dis**, 7, n. 5, p. 498-500, Oct 2016.

HARIRI, N.; THIBAULT, L. High-fat diet-induced obesity in animal models. **Nutr Res Rev**, 23, n. 2, p. 270-299, Dec 2010.

HARVEY, A.; GIBSON, T.; LONERGAN, T.; BRENNER, C. Dynamic regulation of mitochondrial function in preimplantation embryos and embryonic stem cells. **Mitochondrion**, 11, n. 5, p. 829-838, Sep 2011.

HERRING, A.; DONATH, A.; YARMOLENKO, M.; USLAR, E. *et al.* Exercise during pregnancy mitigates Alzheimer-like pathology in mouse offspring. **FASEB J**, 26, n. 1, p. 117-128, Jan 2012.

HO, Y. S.; MAGNENAT, J. L.; BRONSON, R. T.; CAO, J. *et al.* Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. **J Biol Chem**, 272, n. 26, p. 16644-16651, Jun 27 1997.

HUANG, L.; FAN, L.; DING, P.; HE, Y. H. *et al.* Maternal exercise during pregnancy reduces the risk of preterm birth through the mediating role of placenta. **J Matern Fetal Neonatal Med**, 32, n. 1, p. 109-116, Jan 2019.

IBGE. Pesquisa de Orçamentos Familiares 2008-2009. 2010.

JAMES, P. T.; RIGBY, N.; LEACH, R.; INTERNATIONAL OBESITY TASK, F. The obesity epidemic, metabolic syndrome and future prevention strategies. **Eur J Cardiovasc Prev Rehabil**, 11, n. 1, p. 3-8, Feb 2004.

JANSSEN, A. B.; KERTES, D. A.; MCNAMARA, G. I.; BRAITHWAITE, E. C. *et al.* A Role for the Placenta in Programming Maternal Mood and Childhood Behavioural Disorders. **J Neuroendocrinol**, 28, n. 8, Aug 2016.

JHENG, H. F.; HUANG, S. H.; KUO, H. M.; HUGHES, M. W. *et al.* Molecular insight and pharmacological approaches targeting mitochondrial dynamics in skeletal muscle during obesity. **Ann N Y Acad Sci**, 1350, p. 82-94, Sep 2015.

JI, L.; JIANG, P.; LU, B.; SHENG, Y. *et al.* Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. **J Nutr Biochem**, 24, n. 11, p. 1911-1919, Nov 2013.

JOHN, R. M. Prenatal Adversity Modulates the Quality of Maternal Care Via the Exposed Offspring. **Bioessays**, 41, n. 6, p. e1900025, Jun 2019.

JONES, D. P. Disruption of mitochondrial redox circuitry in oxidative stress. **Chem Biol Interact**, 163, n. 1-2, p. 38-53, Oct 27 2006.

JONES, D. W.; MILLER, M. E.; WOFFORD, M. R.; ANDERSON, D. C., Jr. *et al.* The effect of weight loss intervention on antihypertensive medication requirements in the hypertension Optimal Treatment (HOT) study. **Am J Hypertens**, 12, n. 12 Pt 1-2, p. 1175-1180, Dec 1999.

JORNAYVAZ, F. R.; SHULMAN, G. I. Regulation of mitochondrial biogenesis. **Essays Biochem**, 47, p. 69-84, 2010.

KARNIK, S.; KANEKAR, A. Childhood obesity: a global public health crisis. **Int J Prev Med**, 3, n. 1, p. 1-7, Jan 2012.

KATAOKA, S.; NORIKURA, T.; SATO, S. Maternal green tea polyphenol intake during lactation attenuates kidney injury in high-fat-diet-fed male offspring programmed by maternal protein restriction in rats. **J Nutr Biochem**, 56, p. 99-108, Jun 2018.

KAUR, J.; SODHI, R. K.; MADAN, J.; CHAHAL, S. K. *et al.* Forskolin convalesces memory in high fat diet-induced dementia in wistar rats-Plausible role of pregnane x receptors. **Pharmacol Rep**, 70, n. 1, p. 161-171, Feb 2018.

KENNEDY, G. C. The development with age of hypothalamic restraint upon the appetite of the rat. **J Endocrinol**, 16, n. 1, p. 9-17, Nov 1957.

KHACHO, M.; SLACK, R. S. Mitochondrial dynamics in the regulation of neurogenesis: From development to the adult brain. **Dev Dyn**, 247, n. 1, p. 47-53, Jan 2018.

KIM, H.; LEE, S. H.; KIM, S. S.; YOO, J. H. *et al.* The influence of maternal treadmill running during pregnancy on short-term memory and hippocampal cell survival in rat pups. **Int J Dev Neurosci**, 25, n. 4, p. 243-249, Jun 2007.

KIM, Y.; KEOGH, J. B.; CLIFTON, P. M. Polyphenols and Glycemic Control. **Nutrients**, 8, n. 1, Jan 5 2016.

KLEIN, C. P.; DOS SANTOS RODRIGUES, K.; HOZER, R. M.; DE SA COUTO-PEREIRA, N. *et al.* Swimming exercise before and during pregnancy: Promising preventive approach to impact offspring s health. **Int J Dev Neurosci**, 71, p. 83-93, Dec 2018.

KLEIN, C. P.; HOPPE, J. B.; SACCOMORI, A. B.; DOS SANTOS, B. G. *et al.* Physical Exercise During Pregnancy Prevents Cognitive Impairment Induced by Amyloid-beta in Adult Offspring Rats. **Mol Neurobiol**, 56, n. 3, p. 2022-2038, Mar 2019.

KLOP, B.; ELTE, J. W.; CABEZAS, M. C. Dyslipidemia in obesity: mechanisms and potential targets. **Nutrients**, 5, n. 4, p. 1218-1240, Apr 2013.

KOTHARI, V.; LUO, Y.; TORNABENE, T.; O'NEILL, A. M. *et al.* High fat diet induces brain insulin resistance and cognitive impairment in mice. **Biochim Biophys Acta Mol Basis Dis**, 1863, n. 2, p. 499-508, Feb 2017.

KUMAR, S.; KELLY, A. S. Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and Treatment. **Mayo Clin Proc**, 92, n. 2, p. 251-265, Feb 2017.

KUZAWA, C. W.; CHUGANI, H. T.; GROSSMAN, L. I.; LIPOVICH, L. *et al.* Metabolic costs and evolutionary implications of human brain development. **Proc Natl Acad Sci U S A**, 111, n. 36, p. 13010-13015, Sep 9 2014.

LARSSON, N. G.; CLAYTON, D. A. Molecular genetic aspects of human mitochondrial disorders. **Annu Rev Genet**, 29, p. 151-178, 1995.

LI, S.; ZHANG, Y.; SUN, Y.; ZHANG, G. *et al.* Naringenin improves insulin sensitivity in gestational diabetes mellitus mice through AMPK. **Nutr Diabetes**, 9, n. 1, p. 28, Oct 7 2019.

LI, Y. Epigenetic Mechanisms Link Maternal Diets and Gut Microbiome to Obesity in the Offspring. **Front Genet**, 9, p. 342, 2018.

LIMA, M. S.; PEREZ, G. S.; MORAIS, G. L.; SANTOS, L. S. *et al.* Effects of maternal high fat intake during pregnancy and lactation on total cholesterol and adipose tissue in neonatal rats. **Braz J Biol**, 78, n. 4, p. 615-618, Nov 2018.

LIU, D.; DIORIO, J.; TANNENBAUM, B.; CALDJI, C. *et al.* Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. **Science**, 277, n. 5332, p. 1659-1662, Sep 12 1997.

LIU, Y.; FU, X.; LAN, N.; LI, S. *et al.* Luteolin protects against high fat diet-induced cognitive deficits in obesity mice. **Behav Brain Res**, 267, p. 178-188, Jul 1 2014.

LOPATEGI, A.; LOPEZ-VICARIO, C.; ALCARAZ-QUILES, J.; GARCIA-ALONSO, V. *et al.* Role of bioactive lipid mediators in obese adipose tissue inflammation and endocrine dysfunction. **Mol Cell Endocrinol**, 419, p. 44-59, Jan 5 2016.

LU, J.; HOLMGREN, A. The thioredoxin antioxidant system. **Free Radic Biol Med**, 66, p. 75-87, Jan 2014.

LUNT, S. Y.; VANDER HEIDEN, M. G. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. **Annu Rev Cell Dev Biol**, 27, p. 441-464, 2011.

LUSHCHAK, V. I. Classification of oxidative stress based on its intensity. **EXCLI J**, 13, p. 922-937, 2014.

LV, J.; BHATIA, M.; WANG, X. Roles of Mitochondrial DNA in Energy Metabolism. **Adv Exp Med Biol**, 1038, p. 71-83, 2017.

LY, C.; YOCKELL-LELIEVRE, J.; FERRARO, Z. M.; ARNASON, J. T. *et al.* The effects of dietary polyphenols on reproductive health and early development. **Hum Reprod Update**, 21, n. 2, p. 228-248, Mar-Apr 2015.

M, M. A.; MILADI-GORJI, H.; EMAMI-ABARGHOIE, M.; SAFARI, M. *et al.* Maternal Voluntary Exercise during Pregnancy Enhances the Spatial Learning Acquisition but not the Retention of Memory in Rat

Pups via a TrkB-mediated Mechanism: The Role of Hippocampal BDNF Expression. **Iran J Basic Med Sci**, 16, n. 9, p. 955-961, Sep 2013.

MACHLIN, L. J.; BENDICH, A. Free radical tissue damage: protective role of antioxidant nutrients. **FASEB J**, 1, n. 6, p. 441-445, Dec 1987.

MACIEJCZYK, M.; ZEBROWSKA, E.; ZALEWSKA, A.; CHABOWSKI, A. Redox Balance, Antioxidant Defense, and Oxidative Damage in the Hypothalamus and Cerebral Cortex of Rats with High Fat Diet-Induced Insulin Resistance. **Oxid Med Cell Longev**, 2018, p. 6940515, 2018.

MAGRO-MALOSSO, E. R.; SACCONE, G.; DI MASCIO, D.; DI TOMMASO, M. *et al.* Exercise during pregnancy and risk of preterm birth in overweight and obese women: a systematic review and meta-analysis of randomized controlled trials. **Acta Obstet Gynecol Scand**, 96, n. 3, p. 263-273, Mar 2017.

MAILLOUX, R. J. Teaching the fundamentals of electron transfer reactions in mitochondria and the production and detection of reactive oxygen species. **Redox Biol**, 4, p. 381-398, 2015.

MARCELINO, T. B.; DE LEMOS RODRIGUES, P. I.; MIGUEL, P. M.; NETTO, C. A. *et al.* Effect of maternal exercise on biochemical parameters in rats submitted to neonatal hypoxia-ischemia. **Brain Res**, 1622, p. 91-101, Oct 5 2015.

MARCELINO, T. B.; LONGONI, A.; KUDO, K. Y.; STONE, V. *et al.* Evidences that maternal swimming exercise improves antioxidant defenses and induces mitochondrial biogenesis in the brain of young Wistar rats. **Neuroscience**, 246, p. 28-39, Aug 29 2013.

MARSEGLIA, L.; MANTI, S.; D'ANGELO, G.; NICOTERA, A. *et al.* Oxidative stress in obesity: a critical component in human diseases. **Int J Mol Sci**, 16, n. 1, p. 378-400, 2015.

MATSUDA, M.; SHIMOMURA, I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. **Obes Res Clin Pract**, 7, n. 5, p. e330-341, Sep-Oct 2013.

MATTÉ, C. K., Caroline Peres ; August, Pauline Maciel; Hoppe, Juliana Bender. A essência do estado redox celular: implicação para qualidade do sêmen. *In*: MENEGASSI, S. R. O. B., JÚLIO OTAVIO JARDIM (Ed.). **Aspectos Reprodutivos do Touros: Teoria e Prática**: Agrolivros, 2015. p. 141-164.

MAY, J. M. How does ascorbic acid prevent endothelial dysfunction? **Free Radic Biol Med**, 28, n. 9, p. 1421-1429, May 1 2000.

MCCORD, J. M. The evolution of free radicals and oxidative stress. **Am J Med**, 108, n. 8, p. 652-659, Jun 1 2000.

MCCORD, J. M.; FRIDOVICH, I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). **J Biol Chem**, 244, n. 22, p. 6049-6055, Nov 25 1969.

MCGAVOCK, J. M.; ANDERSON, T. J.; LEWANCZUK, R. Z. Sedentary lifestyle and antecedents of cardiovascular disease in young adults. **Am J Hypertens**, 19, n. 7, p. 701-707, Jul 2006.

MCGUIRE, S. F. Understanding the Implications of Birth Weight. **Nurs Womens Health**, 21, n. 1, p. 45-49, Feb - Mar 2017.

MEGA, F.; DE MEIRELES, A. L. F.; PIAZZA, F. V.; SPINDLER, C. *et al.* Paternal physical exercise demethylates the hippocampal DNA of male pups without modifying the cognitive and physical development. **Behav Brain Res**, 348, p. 1-8, Aug 1 2018.

MEI, Y.; THOMPSON, M. D.; COHEN, R. A.; TONG, X. Autophagy and oxidative stress in cardiovascular diseases. **Biochim Biophys Acta**, 1852, n. 2, p. 243-251, Feb 2015.

MELKONIAN, E. A.; SCHURY, M. P. Biochemistry, Anaerobic Glycolysis. *In*: **StatPearls**. Treasure Island (FL), 2020.

MERGENTHALER, P.; LINDAUER, U.; DIENEL, G. A.; MEISEL, A. Sugar for the brain: the role of glucose in physiological and pathological brain function. **Trends Neurosci**, 36, n. 10, p. 587-597, Oct 2013.

MONDA, V.; LA MARRA, M.; PERRELLA, R.; CAVIGLIA, G. *et al.* Obesity and brain illness: from cognitive and psychological evidences to obesity paradox. **Diabetes Metab Syndr Obes**, 10, p. 473-479, 2017.

MORRISON, C. D.; PISTELL, P. J.; INGRAM, D. K.; JOHNSON, W. D. *et al.* High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased Nrf2 signaling. **J Neurochem**, 114, n. 6, p. 1581-1589, Sep 2010.

MOZES, S.; SEFCIKOVA, Z.; RACEK, L. Long-term effect of altered nutrition induced by litter size manipulation and cross-fostering in suckling male rats on development of obesity risk and health complications. **Eur J Nutr**, 53, n. 5, p. 1273-1280, Aug 2014.

MUDD, L. M.; PIVARNIK, J.; HOLZMAN, C. B.; PANETH, N. *et al.* Leisure-time physical activity in pregnancy and the birth weight distribution: where is the effect? **J Phys Act Health**, 9, n. 8, p. 1168-1177, Nov 2012.

MULVIHILL, E. E.; ALLISTER, E. M.; SUTHERLAND, B. G.; TELFORD, D. E. *et al.* Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. **Diabetes**, 58, n. 10, p. 2198-2210, Oct 2009.

MUNAWAR ABBAS; FARHAN SAEED; FAQIR MUHAMMAD ANJUM; MUHAMMAD AFZAAL *et al.* Natural polyphenols: An overview. **International Journal of Food Properties** 20, n. 8, p. 1689-1699, 2016.

MUSRATI, R. A.; KOLLAROVA, M.; MERNIK, N.; MIKULASOVA, D. Malate dehydrogenase: distribution, function and properties. **Gen Physiol Biophys**, 17, n. 3, p. 193-210, Sep 1998.

MUSSIG, K.; REMER, T.; MASER-GLUTH, C. Brief review: glucocorticoid excretion in obesity. **J Steroid Biochem Mol Biol**, 121, n. 3-5, p. 589-593, Aug 2010.

MYLES, I. A. Fast food fever: reviewing the impacts of the Western diet on immunity. **Nutr J**, 13, p. 61, 2014.

NAKANDAKARI, S.; MUNOZ, V. R.; KUGA, G. K.; GASPAR, R. C. *et al.* Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice. **Brain Behav Immun**, 79, p. 284-293, Jul 2019.

NEEL, J. V. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? **Am J Hum Genet**, 14, p. 353-362, Dec 1962.

NELSON, D. L.; COX, M. M. **Princípios de Bioquímica de Lehninger**. 6th ed. Porto Alegre: Artmed, 2014. 9781429234146 (North American ed.)
1429234148 (North American ed.).

NG, M.; FLEMING, T.; ROBINSON, M.; THOMSON, B. *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. **Lancet**, 384, n. 9945, p. 766-781, Aug 30 2014.

NINO CRUZ, G. I.; RAMIREZ VARELA, A.; DA SILVA, I. C. M.; HALLAL, P. C. *et al.* Physical activity during pregnancy and offspring neurodevelopment: A systematic review. **Paediatr Perinat Epidemiol**, 32, n. 4, p. 369-379, Jul 2018.

NORDBERG, J.; ARNER, E. S. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. **Free Radic Biol Med**, 31, n. 11, p. 1287-1312, Dec 1 2001.

NUNEZ ESTEVEZ, K. J.; RONDON-ORTIZ, A. N.; NGUYEN, J. Q. T.; KENTNER, A. C. Environmental influences on placental programming and offspring outcomes following maternal immune activation. **Brain Behav Immun**, 83, p. 44-55, Jan 2020.

O'NEILL, S.; O'DRISCOLL, L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. **Obes Rev**, 16, n. 1, p. 1-12, Jan 2015.

OKEN, E.; NING, Y.; RIFAS-SHIMAN, S. L.; RADESKY, J. S. *et al.* Associations of physical activity and inactivity before and during pregnancy with glucose tolerance. **Obstet Gynecol**, 108, n. 5, p. 1200-1207, Nov 2006.

ONYANGO, I. G.; LU, J.; RODOVA, M.; LEZI, E. *et al.* Regulation of neuron mitochondrial biogenesis and relevance to brain health. **Biochim Biophys Acta**, 1802, n. 1, p. 228-234, Jan 2010.

ORSO, R.; CREUTZBERG, K. C.; WEARICK-SILVA, L. E.; WENDT VIOLA, T. *et al.* How Early Life Stress Impact Maternal Care: A Systematic Review of Rodent Studies. **Front Behav Neurosci**, 13, p. 197, 2019.

PARK, J. W.; KIM, M. H.; EO, S. J.; LEE, E. H. *et al.* Maternal exercise during pregnancy affects mitochondrial enzymatic activity and biogenesis in offspring brain. **Int J Neurosci**, 123, n. 4, p. 253-264, Apr 2013.

PATEL, M. S.; SRINIVASAN, M. Metabolic programming in the immediate postnatal life. **Ann Nutr Metab**, 58 Suppl 2, p. 18-28, 2011.

PEDERSEN, B. K.; SALTIN, B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. **Scand J Med Sci Sports**, 25 Suppl 3, p. 1-72, Dec 2015.

PELLERIN, L.; MAGISTRETTI, P. J. Neuroenergetics: calling upon astrocytes to satisfy hungry neurons. **Neuroscientist**, 10, n. 1, p. 53-62, Feb 2004.

PETERSON, J.; DWYER, J.; BEECHER, G.; BHAGWAT, S. *et al.* Flavanones in oranges, tangerines (mandarins), tangors, and tangelos:
a compilation and review of the data from the analytical literature. **Journal of Food Composition and Analysis** 19, p. 66-73, 2006.

PIKO, L.; TAYLOR, K. D. Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. **Dev Biol**, 123, n. 2, p. 364-374, Oct 1987.

PLAGEMANN, A.; HARDER, T.; RAKE, A.; VOITS, M. *et al.* Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats. **Brain Res**, 836, n. 1-2, p. 146-155, Jul 31 1999.

PLAGEMANN, A.; HARDER, T.; RAKE, A.; WAAS, T. *et al.* Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. **J Neuroendocrinol**, 11, n. 7, p. 541-546, Jul 1999.

PLUMEL, M. I.; STIER, A.; THIERSE, D.; VAN DORSSELAER, A. *et al.* Litter size manipulation in laboratory mice: an example of how proteomic analysis can uncover new mechanisms underlying the cost of reproduction. **Front Zool**, 11, p. 41, 2014.

POPKIN, B. M.; ADAIR, L. S.; NG, S. W. Global nutrition transition and the pandemic of obesity in developing countries. **Nutr Rev**, 70, n. 1, p. 3-21, Jan 2012.

PORTELLA, A. K.; SILVEIRA, P. P.; LAUREANO, D. P.; CARDOSO, S. *et al.* Litter size reduction alters insulin signaling in the ventral tegmental area and influences dopamine-related behaviors in adult rats. **Behav Brain Res**, 278, p. 66-73, Feb 1 2015.

QUICLET, C.; DUBOUCHAUD, H.; BERTHON, P.; SANCHEZ, H. *et al.* Maternal exercise modifies body composition and energy substrates handling in male offspring fed a high-fat/high-sucrose diet. **J Physiol**, 595, n. 23, p. 7049-7062, Dec 1 2017.

QUICLET, C.; SITI, F.; DUBOUCHAUD, H.; VIAL, G. *et al.* Short-term and long-term effects of submaximal maternal exercise on offspring glucose homeostasis and pancreatic function. **Am J Physiol Endocrinol Metab**, 311, n. 2, p. E508-518, Aug 1 2016.

RABBANI, N.; THORNALLEY, P. J. The critical role of methylglyoxal and glyoxalase 1 in diabetic nephropathy. **Diabetes**, 63, n. 1, p. 50-52, Jan 2014.

RAHIMI, R.; AKHAVAN, M. M.; KAMYAB, K.; EBRAHIMI, S. A. Maternal voluntary exercise ameliorates learning deficit in rat pups exposed, in utero, to valproic acid; role of BDNF and VEGF and their receptors. **Neuropeptides**, 71, p. 43-53, Oct 2018.

RAIPURIA, M.; BAHARI, H.; MORRIS, M. J. Effects of maternal diet and exercise during pregnancy on glucose metabolism in skeletal muscle and fat of weanling rats. **PLoS One**, 10, n. 4, p. e0120980, 2015.

RAVELLI, G. P.; STEIN, Z. A.; SUSSER, M. W. Obesity in young men after famine exposure in utero and early infancy. **N Engl J Med**, 295, n. 7, p. 349-353, Aug 12 1976.

RIBEIRO, T. A.; TOFOLO, L. P.; MARTINS, I. P.; PAVANELLO, A. *et al.* Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. **Sci Rep**, 7, n. 1, p. 7634, Aug 9 2017.

RICHTER, E. A.; HARGREAVES, M. Exercise, GLUT4, and skeletal muscle glucose uptake. **Physiol Rev**, 93, n. 3, p. 993-1017, Jul 2013.

ROBINSON, A. M.; BUCCI, D. J. Physical exercise during pregnancy improves object recognition memory in adult offspring. **Neuroscience**, 256, p. 53-60, Jan 3 2014.

ROBINSON, S.; WALTON, R. J.; CLARK, P. M.; BARKER, D. J. *et al.* The relation of fetal growth to plasma glucose in young men. **Diabetologia**, 35, n. 5, p. 444-446, May 1992.

RODRIGUES, A. L.; DE MOURA, E. G.; PASSOS, M. C.; DUTRA, S. C. *et al.* Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. **J Physiol**, 587, n. Pt 11, p. 2647-2661, Jun 1 2009.

RODRIGUES, A. L.; DE MOURA, E. G.; PASSOS, M. C.; TREVENZOLI, I. H. *et al.* Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. **J Nutr Biochem**, 22, n. 2, p. 109-117, Feb 2011.

ROS, P.; DIAZ, F.; FREIRE-REGATILLO, A.; ARGENTE-ARIZON, P. *et al.* Resveratrol Intake During Pregnancy and Lactation Modulates the Early Metabolic Effects of Maternal Nutrition Differently in Male and Female Offspring. **Endocrinology**, 159, n. 2, p. 810-825, Feb 1 2018.

ROSEBOOM, T.; DE ROOIJ, S.; PAINTER, R. The Dutch famine and its long-term consequences for adult health. **Early Hum Dev**, 82, n. 8, p. 485-491, Aug 2006.

ROSEBOOM, T. J.; PAINTER, R. C.; VAN ABEELEN, A. F.; VEENENDAAL, M. V. *et al.* Hungry in the womb: what are the consequences? Lessons from the Dutch famine. **Maturitas**, 70, n. 2, p. 141-145, Oct 2011.

SACCO, S. M.; SAINT, C.; LEBLANC, P. J.; WARD, W. E. Maternal Consumption of Hesperidin and Naringin Flavanones Exerts Transient Effects to Tibia Bone Structure in Female CD-1 Offspring. **Nutrients**, 9, n. 3, Mar 8 2017.

SALARI, A. A.; SAMADI, H.; HOMBERG, J. R.; KOSARI-NASAB, M. Small litter size impairs spatial memory and increases anxiety- like behavior in a strain-dependent manner in male mice. **Sci Rep**, 8, n. 1, p. 11281, Jul 26 2018.

SALIM, S. Oxidative Stress and the Central Nervous System. **J Pharmacol Exp Ther**, 360, n. 1, p. 201-205, Jan 2017.

SANCHEZ-VILLAGRA, M. R.; SULTAN, F. The cerebellum at birth in therian mammals, with special reference to rodents. **Brain Behav Evol**, 59, n. 3, p. 101-113, 2002.

SEBASTIANI, G.; HERRANZ BARBERO, A.; BORRAS-NOVELL, C.; ALSINA CASANOVA, M. *et al.* The Effects of Vegetarian and Vegan Diet during Pregnancy on the Health of Mothers and Offspring. **Nutrients**, 11, n. 3, Mar 6 2019.

SEFCIKOVA, Z.; RACEK, L. Effect of neonatal beta(3)-adrenoceptor agonist CL 316,243 treatment on body fat accumulation and intestinal alkaline phosphatase activity in rats from reduced nests. **Folia Histochem Cytobiol**, 53, n. 4, p. 307-313, 2015.

SEO, J. H.; KIM, T. W.; KIM, C. J.; SUNG, Y. H. *et al.* Treadmill exercise during pregnancy ameliorates posttraumatic stress disorderinduced anxietylike responses in maternal rats. **Mol Med Rep**, 7, n. 2, p. 389-395, Feb 2013.

SHELDON, R. D.; NICOLE BLAIZE, A.; FLETCHER, J. A.; PEARSON, K. J. *et al.* Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis. **J Hepatol**, Aug 29 2015.

SHELDON, R. D.; NICOLE BLAIZE, A.; FLETCHER, J. A.; PEARSON, K. J. *et al.* Gestational exercise protects adult male offspring from high-fat diet-induced hepatic steatosis. **J Hepatol**, 64, n. 1, p. 171-178, Jan 2016.

SHOELSON, S. E.; LEE, J.; GOLDFINE, A. B. Inflammation and insulin resistance. **J Clin Invest**, 116, n. 7, p. 1793-1801, Jul 2006.

SI, X.; LI, Y.; JIANG, Y.; SHANG, W. *et al.* gamma-Aminobutyric Acid Attenuates High-Fat Diet-Induced Cerebral Oxidative Impairment via Enhanced Synthesis of Hippocampal Sulfatides. **J Agric Food Chem**, 67, n. 4, p. 1081-1091, Jan 30 2019.

SIEBEL, A. L.; CAREY, A. L.; KINGWELL, B. A. Can exercise training rescue the adverse cardiometabolic effects of low birth weight and prematurity? **Clin Exp Pharmacol Physiol**, 39, n. 11, p. 944-957, Nov 2012.

SIES, H. Role of metabolic H2O2 generation: redox signaling and oxidative stress. **J Biol Chem**, 289, n. 13, p. 8735-8741, Mar 28 2014.

SINGH, A.; KUKRETI, R.; SASO, L.; KUKRETI, S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. **Molecules**, 24, n. 8, Apr 22 2019.

SMITH, K. B.; SMITH, M. S. Obesity Statistics. **Prim Care**, 43, n. 1, p. 121-135, Mar 2016.

SOKOLOFF, C. D. Regulation of Cerebral Metabolic Rate. *In*: SIEGEL GJ, A. B., Albers RW, et al. (Ed.). **Basic Neurochemistry: Molecular, Cellular and Medical Aspects**. 6º ed., 1999.

SON, G.; HAN, J. Roles of mitochondria in neuronal development. **BMB Rep**, 51, n. 11, p. 549-556, Nov 2018.

SPINDLER, C.; SEGABINAZI, E.; MEIRELES, A. L. F.; PIAZZA, F. V. *et al.* Paternal physical exercise modulates global DNA methylation status in the hippocampus of male rat offspring. **Neural Regen Res**, 14, n. 3, p. 491-500, Mar 2019.

STANFORD, K. I.; LEE, M. Y.; GETCHELL, K. M.; SO, K. *et al.* Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring. **Diabetes**, 64, n. 2, p. 427-433, Feb 2015.

STERN, J. M. Offspring-induced nurturance: animal-human parallels. **Dev Psychobiol**, 31, n. 1, p. 19-37, Jul 1997.

STEVENS, V. J.; OBARZANEK, E.; COOK, N. R.; LEE, I. M. *et al.* Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II. **Ann Intern Med**, 134, n. 1, p. 1-11, Jan 2 2001.

STEWARD, M. M.; SRIDHAR, A.; MEYER, J. S. Neural regeneration. **Curr Top Microbiol Immunol**, 367, p. 163-191, 2013.

SUN, M. K. Roles of neural regeneration in memory pharmacology. **Neural Regen Res**, 13, n. 3, p. 406-407, Mar 2018.

TAN, B. L.; NORHAIZAN, M. E. Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. **Nutrients**, 11, n. 11, Oct 25 2019.

TEICHER, M. H.; KENNY, J. T. Effect of reduced litter size on the suckling behaviour of developing rats. **Nature**, 275, n. 5681, p. 644-646, Oct 19 1978.

THAKER, V. V. Genetic and Epigenetic Causes of Obesity. **Adolesc Med State Art Rev**, 28, n. 2, p. 379-405, Fall 2017.

THANAN, R.; OIKAWA, S.; HIRAKU, Y.; OHNISHI, S. *et al.* Oxidative stress and its significant roles in neurodegenerative diseases and cancer. **Int J Mol Sci**, 16, n. 1, p. 193-217, 2015.

THANNICKAL, V. J.; FANBURG, B. L. Reactive oxygen species in cell signaling. **Am J Physiol Lung Cell Mol Physiol**, 279, n. 6, p. L1005-1028, Dec 2000.

TILOKANI, L.; NAGASHIMA, S.; PAUPE, V.; PRUDENT, J. Mitochondrial dynamics: overview of molecular mechanisms. **Essays Biochem**, 62, n. 3, p. 341-360, Jul 20 2018.

TINLOY, J.; CHUANG, C. H.; ZHU, J.; PAULI, J. *et al.* Exercise during pregnancy and risk of late preterm birth, cesarean delivery, and hospitalizations. **Womens Health Issues**, 24, n. 1, p. e99-e104, Jan-Feb 2014.

TOKARZ, P.; KAARNIRANTA, K.; BLASIAK, J. Role of antioxidant enzymes and small molecular weight antioxidants in the pathogenesis of age-related macular degeneration (AMD). **Biogerontology**, 14, n. 5, p. 461-482, Oct 2013.

TOMASI, D.; WANG, G. J.; VOLKOW, N. D. Energetic cost of brain functional connectivity. **Proc Natl Acad Sci U S A**, 110, n. 33, p. 13642-13647, Aug 13 2013.

TOMIC, V.; SPORIS, G.; TOMIC, J.; MILANOVIC, Z. *et al.* The effect of maternal exercise during pregnancy on abnormal fetal growth. **Croat Med J**, 54, n. 4, p. 362-368, Aug 2013.

TOMIGA, Y.; YOSHIMURA, S.; ITO, A.; NAKASHIMA, S. *et al.* Exercise training rescues high fat diet-induced neuronal nitric oxide synthase expression in the hippocampus and cerebral cortex of mice. **Nitric Oxide**, 66, p. 71-77, Jun 1 2017.

TOUMI, M. L.; MERZOUG, S.; BAUDIN, B.; TAHRAOUI, A. Quercetin alleviates predator stress-induced anxiety-like and brain oxidative signs in pregnant rats and immune count disturbance in their offspring. **Pharmacol Biochem Behav**, 107, p. 1-10, Jun 2013.

TREMMEL, M.; GERDTHAM, U. G.; NILSSON, P. M.; SAHA, S. Economic Burden of Obesity: A Systematic Literature Review. **Int J Environ Res Public Health**, 14, n. 4, Apr 19 2017.

UNGER, R. H. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. **Endocrinology**, 144, n. 12, p. 5159-5165, Dec 2003.

URBAN, N.; GUILLEMOT, F. Neurogenesis in the embryonic and adult brain: same regulators, different roles. **Front Cell Neurosci**, 8, p. 396, 2014.

VAISHNAVI, S. N.; VLASSENKO, A. G.; RUNDLE, M. M.; SNYDER, A. Z. *et al.* Regional aerobic glycolysis in the human brain. **Proc Natl Acad Sci U S A**, 107, n. 41, p. 17757-17762, Oct 12 2010.

VAN GAAL, L. F.; MERTENS, I. L.; DE BLOCK, C. E. Mechanisms linking obesity with cardiovascular disease. **Nature**, 444, n. 7121, p. 875-880, Dec 14 2006.

VAN HAASTEREN, G. A.; VAN TOOR, H.; KLOOTWIJK, W.; HANDLER, B. *et al.* Studies on the role of TRH and corticosterone in the regulation of prolactin and thyrotrophin secretion during lactation. **J Endocrinol**, 148, n. 2, p. 325-336, Feb 1996.

VANHEES, K.; VAN SCHOOTEN, F. J.; VAN WAALWIJK VAN DOORN-KHOSROVANI, S. B.; VAN HELDEN, S. *et al.* Intrauterine exposure to flavonoids modifies antioxidant status at adulthood and decreases oxidative stress-induced DNA damage. **Free Radic Biol Med**, 57, p. 154-161, Apr 2013.

VANNUCCI, R. C.; VANNUCCI, S. J. Glucose metabolism in the developing brain. **Semin Perinatol**, 24, n. 2, p. 107-115, Apr 2000.

VANNUCCI, R. C.; YAGER, J. Y.; VANNUCCI, S. J. Cerebral glucose and energy utilization during the evolution of hypoxic-ischemic brain damage in the immature rat. **J Cereb Blood Flow Metab**, 14, n. 2, p. 279-288, Mar 1994.

VEGA, C. C.; REYES-CASTRO, L. A.; BAUTISTA, C. J.; LARREA, F. *et al.* Exercise in obese female rats has beneficial effects on maternal and male and female offspring metabolism. **Int J Obes (Lond)**, 39, n. 4, p. 712-719, Apr 2015.

VIGITEL. ESTIMATIVAS SOBRE FREQUÊNCIA E DISTRIBUIÇÃO SOCIODEMOGRÁFICA DE FATORES DE RISCO E PROTEÇÃO PARA DOENÇAS CRÔNICAS NAS CAPITAIS DOS 26 ESTADOS BRASILEIROS E NO DISTRITO FEDERAL EM 2014. SAÚDE, M. d. 2014.

VIGITEL. ESTIMATIVAS SOBRE FREQUÊNCIA E DISTRIBUIÇÃO SOCIODEMOGRÁFICA DE FATORES DE RISCO E PROTEÇÃO PARA DOENÇAS CRÔNICAS NAS CAPITAIS DOS 26 ESTADOS BRASILEIROS E NO DISTRITO FEDERAL EM 2018. **Ministério da Saúde**, 2018.

VOSS, M. W.; VIVAR, C.; KRAMER, A. F.; VAN PRAAG, H. Bridging animal and human models of exercise-induced brain plasticity. **Trends Cogn Sci**, 17, n. 10, p. 525-544, Oct 2013.

WADLEY, A. J.; TURNER, J. E.; ALDRED, S. Factors influencing post-exercise plasma protein carbonyl concentration. **Free Radic Res**, 50, n. 4, p. 375-384, 2016.

WAINWRIGHT, P.; PELKMAN, C.; WAHLSTEN, D. The quantitative relationship between nutritional effects on preweaning growth and behavioral development in mice. **Dev Psychobiol**, 22, n. 2, p. 183-195, Mar 1989.

WANG, C.; WEI, Y.; ZHANG, X.; ZHANG, Y. *et al.* A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women. **Am J Obstet Gynecol**, 216, n. 4, p. 340-351, Apr 2017.

WANG, Z.; YUAN, D.; DUAN, Y.; LI, S. *et al.* Key factors involved in obesity development. **Eat Weight Disord**, 23, n. 3, p. 267-274, Jun 2018.

WARD, Z. J.; LONG, M. W.; RESCH, S. C.; GILES, C. M. *et al.* Simulation of Growth Trajectories of Childhood Obesity into Adulthood. **N Engl J Med**, 377, n. 22, p. 2145-2153, Nov 30 2017.

WASINSKI, F.; BACURAU, R. F.; ESTRELA, G. R.; KLEMPIN, F. *et al.* Exercise during pregnancy protects adult mouse offspring from diet-induced obesity. **Nutr Metab (Lond)**, 12, p. 56, 2015.

WASINSKI, F.; ESTRELA, G. R.; ARAKAKI, A. M.; BADER, M. *et al.* Maternal Forced Swimming Reduces Cell Proliferation in the Postnatal Dentate Gyrus of Mouse Offspring. **Front Neurosci**, 10, p. 402, 2016.

WEIHRAUCH-BLUHER, S.; SCHWARZ, P.; KLUSMANN, J. H. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. **Metabolism**, 92, p. 147-152, Mar 2019.

WELLBURN, S.; RYAN, C. G.; AZEVEDO, L. B.; ELLS, L. *et al.* Displacing Sedentary Time: Association with Cardiovascular Disease Prevalence. **Med Sci Sports Exerc**, Nov 10 2015.

WENDE, A. R.; SCHAEFFER, P. J.; PARKER, G. J.; ZECHNER, C. *et al.* A role for the transcriptional coactivator PGC-1alpha in muscle refueling. **J Biol Chem**, 282, n. 50, p. 36642-36651, Dec 14 2007.

WHO. The top 10 causes of death. Fact sheet N°310. : World Health Organization 2014.

WIEBE, H. W.; BOULE, N. G.; CHARI, R.; DAVENPORT, M. H. The effect of supervised prenatal exercise on fetal growth: a meta-analysis. **Obstet Gynecol**, 125, n. 5, p. 1185-1194, May 2015.

WILLIAMSON, G. The role of polyphenols in modern nutrition. **Nutr Bull**, 42, n. 3, p. 226-235, Sep 2017.

WILMOT, E. G.; EDWARDSON, C. L.; ACHANA, F. A.; DAVIES, M. J. *et al.* Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. **Diabetologia**, 55, n. 11, p. 2895-2905, Nov 2012.

WU, H.; LIU, Q.; KALAVAGUNTA, P. K.; HUANG, Q. *et al.* Normal diet Vs High fat diet - A comparative study: Behavioral and neuroimmunological changes in adolescent male mice. **Metab Brain Dis**, 33, n. 1, p. 177-190, Feb 2018.

XAVIER, J. L. P.; SCOMPARIN, D. X.; PONTES, C. C.; RIBEIRO, P. R. *et al.* Litter Size Reduction Induces Metabolic and Histological Adjustments in Dams throughout Lactation with Early Effects on Offspring. **An Acad Bras Cienc**, 91, n. 1, p. e20170971, Mar 21 2019.

YANKOVSKAYA, V.; HORSEFIELD, R.; TORNROTH, S.; LUNA-CHAVEZ, C. *et al.* Architecture of succinate dehydrogenase and reactive oxygen species generation. **Science**, 299, n. 5607, p. 700-704, Jan 31 2003.

YAU, S. Y.; LEE, T. H.; FORMOLO, D. A.; LEE, W. L. *et al.* Effects of Maternal Voluntary Wheel Running During Pregnancy on Adult Hippocampal Neurogenesis, Temporal Order Memory, and Depression-Like Behavior in Adult Female and Male Offspring. **Front Neurosci**, 13, p. 470, 2019.

YE, E. Q.; CHACKO, S. A.; CHOU, E. L.; KUGIZAKI, M. *et al.* Greater whole-grain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. **J Nutr**, 142, n. 7, p. 1304-1313, Jul 2012.

ZAMORA, M.; VILLENA, J. A. Targeting mitochondrial biogenesis to treat insulin resistance. **Curr Pharm Des**, 20, n. 35, p. 5527-5557, 2014.

ZAVORSKY, G. S.; LONGO, L. D. Exercise guidelines in pregnancy: new perspectives. **Sports Med**, 41, n. 5, p. 345-360, May 1 2011.

ZHONG, J.; XU, C.; REECE, E. A.; YANG, P. The green tea polyphenol EGCG alleviates maternal diabetes-induced neural tube defects by inhibiting DNA hypermethylation. **Am J Obstet Gynecol**, 215, n. 3, p. 368 e361-368 e310, Sep 2016.

ZHOU, L.; YOSHIMURA, Y.; HUANG, Y.; SUZUKI, R. *et al.* Two independent pathways of maternal cell transmission to offspring: through placenta during pregnancy and by breast-feeding after birth. **Immunology**, 101, n. 4, p. 570-580, Dec 2000.

ZIELINSKY, P.; BUSATO, S. Prenatal effects of maternal consumption of polyphenol-rich foods in late pregnancy upon fetal ductus arteriosus. **Birth Defects Res C Embryo Today**, 99, n. 4, p. 256-274, Dec 2013.

ZIELINSKY, P.; PICCOLI, A. L., Jr.; MANICA, J. L.; NICOLOSO, L. H. *et al.* Maternal consumption of polyphenol-rich foods in late pregnancy and fetal ductus arteriosus flow dynamics. **J Perinatol**, 30, n. 1, p. 17-21, Jan 2010.

ZIELINSKY, P.; PICCOLI, A. L., Jr.; MANICA, J. L.; NICOLOSO, L. H. *et al.* Reversal of fetal ductal constriction after maternal restriction of polyphenol-rich foods: an open clinical trial. **J Perinatol**, 32, n. 8, p. 574-579, Aug 2012.

ZIKO, I.; DE LUCA, S.; DINAN, T.; BARWOOD, J. M. *et al.* Neonatal overfeeding alters hypothalamic microglial profiles and central responses to immune challenge long-term. **Brain Behav Immun**, 41, p. 32-43, Oct 2014.

VIII. Material suplementar

A produção de espécies reativas de oxigênio e nitrogênio, medida por meio da oxidação da diclorofluoresceína (DCFH) foi determinada de acordo com Lebel et al. (1992). Cinquenta µL de amostra biológica foi incubada à 37ºC, no escuro, por 30 min, com 240 µL de 2',7'-diclorofluoresceína diacetato (H2DCF-DA) em placa de 96 poços. H2DCF-DA é clivada por esterases celulares e o H2DCF formado é oxidado pelas espécies reativas presentes na amostra, produzindo o composto fluorescente DCF, que é determinado fluorimetricamente utilizando os comprimentos de onda de excitação de 488 nm e de emissão de 525 nm. Uma curva padrão foi realizada em paralelo com as amostras, utilizando DCF como padrão (0,25 – 10 µM). Os resultados foram expressos como nmol DCF/mg proteína.

As atividades das enzimas antioxidantes superóxido-dismutase (Boveris 1984), catalase (Aebi 1984) foram determinadas espectrofotometricamente em placa de 96 poços. A atividade da SOD foi determinada por meio da quantificação da inibição da auto-oxidação da adrenalina, processo depende de superóxido, que foi medido à 480 nm. A atividade da CAT foi determinada pela redução na absorvância medida à 240 nm, resultado da redução na concentração do substrato dessa enzima, peróxido de hidrogênio.

Resultados:

No cerebelo da prole não houve alteração na oxidação de DCF ($F(5,36)=1.059;p=0.3988$), assim como na atividade das enzimas SOD

(F(5,39)=2.325;p=0.0611), e CAT (F(5,38)=1.758;p=0.1452)(gráficos não mostrados).

No hipotálamo não houve alteração na atividade das enzimas SOD (F(5,36)=1.242;p=0.3100), e CAT (F(5,37)=1.703;p=0.1581)(gráficos não mostrados).

IX. Carta de aprovação CEUA

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 31307
Título: Efeito da prática de exercício físico aliado à suplementação com naringenina no período gestacional sobre parâmetros comportamentais e bioquímicos na prole submetida à superalimentação pós-natal

Vigência: 02/05/2016 à 01/05/2020

Pesquisadores:

Equipe UFRGS:

CRISTIANE MATTE - coordenador desde 02/05/2016
Pauline Maciel August - Aluno de Doutorado desde 02/05/2016

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 30/05/2016 - SALA 330 DO ANEXO I - PRÉDIO DA REITORIA DA UFRGS/CAMPUS CENTRO/UFRGS, em seus aspectos éticos e metodológicos, para a utilização de 216 ratos Wistar machos adultos, 432 ratas Wistar fêmeas adultas e 896 filhotes machos provenientes destes acasalamentos, no período de 02/05/2016 a 01/05/2020, provenientes do biotério do Departamento de Bioquímica -ICBS/UFRG, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Quinta-Feira, 9 de Junho de 2016

MARCELO MELLER ALIEVI
Coordenador da comissão de ética