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ALLAN DE OLIVEIRA LIRA

# DESNUTRIÇÃO PROTEICA PERINATAL INDUZ MUDANÇAS NA EXPRESSÃO DE GENES METABÓLICOS DE RATOS: potencial efeito da atividade física materna

RECIFE

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Tese apresentada ao Programa de Pós-Graduação em Nutrição do Centro de Ciências da Saúde da Universidade Federal de Pernambuco para obtenção do título de Doutor em Nutrição.

**Área de concentração:** Bases Experimentais de Nutrição.

**Orientador:** Profª. Drª. Carol Virginia Góis Leandro

**Co-Orientadora:** Profª. Drª. Raquel da Silva Aragão

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Aprovada em: 30/11/2020.

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“O problema do mundo é que os estúpidos são excessivamente confiantes, e os inteligentes são cheios de dúvidas.” (RUSSEL, B. Mortals and Others 1931-1935).

## RESUMO

A exposição perinatal a um ambiente de baixo valor nutritivo predispõe a descendência ao desenvolvimento de doenças metabólicas na idade adulta, em ambos modelos experimentais e em estudos com humanos. As respostas adaptativas à má nutrição materna vêm sendo descritas em vários tecidos metabólicos, mas pouco foi explorado acerca do fígado e do tecido adiposo. O objetivo do estudo é avaliar o impacto de uma dieta de baixo valor proteico sobre a expressão de genes relacionados ao metabolismo no fígado e tecido adiposo de ratos, e buscar evidências na literatura de como a atividade física materna pode melhorar o prognóstico. No presente estudo, avaliamos a expressão de genes do ciclo glicolítico, lipolítico e adipogênico no fígado e tecido adiposo de ratos aos 30 dias e 90 dias de vidas que foram expostos a dieta hipoproteica durante a gestação e lactação. Também foram conduzidas buscas para uma revisão sistemática seguindo o protocolo da PRISMA (Preferred Reporting Items for Systematic-reviews and Meta-analysis) utilizando as seguintes palavras de busca: “Physical activity” ou “exercise” e “gestation” ou “pregnancy” e “offspring” ou “litter”. Foram critérios de inclusão, a descrição do protocolo de atividade física materna antes e/ou durante a gestação e a avaliação no metabolismo da mãe e/ou dos filhotes. Foram observados no tecido adiposo uma menor expressão na PDK4, CT e CPT1b aos 30 dias de filhotes desnutridos, mas que se normalizaram aos 90 dias. No fígado, alguns resultados foram similares, mas também foi observado maior expressão gênica de CT e FAS aos 30 dias nos filhotes desnutridos. A revisão sistemática evidenciou que a atividade física materna diminui a concentração de triglicerídeos nas ratas. Nos filhotes, foi observado uma melhora na glicemia, sensibilidade a insulina e perfil lipídico. Dessa forma, fica evidenciado que a desnutrição proteica perinatal provoca alterações na expressão gênica de tecidos metabólicos, e que a atividade física materna poderia, possivelmente, atuar na prevenção dessas alterações.



Palavras-chave: Desnutrição. Gestação. Metabolismo. Plasticidade fenotípica. Tecido adiposo. Fígado. Ratos.

## ABSTRACT

Perinatal exposure to a poor nutritional environment predisposes the progeny to the development of metabolic disease at the adult age, both in experimental models and humans. Numerous adaptive responses to maternal protein restriction have been reported in metabolic tissues, but little have explored the liver and the adipose tissue. The aim of this study is to evaluate the impact of a low-protein diet over the metabolism-related gene expression on the liver and the adipose tissue in rats, and search for evidences in the literature of how the maternal physical activity could improve the prognosis. In the present study, we evaluated the gene expression of genes from the glycolytic cycle, as well as lipolytic and adipogenic genes from the liver and the adipose tissue of rats at 30 and 90 days of age that were exposed previously to a low-protein diet during the gestation and suckling period. We also conducted a search for a systematic review with the guidance of the PRISMA protocol ((Preferred Reporting Items for Systematic-reviews and Meta-analysis) utilizing the following search-terms: “physical activity” or “exercise” and “gestation” or “pregnancy” and “offspring” or “litter”. Eligibility criteria included the full description of physical activity protocol before and/or gestation and the evaluation of metabolic parameters on the mothers and/or pups. In the adipose tissue, we observed a downregulation of PDK4, CT, and CPT1b at 30 days in undernourished pups, that were stabilized at the 90 days. In the liver, we found similar results but were also observed an upregulation of CT and FAS at 30 days in undernourished pups. The systematic review showed evidences that the maternal physical activity diminished the concentration of triglycerides in the mothers. In the pups, the findings included a betters glycaemia, insulin sensibility, and lipidic profile. As such, we know that a low-protein diet causes transcriptional changes on metabolic tissues, and that the maternal physical activity could possibly act to prevent those alterations.



Keywords: Malnutrition. Gestation. Metabolism. Phenotypic plasticity. Adipose tissue. Liver. Rats.

## LISTA DE ILUSTRAÇÕES

Artigo 1



Artigo 2

## LISTA DE TABELAS

Artigo 2

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| β-HAD | Beta-hidroxiacil-coenzima A desidrogenase |
| CPT1β | Carnitina palmitoil transferase 1 beta |
| CS | Citrato sintase |
| DNA | Ácido desoxirribonucleico |
| FAS | Ácido graxo sintase |
| FOXO1 | Proteína forkhead box O1 |
| G-6-P | Glicose-6-fosfatase |
| GLUT4 | Transportador de glicose tipo 4 |
| HNF4 | Fator nuclear de hepatóticos 4 |
| Myod1 | Proteína de determinação de mioblastos 1 |
| PCK1 | fosfoenolpiruvato carboxiquinase 1 |
| PDK4 | Piruvato desidrogenase lipoamida quinase isozima 4 |
| PFK | Fosfofrutoquinase |
| PGC1α | Coativador 1-alfa do receptor gama ativado por proliferador de peroxissoma |
| PKB | Proteína quinase b |
| PKLR | Piruvato quinase |
| PPARα | Receptor ativado por proliferador de peroxissoma alfa |
| RNAm | Ácido ribonucleico mensageiro |
| $VO_2$ | Consumo de oxigênio |
| $VO_{2max}$ | Consumo máximo de oxigênio |

**SUMÁRIO**

# 1 INTRODUÇÃO

A má nutrição nos períodos iniciais da vida não apenas impacta negativamente o desenvolvimento fetal e neonatal, mas também provoca efeitos duradouros, resultando numa maior susceptibilidade ao desenvolvimento de doenças cardiovasculares e metabólicas na idade adulta (BARKER 2007). Evidências de estudos epidemiológicos e de modelos com experimentação animal dão suporte a ideia de que a desnutrição perinatal predispõe o indivíduo ao aparecimento de doenças metabólicas na idade adulta (DE BRITO ALVES, NOGUEIRA *et al*. 2014; NOGUEIRA, ANDRADE *et al*. 2019; FRAGOSO, CARVALHO JUREMA SANTOS *et al*. 2020).

O vínculo entre uma nutrição pobre no início da vida com o aumento do risco de desenvolvimento de doenças crônicas na idade adulta pode ser mediado por alterações bioquímicas persistentes nos principais tecidos responsivos à insulina (DE BRITO ALVES, TOSCANO *et al*. 2017). A exposição de camundongos a uma dieta hipoproteica durante a gestação e lactação mostrou impactar características morfológicas, distribuição de tecido adiposo branco, além de reduzir a expressão de proteínas responsáveis pela sinalização de insulina, incluindo a IRS1, as subunidades da PI3K, p110b e p85a, e AKT1 em camundongos machos (JONES e OZANNE 2009).

Estudos prévios reportaram que a expressão de genes e de proteínas de enzimas relacionadas ao metabolismo da glicose e dos ácidos graxos no músculo esquelético foram alteradas a curta (30 dias de vida) e longa (90 dias de vida) duração em ratos expostos a dieta hipoproteica perinatal (DE BRITO ALVES, TOSCANO *et al*. 2017). Interessantemente, foi observado, em ambos músculos sóleo e EDL de ratos, uma diminuição duradoura na expressão da piruvato desidrogenase quinase 4 (PDK4) induzida pela dieta hipoproteica perinatal. A PDK4 é uma enzima que leva a desativação alostérica do complexo piruvato desidrogenase via fosforilação, dessa forma redireciona o fluxo metabólico do ciclo de Krebs para vias anabólicas (PETTERSEN, TUSUBIRA *et al*. 2019).

Também foi observado que o consumo de uma dieta hipoproteica perinatal contribuiu para o perfil fenotípico de gordura apresentado no fígado durante a vida adulta de ratos, com magnitude relacionada ao período de exposição à dieta hipoproteica (CAMPISAN, ECHARTE *et al*. 2017). Portanto as avaliações no fígado e tecido adiposo são importantes para obter uma maior descrição dos distúrbios transcricionais promovidos pela dieta hipoproteica perinatal nos tecidos responsivos à insulina, assim como a sua associação a

outros fatores ambientais reconhecidamente benéficas à homeostase metabólica, como a atividade física.

É referida como atividade física qualquer movimento realizado pelo músculo esquelético que demande um gasto energético acima do gasto energético basal (LEANDRO, AMORIM *et al*. 2009). Quando a atividade física é feita sistematicamente, marcada pela intensidade do esforço, frequência, volume e tempo, passa a ser classificada como exercício físico ou treinamento físico, caso haja um cronograma de atividades com um objetivo claro e reavaliações periódicas (LEANDRO, AMORIM *et al.* 2009). As respostas orgânicas a esses estímulos dependem de sua intensidade, frequência, volume, tipo de atividade e tempo investido (Leandro, Amorim et al. 2009). A forma mais comum de mensurar a intensidade de uma atividade física é através do $VO_{2máx}$ (consumo máximo de oxigênio) ou $FC_{máx}$ (frequência cardíaca máxima) (Leandro, Amorim et al. 2009). A atividade física é considerada de baixa intensidade se realizada entre 20-50% do $VO_{2máx}$ ou da $FC_{máx}$ do indivíduo, de moderada intensidade entre 50-75%, e de alta intensidade acima de 75% do $VO_{2max}$/$FC_{máx}$ (LEANDRO, AMORIM *et al*. 2009). Atualmente, a prática de atividade física é encorajada pela comunidade científica como um estilo de vida saudável para todas as pessoas, incluído mulheres grávidas (ACOG, 2015).

A recomendação do American College of Obstetricians and Gynecologists é que mulheres com gestação de baixo risco podem praticar atividades físicas de baixa a moderada intensidade (20-50% $VO_{2máx}$) por 30 minutos ao dia sem perigo (2015). De fato, a atividade física de intensidade moderada de forma regular está associada a baixa susceptibilidade de desenvolvimento da diabetes gestacional, por aumentar a sensibilidade a insulina e pela captação de glicose não-dependente de insulina (WOJTASZEWSKI, HANSEN *et al*. 2000; RICHTER, NIELSEN *et al*. 2004). Outros benefícios da atividade física materna são bem conhecidos, isso inclui um melhor condicionamento aeróbico e cardiovascular (DEMPSEY, BUTLER *et al*. 2004; RUCHAT, DAVENPORT *et al*. 2012; MISHRA e KISHORE 2018). Não obstante, os potenciais resultados de tal prática sobre a descendência ainda precisam ser melhor elucidados.

Um estilo de vida ativo durante a gestação modula adaptações fisiológicas para o crescimento fetal ao aumentar a disponibilidade de nutrientes e oxigênio através da placenta (CLAPP, SCHMIDT *et al*. 2004; HAAKSTAD, VOLDNER *et al*. 2007). No entanto, os efeitos da atividade física materna sobre o crescimento e oxigenação fetal são dependentes da duração e da intensidade do exercício (CLAPP, KIM *et al*. 2002; CLAPP 2003). Um estudo de 2013 mostrou que a prática de atividade física voluntária durante a gestação e lactação

aumenta a tolerância a glicose e a sensibilidade a insulina em ratos adultos (CARTER, QI *et al*. 2013). Embora os efeitos de exercícios submáximos durante a gestação na saúde da descendência sejam contraditórios, já vem sendo provado que exercícios controlados de baixa intensidade, baixo impacto e volume são seguros para humanos e também para animais (SZYMANSKI e SATIN 2012).

Os modelos experimentais de atividade física nos ajudar a entender melhor como ocorre a interação entre esse estímulo ambiental e as respostas adaptativas dos animais. Entretanto, os resultados de diferentes estudos tende a variar devido a diferentes metodologias utilizadas. Dessa forma, decidimos conduzir uma revisão sistemática de modo a juntar tudo o que foi feito até então em termos de protocolo atividade física materna, e identificar as diferentes repercussões desses experimentos sobre as respostas adaptativas metabólicas das mães e dos filhotes.

O presente estudo foi realizado em colaboração com o laboratório *Cardio-Metabolism, Diabetes and Nutrition* (CarMeN) da Université Lyon 1, na França; com supervisão das orientadoras Carol Virginia Góis Leandro e Raquel da Silva Aragão no Brasil, e supervisão do pesquisador Dr. Luciano Pirolla na França. A pesquisa resultou no artigo intitulado "*Maternal low protein diet induces persistent expression changes in metabolic genes in male rats*" publicado na revista *World Journal of Diabetes* e posteriormente na revisão sistemática cujo título é "*Physical activity during pregnancy and metabolic consequences on mothers and/or offspring: a systematic review*".

## 2 HIPÓTESE

A desnutrição proteica perinatal modula a expressão de genes das vias anabólicas do metabolismo no fígado e no tecido adiposo da descendência, que pode ser atenuada com a prática de atividade física durante a gestação.

# 3 OBJETIVOS

Avaliar o impacto de uma dieta hipoproteica perinatal sobre genes relacionados ao metabolismo de ratos no fígado e no tecido adiposo e buscar evidências do papel atenuador da atividade física materna.

## 3.1 OBJETIVOS ESPECÍFICOS

- No estudo experimental: Avaliar a expressão de RNAm da PDK4, CS, CPT1b, Acetil-CoA carboxilase, FAS, leptina, receptor de insulina, PFK, b-HAD, PPARα, FOXO1, HNF4, G-6-P, PCK1 e PKLR aos 30 dias e 90 dias de vida no tecido adiposo e fígado de ratos que foram submetidos à dieta hipoproteica perinatal.

- Na revisão sistemática: Procurar na literatura estudos que avaliaram os efeitos da atividade física durante a gestação/lactação sobre diferentes parâmetros metabólicos das mães e/ou filhotes.

# 4 MÉTODOS

O presente estudo conta com duas metodologias diferentes apresentadas em diferentes subseções. A primeira metodologia se refere aos estudos experimentais realizados em laboratório. Enquanto que a segunda detalha os passos tomados para a montagem da nossa revisão sistemática.

## 4.1 ESTUDO EXPERIMENTAL

Ratos albinos (*Rattus novergicus*) foram obtidos do biotério do Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, e alojados numa gaiola padrão de biotério num ambiente a uma temperatura de 22 ± 1 °C com ciclo claro-escuro controlado (escuro 18:00-06:00). Foram administradas *ad libitum* água e ração padrão de laboratório (52% carboidrato, 21% proteína e 4% lipídeos – Labina, Purina Agriband, São Paulo, Brasil). Os grupos foram divididos de acordo com a dieta de suas mães durante a gestação e lactação: o controle foram filhotes de mães que se alimentaram com uma dieta a 17% de caseína (n = 5, grupo normoproteico, NP) e o grupo hipoproteico de filhotes cujas mães receberam dieta com baixo valor proteico, 8% de caseína (n = 5, grupo hipoproteico, LP). As dietas foram preparadas no laboratório de Nutrição Experimental do Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, seguindo o guia de dieta da American Institute of Nutrition – AIN-93 (REEVES 1993).

Durante o período de lactação, os filhotes foram mantidos em ninhadas de 8 indivíduos de ambos os sexos para assegurar uma nutrição padronizada até o desmame. Após o período de lactação (21 dias de vida), três a quatro machos de cada ninhada foram alojados coletivamente em gaiolas padrão e receberam água e ração padrão de laboratório *ad libitum*. Os grupos experimentais consistiram de um ou dois ratos machos de cada mãe. Filhotes fêmeas não foram incluídas no estudo. Todas as análises experimentais foram feitas no tecido adiposo e fígado de ratos machos sacrificados aos 30 dias e aos 90 dias de vida por decapitação. Todos os animais foram eutanasiados entre 14:00-17:00 após 4 ou 5 horas de jejum. O fígado e tecido adiposo visceral foram cuidadosamente dissecados, congelados em nitrogênio líquido e armazenados a -80°C até a extração do RNA.

A manipulação e os cuidados com os animais seguiram o estatuto da Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL) e o Colégio Brasileiro de Experimentação Animal (COBEA). O protocolo experimental foi aprovado pelo Comitê de Ética no uso de

animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (processo n°23073.062778/2014-38).

### 4.1.1 Extração de RNA, transcrição reversa e qPCR em tempo real

O RNA total foi extraído do fígado e do tecido adiposo usando o reagente TriPure [Sigma-Aldrich (Roche), St. Quentin Fallavier, França] de acordo com as instruções do fabricante. Foi adicionado 10 µL de TriPure por miligrama de tecido; a suspensão resultante foi homogeneizada usando o Precellys Lysing Kit (Bertin, Montigny-le-Bretonneux, França) seguindo as instruções do fabricante. Então 1/4 do volume de clorofórmio foi adicionado. Eles foram vortexados 3 x 15 segundos, encubados em temperatura local por 5 minutos e centrifugados por 15 minutos a 15000g numa temperatura de 4°C.

O RNA foi precipitado após a adição de 1/2 do volume em isopropanol (Carlo Elba, Val-de-Reuil, França). Os sobrenadantes foram usados para extração de proteína e os *pellets* de RNA foram lavados sequencialmente com 70% e 95% etanol (Carlo Elba), secados e dissolvidos em 100 µL de água livre de RNase. A concentração de RNA e sua pureza (260/280 nm grau de absorbância) foi determinado usando um Nanodrop 2000 (Thermo-Fischer).

A transcrição reversa foi feita usando um kit RT-Takara (Primescript TM, Takara), com 1 µL de RNA como padrão, seguindo as instruções do fabricante. As amostras foram aquecidas por 10 minutos a uma temperatura de 65°C. Posteriormente, as amostras foram misturadas com 4 µL de substância tampão PrimeScript, 1 µL oligodT (50 µM), 4 µL de hexâmaro aleatório e 1 µL do mix de enzimas PrimeScript RT, seguido de 15 minutos de incubação a 37°C e 15 segundos a 85°C. O RNA foi removido por incubação de 1 µL de RNase H por 20 minutos a 37°C. As reações da transcrição reversa tiveram um volume final de 200 µL após adição de água livre de RNase e posteriormente foram armazenadas numa temperatura de -20°C. O PCR em tempo real foi realizado utilizando um termociclador em tempo real (Rotor-Gene Real-Time PCR System, Labgene Scientific Instruments, Archamps, França).

As reações foram encubadas a 95°C por 10 minutos, seguidos por 40 ciclos de desnaturação (95°C, 10s), anelamento (58-62°C, a depender do primer, 30s) e polimerização (72°C, 30s). O nível de expressão de RNAm da piruvato desidrogenase lipoamida quinase isozima 4 (PDK4), citrato sintase (CS), carnitina palmitoil transferase 1b (CPT1b), acetilcoenzima A carboxilase, ácido graxo sintase (FAS), leptina, receptor de insulina, fosfofrutoquinase (PFK), beta-hidroxiacil-coenzima A desidrogenase, receptor ativado por proliferador de peroxissoma alfa (PPARα), proteína forkhead box O1 (FOXO1), fator nuclear de

hepatóticos 4 (HNF4), glicose-6-fosfatase (G-6-P), fosfoenolpiruvato carboxiquinase (PCK1) e piruvato quinase (PKLR) foram mensurados no RNA total extraído do tecido adiposo e do fígado. Os resultados da qPCR de cada gene (incluindo o gene de manutenção) foram expressos em unidades arbitrárias derivadas de uma curva de calibração padrão de uma amostra de referência. As amostras de referência foram geradas ao mixar 10 µL alíquotas de 10 amostras de cDNA, 5 do grupo NP e 5 do grupo LP. A qPCR de cada amostras foram duplicadas. Os dados da expressão gênica foram normalizados usando a proteína ribossomal L19 (RPL19) como o gene de manutenção. Como controle extra, os amplicons de qPCR foram analisados por gel de agarose para sua validação.

### 4.1.2 Análise estatística

A análise estatística foi conduzida pelo programa GraphPad Prism 5 para Windows (GraphPad Software®. Inc., La Jolla, CA, Estados Unidos). Uma análise de dados exploratória foi feita para identificar informações imprecisas e a presença de *outliers* e para testar a distribuição normal dos dados. Os testes de normalidade Kolmogorov-Smirnov e Shapiro-Wilk foram aplicados em todas as amostras. A significância estatística foi avaliada através do teste de análise de variância ANOVA *two-way* com a dieta materna (hipoproteica e normoproteica) e idade (30 dias e 90 dias) como fatores. O teste de Bonferroni foi utilizado como *post hoc*. Os valores são apresentados em média e erro padrão da média. O valor de $P < 0.05$ foi considerado estatisticamente significativo.

## 4.2 REVISÃO SISTEMÁTICA

A revisão sistemática seguiu as recomendações da PRISMA (Preferred-Reporting-Items-for-Systematic-Reviews-and-Meta-Analysis). Os critérios de elegibilidade foram definidos previamente para prevenir o risco de viés pelos revisores. Devido à natureza da nossa questão e pela facilidade em se trabalhar com o animal, somente estudos originais publicados e conduzidos exclusivamente com ratas submetidas a um protocolo de atividade física antes e/ou durante a gestação foram incluídos. Outros critérios de elegibilidade incluíram a descrição completa do protocolo de atividade física utilizado, bem como parâmetros de avaliação no metabolismo da mãe e/ou dos filhotes acerca dos efeitos provocados pelo protocolo de atividade física materno, sem especificações. Estudos que não atenderam aos critérios de elegibilidade ou não estiveram disponíveis nas bases de dados foram excluídos.

### 4.2.1 Termos de busca

As buscas foram conduzidas entre Junho e Julho de 2020 usando as seguintes bases de dados: Pubmed/Medline, Science Direct (Elsevier), Springer Link (Springer Nature) e Scopus. Os termos de buscas e cada uma de seus termos de entrada (sinônimos, derivação de tema, e palavras próximas) achadas através das bases de dados foram combinadas pelo operador booleano “OU”, e pesquisados separadamente como um “conjunto de busca”. Os conjuntos de buscas foram então combinados com o operador booleano “E”, o que nos deu a coleção final de possíveis estudos a serem analisados.

### 4.2.2 Seleção de artigos

A fase de triagem da seleção dos artigos foi realizada por dois revisores, na qual o título e o resumo de cada artigo foi lido. Todos os artigos originais que utilizaram um protocolo de atividade física materna foram selecionados para serem avaliados. Após a fase inicial, a elegibilidade dos artigos foi feita por ambos revisores após a leitura completa de cada artigo para possível inclusão nessa revisão sistemática. Quando houve discordâncias entre os dois revisores acerca da elegibilidade de um artigo, um terceiro revisor foi consultado.

### 4.2.3 Extração de dados

A extração dos dados foi conduzida por um revisor, e então checada por dois revisores diferentes. Os dados extraídos incluem a linhagem do animal, a idade ou peso desses animais, o desenho experimental (o protocolo de atividade física e algum outro tipo de intervenção, se utilizado), e os resultados da avaliação no metabolismo da mãe e/ou do filhote.

## 5 RESULTADOS

Os resultados da pesquisa encontram-se apresentados em forma de artigo, os quais estão dispostos nos Apêndices A e B.

## 6 CONCLUSÃO

Os nossos resultados permitem afirmar que a desnutrição proteica perinatal provocou mudanças na expressão de genes relacionados ao metabolismo no fígado e no tecido adiposo de ratos. As mudanças observadas nos ratos hipoproteicos sugerem uma maior atividade lipogênica no tecido adiposo e da gliconeogênese no fígado, o que deve promover um efeito compensatório em resposta à falta do nutriente no início da vida. Na revisão sistemática, os estudos evidenciaram que a atividade física materna de intensidade baixa a moderada altera a composição corporal das mães, com menor concentração de ácidos graxos. Nos filhotes, foi observado uma melhor situação glicêmica, sensibilidade a insulina e perfil lipídico. Esses resultados sugerem que a atividade física materna poderia ter um papel de impacto em atenuar ou até prevenir algumas das respostas adaptativas observadas nos ratos expostos a desnutrição proteica perinatal.

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# APÊNDICE A – MATERNAL LOW PROTEIN DIET INDUCES PERSISTENT EXPRESSION CHANGES IN METABOLIC GENES IN MALE RATS

**Name of Journal:** *World Journal of Diabetes*
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***Basic Study***

**Maternal low protein diet induces persistent expression changes in metabolic genes in male rats**

de Oliveira Lira A *et al*. Perinatal undernutrition and metabolism

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**Abstract**

BACKGROUND

Perinatal exposure to a poor nutritional environment predisposes the progeny to the development of metabolic disease at the adult age, both in experimental models and humans. Numerous adaptive responses to maternal protein restriction have been reported in metabolic tissues. However, the expression of glucose/fatty acid metabolism-related genes in adipose tissue and liver needs to be described.

AIM

To evaluate the metabolic impact of perinatal malnutrition, we determined malnutrition-associated gene expression alterations in liver and adipose tissue.

METHODS

In the present study, we evaluated the alterations in gene expression of glycolytic/Krebs cycle genes (Pyruvate dehydrogenase kinase 4 and citrate synthase), adipogenic and lipolytic genes and leptin in the adipose tissue of offspring rats at 30 d and 90 d of age exposed to maternal isocaloric low protein (LP) diet throughout gestation and lactation. We also evaluated, in the livers of the same animals, the same set of genes as well as the gene expression of the transcription factors peroxisome proliferator-activated receptor gamma coactivator 1, forkhead box protein O1 and hepatocyte nuclear factor 4 and of gluconeogenic genes.

RESULTS

In the adipose tissue, we observed a transitory (*i.e.*, at 30 d) downregulation of pyruvate dehydrogenase kinase 4, citrate synthase and carnitine palmitoyl transferase 1b gene expression. Such transcriptional changes did not persist in adult LP rats (90 d), but we observed a tendency towards a decreased gene expression of leptin ($P$ = 0.052). The liver featured some gene expression alterations comparable to the adipose tissue, such as pyruvate dehydrogenase kinase 4 downregulation at 30 d and displayed other tissue-specific changes, including citrate synthase and fatty acid synthase upregulation, but pyruvate kinase downregulation at 30 d in the LP group and carnitine palmitoyl transferase 1b downregulation at 90 d. These gene alterations, together with previously described changes in gene expression in skeletal muscle, may account for the metabolic adaptations in response to maternal LP diet and highlight the occurrence of persistent transcriptional defects in key metabolic genes that may contribute to the development of metabolic alterations during the adult life as a consequence of perinatal malnutrition.

CONCLUSION

We conclude that perinatal malnutrition relays long-lasting transcriptional alterations in metabolically active organs, *i.e.*, liver and adipose tissue.

**Key words:** Metabolic adaptation; Phenotype plasticity; Liver; Adipose tissue; Metabolism; Maternal protein undernutrition; Rats

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**Core tip:** Perinatal exposure to a poor nutritional environment predisposes to metabolic disease. Here, the expression of metabolism-related genes in adipose tissue and liver was investigated. We evaluated the alterations in gene expression of glycolytic/Krebs cycle genes, adipogenic and lipolytic genes in adipose tissue of offspring rats at 30 d/90 d of age, exposed to maternal low protein diet throughout gestation/lactation. We also evaluated expression of liver transcription factors and gluconeogenic genes. Persistent gene alterations were observed that may account for the metabolic adaptations in response to maternal low protein diet, highlighting the occurrence of persistent transcriptional defects as a consequence of perinatal malnutrition.

## INTRODUCTION

Perinatal malnutrition occurring during pregnancy and lactation not only has a negative impact on fetal development and neonatal growth but also relays long-lasting adverse effects resulting in increased susceptibility to cardiovascular and metabolic diseases in adulthood, as posited by the developmental origin of health and disease hypothesis[1,2]. Evidence from epidemiological cohorts, with the Dutch Famine Birth Cohort Study being the most relevant[3], and experimental animal models[4-6] support the idea that a poor nutritional environment during fetal and early postnatal life predisposes to cardiovascular and metabolic disease in adulthood. The occurrence of persistent epigenetic alterations has been proposed as one of the mechanisms linking *in utero* nutritional deprivation to increased risk of disease in adulthood. Individuals who were prenatally exposed to the Dutch famine during the 1944-1945 were shown six decades later to have lower DNA methylation of the imprinted *IGF2* gene in comparison to their siblings not exposed to the famine period[7]. Observational studies have suggested a link between poor fetal growth and the development of impaired glucose tolerance at the adult age in both sexes[8], and final evidence that maternal nutrition during gestation affects glucose metabolism in adult life was provided by the observation that an oral glucose load in adults exposed prenatally to the Dutch famine led to higher glycemic concentrations as compared to individuals being born around the same years but not exposed *in utero* to the 1944-1945 famine[9].

Work in animal models suggested that the link between early-life malnutrition and the increased risk of developing metabolic disease in adulthood may be mediated by persistent biochemical alterations in the main insulin-responsive tissues, including glycolytic and oxidative skeletal muscle fibers[10]. Exposure of mice to a maternal protein-restricted diet during gestation and lactation was shown to impact the morphological features and body distribution of white adipose tissue and to reduce the protein expression levels of most of the key insulin signaling proteins, including IRS1, the PI3K subunits p110 and p85, Akt1 (v-akt murine thymoma viral oncogene homolog 1) and its phosphorylated form on serine 473 in male offspring[11], resulting in an altered distribution and morphology of white adipose tissue[12]. In a similar way, perinatal low protein (LP) diet consumption contributed to fatty liver phenotype at the adult age, which the magnitude was related to the period of exposure to the LP diet[13].

The occurrence of alterations of glycemic control in humans, associated to the observation that perinatal malnutrition induces defects in the insulin signaling pathways in rodent models, prompted us to evaluate whether the main metabolic pathways are affected by a LP diet administered to dams during pregnancy and lactation. In a previous study, we reported that gene and protein expression of enzymes participating in glucose and fatty acid metabolism in skeletal muscle were altered at short-term (30 d) and long-term (90 d) timepoints in male rat offspring exposed to a maternal LP diet during gestation and lactation[10]. Interestingly, we observed, both in soleus and extensor digitorum longus

skeletal muscle, a LP-induced, long-lasting downregulation of pyruvate dehydrogenase kinase 4 (*PDK4*), an enzyme leading to allosteric deactivation of the pyruvate dehydrogenase complex *via* phosphorylation and hence a redirection of the metabolic flux from catabolic Krebs cycle to anabolic pathways[14].

To obtain a wider description of the effects of perinatal LP diet on insulin-responsive tissues, the main goal of the present study was to evaluate the short-term and long-term effects of a LP diet during gestation and lactation on the expression of key genes involved in the metabolism of glucose and fatty acid in the liver and adipose tissue of male rat offspring. We demonstrate the occurrence of long lasting alterations of gene expression in both tissues, up to 90 d of age, that reflect the persistence of altered metabolism in the offspring consequent to *in utero* and early-life exposure to deleterious nutritional conditions.

## MATERIALS AND METHODS

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Centre (protocol 23076 062778/2014-38), Federal University of Pernambuco, Brazil. All efforts were made to minimize animal discomfort and the number of animals used; in addition, we followed the Guidelines for the Care and Use of Laboratory Animals.

### *Animals*

Male albino Wistar rats (*Rattus novergicus*) were obtained from the Academic Center of Vitoria de Santo Antão animal facility, Federal University of Pernambuco, Brazil. Animals were housed at 22 ± 1 °C with a controlled light–dark cycle (dark 18:00–06:00 h). Standard laboratory chow (52% carbohydrate, 21% protein and 4% lipids-Labina, Purina Agriband, São Paulo, Brazil) and water were administered *ad libitum*. Groups were divided according to their mother's diet: control pups from dams fed a 17% protein diet ($n$ = 5, normal protein group, NP), and LP pups from dams fed an 8% casein diet ($n$ = 5, low protein, LP) during gestation and lactation. Diets were prepared at the Laboratory of Experimental Nutrition-Center of Vitoria de Santo Antão, Federal University of Pernambuco, according to the American Institute of Nutrition–AIN-93 dietary guidelines[15].

During suckling, offspring was maintained as litters of eight pups of both sexes to ensure standardized nutrition until weaning. At weaning (21 d postpartum), three to four male offspring of each litter were housed in collective cages and received standard diet and water *ad libitum*. The experimental groups consisted of one or two male rats from each mother. Female offspring were not included in the present study. All experimental analyses were performed in adipose tissue and liver collected from male rats sacrificed either at 30 d old or 90 d old by decapitation. All rats were euthanized between 14:00-17:00 after a 4-5 h fasting period. The liver and visceral adipose tissue were carefully dissected, snap-frozen in liquid nitrogen and stored at –80 °C until RNA extraction.

### *RNA extraction, reverse transcription and quantitative PCR*

Total RNA was extracted from liver and visceral adipose tissue with Tripure reagent [Sigma-Aldrich (Roche), St. Quentin Fallavier, France] according to the manufacturer's instructions. Briefly, 10 µL of Tripure per milligram of tissue was added, and the resulting suspension was homogenized using a Precellys Lysing kit (Bertin, Montigny-le-Bretonneux, France) according to the manufacturer's instructions. After grinding, 1/4 volume of chloroform was added. They were vortexed 3 × 15 s, incubated at room temperature for 5 min and centrifuged for 15 min at 15000 g at 4 °C.

RNA was precipitated by addition of 1/2 volume of isopropanol (Carlo Erba, Val-de-Reuil, France) and centrifugation (15 min at 15000 g at 4 °C). Supernatants were used for protein extraction and RNA-containing pellets were washed sequentially with 70% and 95% ethanol (Carlo Erba), dried and dissolved in 100 µL RNase-free water. RNA concentration and purity (260/280 nm absorbance ratio) was determined on a Nanodrop 2000 (Thermo-Fisher).

Reverse transcription was performed using an RT-Takara kit (Primescript TM, Takara) using 1 µg of RNA as template and following the manufacturer's instructions. Briefly, samples were heated for 10 min at 65 °C. Samples were mixed with 4 µL PrimeScript Buffer 5 ×, 1 µL oligodT (50 µM), 4 µL random hexamers and 1 µL of PrimeScript RT Enzyme Mix followed by a 15 min incubation at 37 °C and 15 s at 85 °C. RNA was removed by incubation with 1 µL of RNase H for 20 min at 37 °C. Reverse transcription reactions were brought to 200 µL final volume by adding RNase free water and stored at -20 °C. Real-time quantitative PCR (qPCR) amplification was performed using a Rotor-Gene Real-Time PCR System (Labgene Scientific Instruments, Archamps, France). Sequences of primers used in this study are available upon request.

Reactions were incubated at 95 °C for 10 min, followed by 40 cycles of denaturation (95 °C, 10 s), annealing (58-62 °C depending on the primer sets, 30 s) and elongation (72 °C, 30 s). mRNA expression levels of *PDK4*, citrate synthase (*CS*), carnitine palmitoylacyltransferase 1b, acetyl-CoA carboxylase, fatty acid synthase, leptin, insulin receptor, phosphofructokinase, beta hydroxyacyl-coenzyme-A dehydrogenase, peroxisome proliferator-activated receptor-alpha coactivator 1 alpha, peroxisome proliferator-activated receptor-alpha, forkhead box protein O1, hepatocyte nuclear factor 4, glucose 6-phosphatase, phosphoenolpyruvate carboxykinase and pyruvate kinase L/R were measured on total RNA extracted from adipose tissue and liver. qPCR results from each gene (including the housekeeping gene) were expressed as arbitrary units derived from a standard calibration curve derived from a reference sample. Reference samples were generated by mixing 10 µL aliquots from ten cDNA samples, five from the NP group and five from the LP group. qPCR for each sample was carried out in duplicate. Gene expression data were normalized using ribosomal protein L19 as a housekeeping gene. As a further control, qPCR amplicons were analyzed by agarose gel to validate the amplicon size.

***Statistical analysis***

Statistical analysis was conducted with GraphPad Prism 5 program for Windows (GraphPad Software®. Inc., La Jolla, CA, United States). Exploratory data analysis was used to identify possible inaccurate information and the presence of outliers and to test the assumption of normality in all data distributions. Kolmogorov-Smirnov and Shapiro-Wilk normality tests were applied in total sample. Statistical significance was evaluated using analysis of variance ANOVA two-way test with maternal diet (low/normal protein) and age (30 d and 90 d) as factors. Bonferroni's post hoc test was used. The values are presented as mean and standard error means, and $P$ values < 0.05 were considered statistically significant. $P$ values < 0.05 are denoted as "a" in figures; $P$ values < 0.01 are denoted as "b" in figures.

## RESULTS

### *Perinatal LP diet in rats programs a lower body weight in the offspring*

We applied a model of perinatal protein restriction to rat dams throughout pregnancy and lactation followed by a switch to a NP diet after weaning as schematically represented in Figure 1.

In spite of the administration of a NP diet after weaning, the LP group displayed a lower bodyweight both at 30 d [NP: 106.3 ± 17.1 g, LP: 87.3 ± 6.0 g; $P$ < 0.05, unpaired $t$-test, $n$ = 12 (NP) and 6 (LP)] and 90 d of age (NP: 326.7 ± 22.6 g, LP: 306.2 g; $P$ < 0.05, unpaired $t$-test, $n$ = 8 for both groups).

### *Perinatal LP diet reprograms gene expression patterns in the adipose tissue*

The gene expression of two key enzymes linking the glycolytic pathway to the Krebs cycle, *PDK4* and *CS*, was evaluated in adipose tissue at different ages (Figure 2).

In NP rats, *CS* and *PDK4* genes displayed a time-dependent downregulation, with mRNA expression at 90 d being lower than at 30 d ($P$ = 0.057 for *PDK4* and < 0.01 for *CS*; Figure 2). In comparison, the maternal LP diet, by inducing a significant downregulation of both genes at 30 d, abolished the time-dependent downregulation of both genes. These results indicate that the glycolytic flux may be altered in LP offspring.

On the contrary, genes related to fatty acid metabolism, while showing a time-dependent downregulation with lower expression at 90 d, were not affected by the administration of a LP diet during pregnancy and lactation (Figure 3).

These results indicate that the glycolytic flux may be altered in the adipose tissue of LP offspring while fatty acid metabolism is not affected. As body weight of LP offspring remained lower at 30 d and 90 d as compared to NP offspring, we also evaluated the gene expression levels of leptin and observed a quasi-significant decrease of the hormone's gene expression ($P$ = 0.052; Figure 4).

### *Perinatal LP diet reprograms gene expression patterns of multiple pathways in the liver*

The expression of genes of the glycolytic pathway and Krebs cycle was evaluated in the liver at 30 d and 90 d (Figure 5). As observed in adipose tissue, *PDK4* expression was reduced in the LP group at 30 d. Conversely, *CS* was significantly upregulated at 30 d in the LP group but then returned to levels

comparable to the NP group at 90 d. The phosphofructokinase gene and insulin receptor gene did not show any difference between the two groups.

In the liver, genes related to fatty acid metabolism were also significantly modulated by the perinatal exposure to a LP diet (Figure 6). Fatty acid synthase, that displayed a strong age-dependent downregulation in both groups, is strongly upregulated in the LP group at 30 d, but then returned to levels comparable to the NP group at 90 d. On the contrary, carnitine palmitoylacyltransferase 1b (fatty acid transporter) showed a significant age-dependent increase in the NP group and was strongly downregulated at 90 d in the LP group.

Transcriptional patterns in the liver are orchestrated by a group of key transcription factors that include peroxisome proliferator-activated receptor-alpha coactivator 1 alpha, forkhead box protein O1 and hepatocyte nuclear factor 4. None of these genes were significantly affected in the LP group, and the time-dependent decrease of the gene expression of peroxisome proliferator-activated receptor-alpha coactivator 1 alpha was maintained in both LP and NP groups (Figure 7).

The liver is the quantitatively major organ responsible for gluconeogenesis, providing glucose supply during starvation. We evaluated the impact of the LP diet on gluconeogenic genes by measuring mRNA levels of glucose 6-phosphatase and *PEPKC* without detecting any LP-induced defect. However, pyruvate kinase L/R was significantly downregulated in the LP group at 30 d, suggesting an accumulation of phosphoenolpyruvate and potentially a higher gluconeogenic rate at 30 d due to higher abundance of the precursor (Figure 8).

## DISCUSSION

The developmental origin of health and disease model supports the idea that exposure in the critical periods of development, represented by the prenatal and early postnatal life, to a poor nutritional status, toxic substances, drugs or other kind of stress can predispose to the development of disease states, including metabolic syndrome and diabetes during adult life[2]. In particular, early-life undernutrition, especially when associated to a nutritional transition leading to obesity, is associated to a higher incidence of diabetes in adult life[16]. One of the mechanisms proposed to explain such long lasting effects is the development of epigenetic alterations that sustain alterations of gene expression patterns from the young age into adulthood[17,18] by causing persistent alterations of DNA methylation patterns, histone post-translational modifications and microRNA patterns[19,20].

In a previous study, using a rat model of perinatal protein undernutrition throughout gestation and lactation, there were changes in the gene and protein expression levels of key enzymes of glycolysis and fatty acid oxidation pathways in the skeletal muscle of the progeny, observing both postnatal acute effects (at 30 d of age) and chronic effects (at 90 d of age), the latter being representative of adaptive processes[14]. Specifically, oxidative soleus muscle responded to a LP maternal diet by downregulating hexokinase 2 and *PDK4* up to 90 d of age. For glycolytic extensor digitorum longus,

the effects of a LP maternal diet were more pronounced at 30 d of age with a similar downregulation of genes coding for enzymes of the glycolytic pathway[10].

To obtain a more exhaustive description of the transcriptional disturbances induced by a perinatal LP diet in insulin-responsive tissues, we have now investigated the transcriptional changes resulting from a prenatal and postnatal exposure to LP in visceral adipose tissue and liver.

A key finding of our study is the short-term downregulation of *PDK4*, observed both in liver and adipose tissue and previously detected in soleus and extensor digitorum longus. PDK4 by phosphorylating the pyruvate dehydrogenase complex inhibits its activity and the resulting production of acetyl CoA. In the LP condition, PDK4 downregulation would therefore favor the activity of the pyruvate dehydrogenase complex and increase the glycolytic flux into the Krebs cycle[21,22].

Our observations pointing to a *PDK4* modulation in the LP diet condition underscores the central role of this enzyme in regulating metabolic flexibility, which was also observed in the heart[23,24]. Interestingly, the first enzyme of the Krebs cycle, CS appears to be upregulated in the liver, while in the adipose tissue is downregulated, thus favoring the use of newly synthesized acetyl CoA as a lipogenic substrate[21]. To keep with this hypothesis, we observed a parallel decrease, at 30 d of age, of carnitine palmitoylacyltransferase 1b, the rate-controlling enzyme of long-chain fatty acid beta-oxidation pathway. At the more advanced age of 90 d, such transcriptional changes in the adipose tissue were lost, but a decrease in the expression of the gene coding for leptin was observed, which neared statistical significance ($P$ = 0.052, Figure 4). We hypothesize that decreased leptin gene expression observed at 90 d of age in the LP group may be a compensatory mechanism to induce higher food uptake in the LP animals as this group has a significantly lower body weight both at 30 d and 90 d of age.

The liver also shows gene expression alterations that would favor anabolic pathways. At 30 d, fatty acid synthase is upregulated in the LP group suggesting increased hepatic lipogenesis. At the same time, gluconeogenesis may also be increased in the LP group. While phosphoenolpyruvate carboxykinase and glucose 6-phosphatase were not upregulated, we observed downregulation of the pyruvate kinase L/R at 30 d, which may favor the accumulation of phosphoenolpyruvate and thus funneling of this glycolytic intermediate into gluconeogenesis.

Taken together, the gene expression changes that we have observed in the liver and adipose tissue in male rats submitted to perinatal LP undernutrition suggest the occurrence of improved lipogenesis (in adipose tissue) and gluconeogenesis (in liver) that may provide a compensatory effect to counteract the early-life exposure to the perinatal LP diet.

## ARTICLE HIGHLIGHTS

***Research background***

Perinatal exposure to a poor nutritional environment predisposes the progeny to the development of metabolic disease at the adult age, both in experimental models and humans. Numerous adaptive responses to maternal protein restriction have been reported in metabolic tissues. However, the expression of glucose/fatty acid metabolism-related genes in adipose tissue and liver needs to be described.

***Research motivation***

To evaluate the metabolic impact of perinatal malnutrition, we determined malnutrition-associated gene expression alterations in liver and adipose tissue.

***Research objectives***

In the present study, we evaluated the alterations in gene expression of glycolytic/Krebs cycle genes (pyruvate dehydrogenase kinase 4 and citrate synthase), adipogenic and lipolytic genes and leptin in the adipose tissue of offspring rats at 30 d and 90 d of age exposed to maternal isocaloric low protein (LP) diet throughout gestation and lactation. We also evaluated these genes in the livers of the same animals as well as the gene expression of the transcription factors peroxisome proliferator-activated receptor gamma coactivator 1, forkhead box protein O1 and hepatocyte nuclear factor 4 and of gluconeogenic genes.

***Research methods***

Research methods included animal husbandry, RNA extraction, reverse transcription and quantitative PCR and appropriate statistical analysis.

***Research results***

In the adipose tissue, we observed a transitory (*i.e.*, at 30 d) downregulation of pyruvate dehydrogenase kinase 4, citrate synthase and carnitine palmitoylacyltransferase 1b gene expression. Such transcriptional changes did not persist in adult LP rats (90 d), but we observed a tendency towards a decreased gene expression of leptin ($P$ = 0.052). The liver featured some gene expression alterations comparable to the adipose tissue, such as pyruvate dehydrogenase kinase 4 downregulation at 30 d, and displayed other tissue-specific changes, including citrate synthase and fatty acid synthase upregulation, but pyruvate kinase downregulation at 30 d in the LP group and carnitine palmitoylacyltransferase 1b downregulation at 90 d. These gene alterations, together with previously described changes in gene expression in skeletal muscle, may account for the metabolic adaptations in response to maternal LP diet and highlight the occurrence of persistent transcriptional defects in key metabolic genes that may contribute to the development of metabolic alterations during the adult life as a consequence of perinatal malnutrition.

***Research conclusions***

We conclude that perinatal malnutrition relays long-lasting transcriptional alterations in metabolically active organs, *i.e.*, the liver and adipose tissue.

***Research perspectives***

Our observations lay the basis for possible future research directed to human studies.

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**Figure Legends**

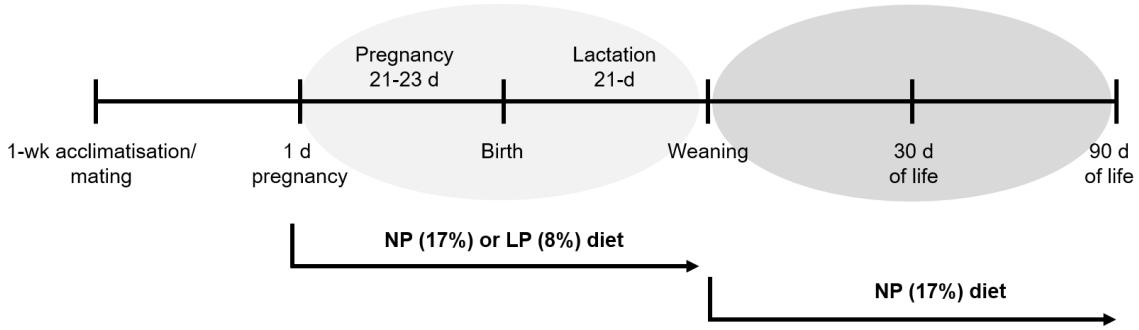


**Figure 1 Schematic diagram of the experimental protocol in Wistar rats.** Rats were exposed to maternal low protein diet during gestation and lactation and then switched to a normal protein diet. NP: Normal protein; LP: Low protein.

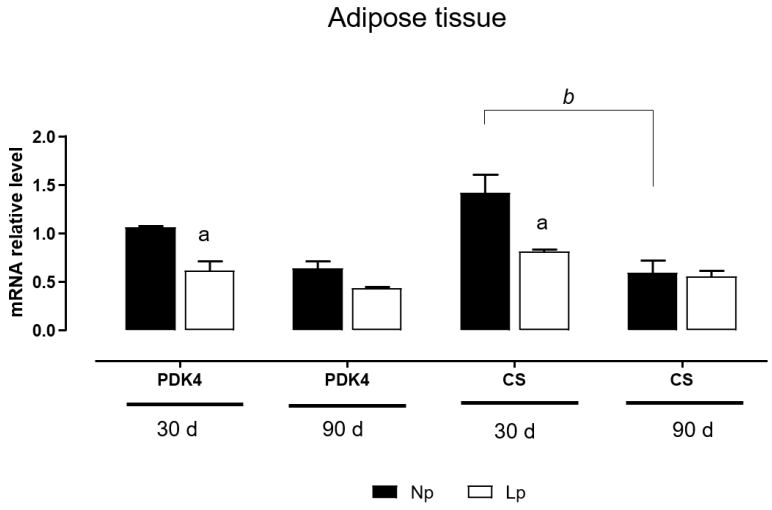


**Figure 2 Expression of pyruvate dehydrogenase kinase 4 and citrate synthase mRNA in adipose tissue of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and their RNA extracted from adipose tissue was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post-hoc test was used. Differences between diet groups are indicated by an asterisks; differences between ages are indicated by bars. $^{a}P < 0.05$, $^{b}P < 0.01$. NP: Normal protein; LP: Low protein; PDK4: Pyruvate dehydrogenase kinase 4; CS: Citrate synthase.

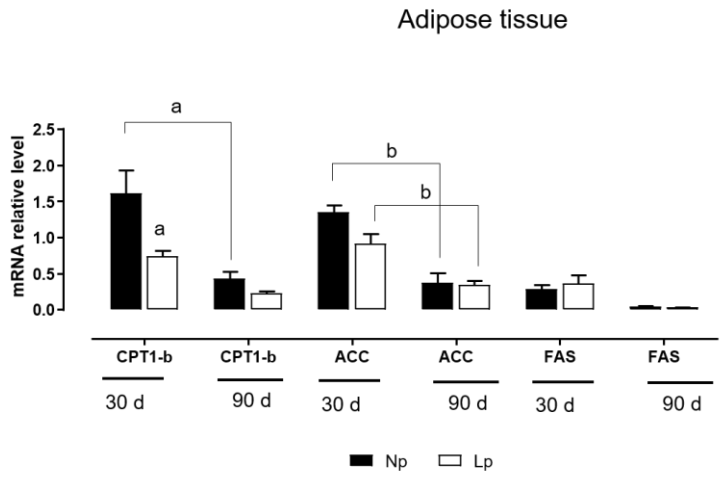


**Figure 3 Expression of adipogenic and lipolytic genes in the adipose tissue of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression of carnitine palmitoylacyltransferase 1, acetyl-CoA carboxylase and fatty acid synthase in adipose tissue was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by an asterisks; differences between ages are indicated by bars. $^{a}P < 0.05$, $^{b}P < 0.01$. NP: Normal protein; LP: Low protein; FAS: Fatty acid synthase; CPT1-b: Carnitine palmitoylacyltransferase 1b; ACC: Acetyl-CoA carboxylase.

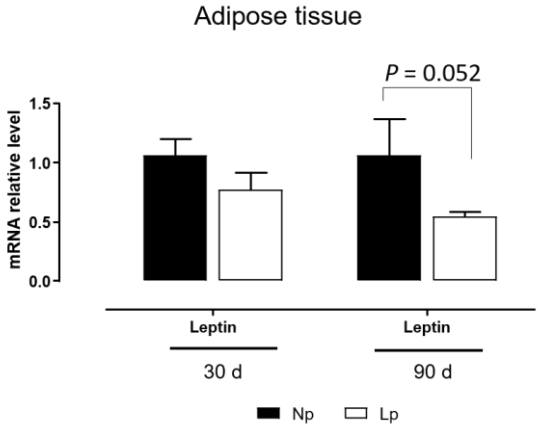


**Figure 4 Gene expression of leptin in adipose tissue of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression of leptin from adipose tissue was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d,

90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by asterisks. NP: Normal protein; LP: Low protein.

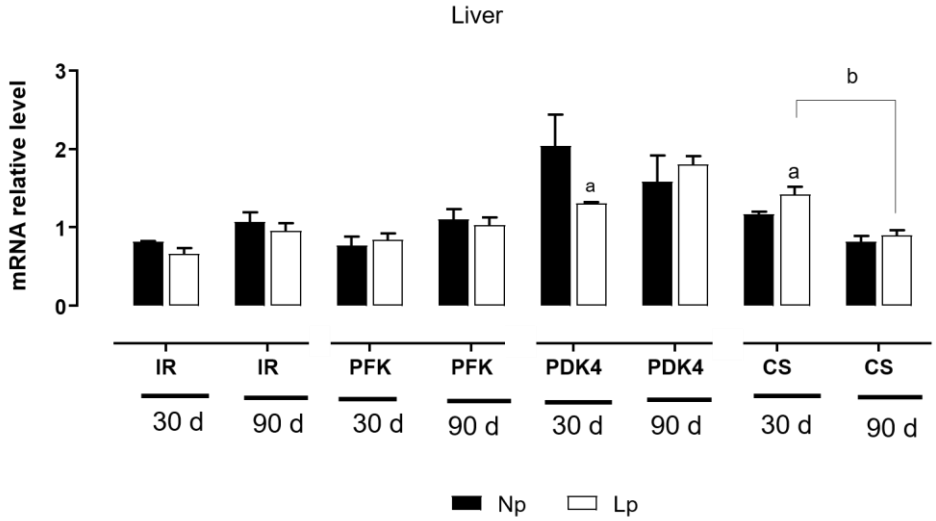


**Figure 5 Expression of insulin receptor, glycolytic genes and Krebs cycle genes in the liver of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression of insulin receptor, phosphofructokinase, pyruvate dehydrogenase kinase 4 and citrate synthase was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by asterisks; differences between ages are indicated by bars. $^{a}P < 0.05$, $^{b}P < 0.01$. NP: Normal protein; LP: Low protein; CS: Citrate synthase; PDK4: Pyruvate dehydrogenase kinase 4; PFK: Phosphofructokinase; IR: Insulin receptor.

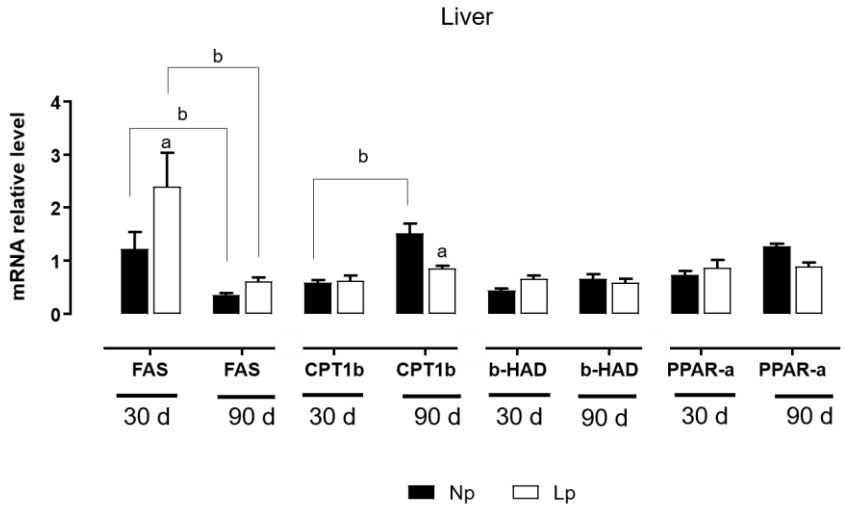


**Figure 6 Expression of genes related to lipid metabolism in the liver of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression was analyzed by

quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by asterisks; differences between ages are indicated by bars. [a]$P < 0.05$, [b]$P < 0.01$. NP: Normal protein; LP: Low protein; FAS: Fatty acid synthase; CPT1-b: Carnitine palmitoylacyltransferase 1b; b-HAD: Beta hydroxyacyl-coenzyme-A dehydrogenase; PPAR-a: Peroxisome proliferator-activated receptor-alpha.

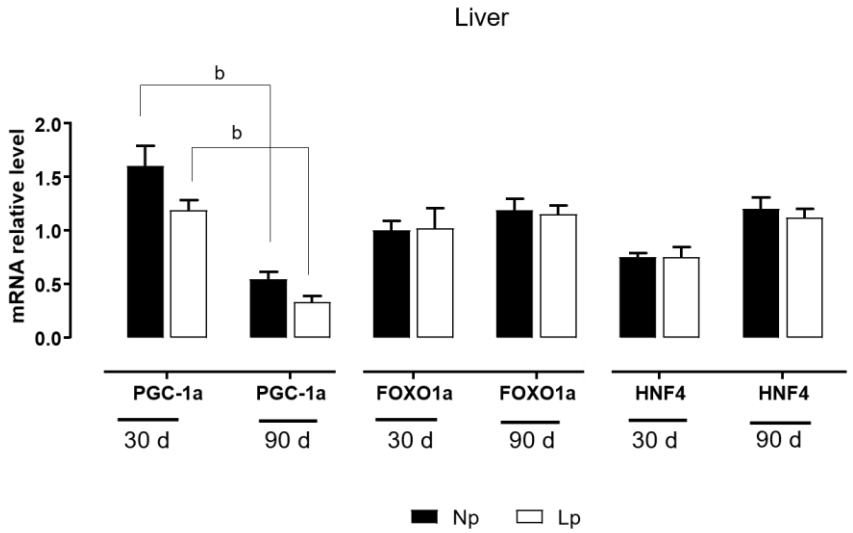


**Figure 7 Expression of key transcription factors in the liver of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression of peroxisome proliferator-activated receptor-alpha coactivator 1 alpha, forkhead box protein O1 and hepatocyte nuclear factor 4 was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by asterisks; differences between ages are indicated by bars. [a]$P < 0.05$, [b]$P < 0.01$. NP: Normal protein; LP: Low protein; PGC-1α: Peroxisome proliferator-activated receptor-alpha coactivator 1 alpha; FOXO1a: Forkhead box protein O1; HNF4: Hepatocyte nuclear factor 4.

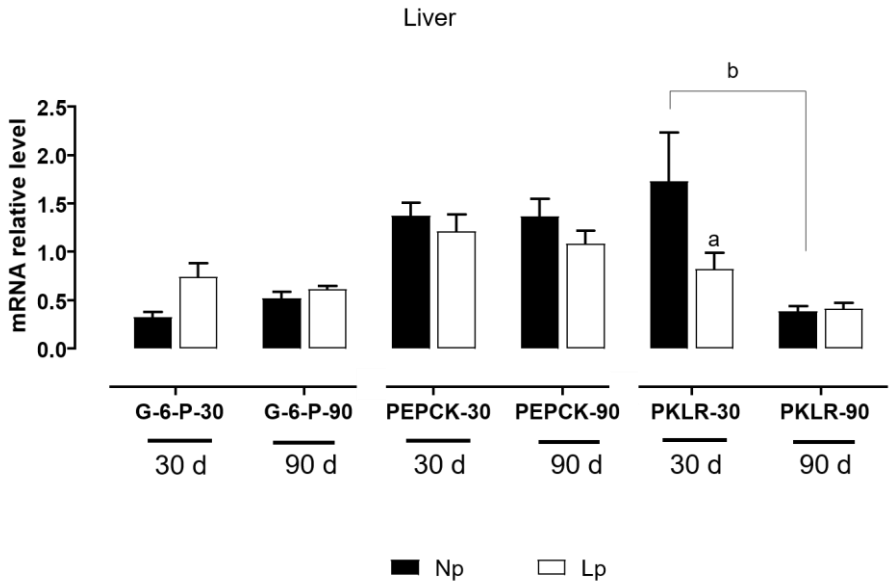


**Figure 8 Expression of gluconeogenic genes in the liver of rats.** Rats were exposed to maternal low protein diet during gestation and lactation, and gene expression of glucose 6-phosphatase, phosphoenolpyruvate carboxykinase and pyruvate kinase L/R was analyzed by quantitative reverse transcription PCR. Data are shown as mean ± standard error means and analyzed by two-way ANOVA with the mother's diet (normal protein; low protein) and age (30 d, 90 d) as variable factors. Bonferroni's post hoc test was used. Differences between diet groups are indicated by asterisks; differences between ages are indicated by bars. $^{a}P < 0.05$, $^{b}P < 0.01$. NP: Normal protein; LP: Low protein; G6Pase: Glucose 6-phosphatase; PEPCK: Phosphoenolpyruvate carboxykinase; PKLR: Pyruvate kinase L/R.

# APÊNDICE B – PHYSICAL ACTIVITY DURING PREGNANCY AND METABOLIC CONSEQUENCES ON MOTHERS AND/OR OFFSPRING: A SYSTEMATIC REVIEW

**Title: Physical activity during pregnancy and metabolic consequences on mothers and/or offspring: a systematic review**

**Short-title:** Maternal physical activity and metabolism

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**Abstract**

Maternal physical activity is associated to a better aerobic condition, cardiovascular fitness and metabolic homeostasis. These adaptations can potentially alter foetus development through epigenetic mechanisms supported by a higher nutritional supply through foetal-placental interaction. The objective of this review is to evidence the protocols of maternal physical activity that have been used and discuss its interaction to the mother and/or offspring's metabolism. This review was conducted according to the Preferred Reporting Items for Systematic-reviews and Meta-Analysis (PRISMA) guidelines. Search-terms included *physical activity* or *exercise* and *gestation* or *pregnancy* and *litter* or *offspring*. Studies that utilized any protocol of physical activity before and/or during gestation and/or lactation and evaluated its metabolic outcomes on the mothers and/or offspring were included. Information on strain, age or body weight, description of the protocol of physical activity, and other additional interventions in association were extracted from the studies. Eleven studies were included after a search conducted between June and July 2020. Results show that low-to-moderate physical activity before and during gestation reduced the mother's triglyceride concentrations. The maternal physical activity also ameliorates the offspring's glycaemia and insulin sensibility, although it was associated with a reduced body weight at birth as well.

**Keywords:** Exercise, pregnancy, offspring, rats, metabolism

## Introduction

Physical activity is referred to any movement made by the skeletal muscle system that demands an energy expenditure above the basal energy expenditure (Leandro, Amorim et al. 2009). When the physical activity is realized systematically, marked out by effort intensity, frequency, volume, and time, it is classified as physical exercise or physical training (if there is a chronogram with an objective and an end for the last) (Leandro, Amorim et al. 2009). Organic responses to these stimuli depends on its intensity, frequency, volume, and time invested. The most common way of measuring physical activity intensity is through $VO_{2max}$ (maximum oxygen consumption) or $HR_{max}$ (maximum heart rate)(Leandro, Amorim et al. 2009). A physical activity is considered of low-intensity when realized at 20-50% of an individual $VO_{2max}$ or $HR_{max}$, moderate intensity at 50-75%, and high intensity above 75% $VO_2/HR_{max}$ (Leandro, Amorim et al. 2009). Currently, the practices of physical activity is encouraged by the scientific community as a healthy life-style for all people, including pregnant women (2015).

The recommendations of the American College of Obstetricians and Gynecologists (2015) is that women with low-risk gestation may safely practice low-to-moderate physical exercise (30% to 65% of $VO_{2max}$) for 30 minutes per day. In fact, regular moderate exercise in pregnant women is associated with a low susceptibility to develop gestational diabetes by enhancing insulin sensibility and fostering non-insulin-stimulated glucose uptake (Wojtaszewski, Hansen et al. 2000; Richter, Nielsen et al. 2004). The benefits from the physical activity on the mother are now well known, which includes an improved aerobic condition, and cardiovascular fitness (Dempsey, Butler et al. 2004; Ruchat, Davenport et al. 2012; Mishra and Kishore 2018). Nonetheless, the potential outcomes on offspring are still in need to be better elucidated.

An active maternal lifestyle modulates physiological adaptations to the foetal placental growth by increasing nutrient and oxygen availability to the foetus (Clapp, Schmidt et al. 2004; Haakstad, Voldner et al. 2007). However, the effects of maternal exercise on foetal oxygenation and foetal placental growth are dependent on duration and intensity of the exercise (Clapp, Kim et al. 2002; Clapp 2003). A recent study shows that voluntary physical activity during pregnancy and lactation improves glucose tolerance and insulin sensibility in adult offspring (Carter, Lewis et al. 2012). Although the effects of submaximal exercises during gestation on offspring health are contradictory, it has been proven that controlled mild pace exercises during pregnancy is safe for humans and animals (Szymanski and Satin 2012).

The experimental models of physical activity help us to better understand the interaction between the environment and the animal's metabolic adaptations. However, results from different studies tends to vary due to different methodological procedures. As such, we decided to conduct a systematic review in order to put together what has been done in terms of protocols of maternal physical activity, and identify the repercussions of those experiments on the metabolism of the mothers and/or pups. This review reports maternal and offspring outcomes in an effort to recognise possible relationships with the physical activity and facilitate future researches.

**Methods**

This is a systematic review relating to maternal physical activity and its metabolic responses on rats undertaken according with the Preferred-Reporting-Items-for-Systematic-Reviews-and-Meta-Analysis (PRISMA) statement. The focus of the review was determined before the research by the question: what kind of protocols of physical activity during pregnancy have

been utilized and what are the impacts that they might cause on the mothers and their offspring?

*Eligibility criteria*

Eligibility criteria were defined previously to prevent the risk of bias from the reviewers. Due to the nature of our question, this review included published original studies that were conducted exclusively with female rats submitted to a protocol of physical activity before and/or during gestation and/or lactation. The evaluation of metabolic parameters on mothers and/or offspring was also a requirement for eligibility. Studies without a clear description of the protocol of physical activity and how these activities were executed were excluded as well as those that were not available on the databases.

*Search terms*

The search was conducted between June and July, 2020 using the electronic databases: Pubmed/Medline, Science Direct (Elsevier), Springer Link (Springer Nature), and Scopus. The search terms and each of their entry terms (synonyms, derivation of theme, and closely related words) found across the different databases were combined with the Boolean operator 'OR', and search separately as 'search sets'. The search sets were then combined with the Boolean operator 'AND', which gave us the final collection of possible articles (Table 1).

*Articles selection*

The screening phase of the selection was made by two different reviewers on which title and abstract of every article was read. Every original article that utilized a protocol of maternal physical activity was selected for eligibility. After the initial phase, the eligibility of the articles was made by both reviewers from reading the full-text for possible inclusion in this systematic review. When there was a disagreement between the two reviewers about the eligibility of an article, a third reviewer was consulted.

**Table 1.** Search strategy results

| Search sets | Databases | | | |
|---|---|---|---|---|
| | **Pubmed** | **Science Direct** | **Springer Link** | **Scopus** |
| Exercise OR Physical activity OR Physical exercise OR Exercise training | 618,145 | 562,808 | 158,928 | 1,114,142 |
| Mother OR Pregnancy OR Gestation | 1,227,160 | 655,347 | 463,181 | 1,483,288 |
| Animal model OR Laboratory animal model OR Experimental animal model | 805,736 | 284,668 | 408,269 | 1,216,672 |
| Offspring OR Litter | 98,746 | 223,710 | 158,468 | 391,833 |
| Combined search result | 171 | 363 | 1,547 | 2,122 |

*Data extraction*

The extraction of the data was conducted by one reviewer, and then checked by two different reviewers. The extracted data included the strain of the utilized animals, the age or weight of those animals, the experimental design (protocol of maternal physical activity used with or without an additional intervention), and the metabolic outcomes on the mothers and/or the litters.

## Results

*Study selection and extracted data*

The combined result of the search on the databases returned a total of 4,203 articles. After the exclusion of duplicates and the initial screening, 100 articles remained as possible relevant studies to be selected. Followed by the full-text reading and the application of the eligibility criteria, 11 articles were included in this systematic review (Figure 1).

Extracted methodological data of the studies are detailed in Table 2. The publication period of the selected studies was between 1989 to 2017, with most of them published in the past 10 years. Rat strains utilized was not so varied: Wistar rats (n=8), Sprague-Dawley (n=3). The studies utilized female rats at the age of 60 to 100 days old, and mostly weighting around the range of 170g-200g. The method of physical activity chosen by most studies was the program of running on a motor-driven treadmill (n=6), followed by the swimming program on a cage with water (n=3), and the voluntary physical activity on a running wheel (n=2). Only 3 studies did not have any other type of methodological intervention aside from the maternal physical activity. Diabetes induction prior gestation was utilized by 5 studies, 3 other studies

utilized 3 different types of intervention as followed: overnutrition, obesity, and undernutrition.

*Outcomes synthesis*

Data on the maternal and offspring responses to physical activity during gestation are detailed in Table 3. The studies presented either no difference in body weight gain or reduced body weight gain during pregnancy on active mothers (Uriu-Hare, Keen et al. 1989; Denadai, Da Cruz Piçarro et al. 1994; Vanheest and Rodgers 1997; Damasceno, Silva et al. 2012; Falcão-Tebas, Tobias et al. 2012; Quiclet, Siti et al. 2016; Corvino, Damasceno et al. 2017). Five studies showed no difference in the glycaemia of mothers that performed physical activity during gestation to those who did not (Uriu-Hare, Keen et al. 1989; Denadai, Da Cruz Piçarro et al. 1994; Vanheest and Rodgers 1997; Damasceno, Silva et al. 2012; Corvino, Damasceno et al. 2017). Only one study utilizing the protocol of voluntary physical activity presented a better glycaemia on mothers (Raipuria, Bahari et al. 2015). Five studies observed improvements on the active mother's lipidic profile (Vanheest and Rodgers 1997; Corvino, Volpato et al. 2015; Raipuria, Bahari et al. 2015; Quiclet, Siti et al. 2016; Ribeiro, Tofolo et al. 2017). Four studies also evidenced either a higher glucose tolerance and/or insulin sensibility on mothers that underwent a program of physical activity during pregnancy (Corvino, Volpato et al. 2015; Raipuria, Bahari et al. 2015; Corvino, Damasceno et al. 2017; Ribeiro, Tofolo et al. 2017).

One study found an increased in fetal weight of pups from active obese mothers (Uriu-Hare, Keen et al. 1989). However, four different studies evidenced a reduced body weight of pups born from mothers that undertook a program of physical activity during gestation (Denadai, Da Cruz Piçarro et al. 1994; Damasceno, Silva et al. 2012; Raipuria, Bahari et al.

2015; Corvino, Damasceno et al. 2017). One study found that pups from exercised diabetic mothers had a retarded growth rate in comparison to pups from sedentary diabetic mothers (Corvino, Volpato et al. 2015), and another study related that a high intensity exercise during gestation diminishes the number of pups per litter (Denadai, Da Cruz Piçarro et al. 1994). One study did not show any differences in body weight of pups born from active mothers or sedentary mothers (Vanheest and Rodgers 1997). Two studies did not encounter any glycaemia changes on the pups (Uriu-Hare, Keen et al. 1989; Quiclet, Siti et al. 2016). Yet physical activity during pregnancy was related to improvements on pup's glycaemia in five different studies (Vanheest and Rodgers 1997; Falcão-Tebas, Tobias et al. 2012; Carter, Qi et al. 2013; Raipuria, Bahari et al. 2015; Ribeiro, Tofolo et al. 2017) and one study found that pups born from active mothers have a better insulin sensitivity (Ribeiro, Tofolo et al. 2017). One study also evidenced a normalization of the mRNA expression of PGC1α, GLUT4, and MyoD1 of pups from active obese mothers in comparison to the reduced values of the pups from sedentary obese mothers (Raipuria, Bahari et al. 2015). An improved lipidic profile of the pups was also seen in three different studies (Falcão-Tebas, Tobias et al. 2012; Raipuria, Bahari et al. 2015; Ribeiro, Tofolo et al. 2017).

## Discussion

The aim of the present review was to compile the studies that have been utilizing the protocols maternal physical activity as an intervention, and analyse its responses on the mothers and/or offspring. Although different among them, three main types of protocols of physical activity were used (swimming, voluntary physical activity and running on a treadmill) and were mostly associated with another protocol to induce either gestational diabetes, undernutrition, and obesity, which resulted in a number of contrasting outcomes.

Most of the studies analysed did not show any difference in body weight gain throughout pregnancy among active and sedentary rats, but a few have observed that active mothers were lighter than its pairs. These results are in agreement with previous studies showing that undernutrition, long duration activities and submaximal exercises are associated with a reduced body weight gain (Bayol, Jones et al. 2004; Santana Muniz, Beserra et al. 2014). It can be explained by the fact that the energetic costs for those animals were way higher than the sedentary rats. Meanwhile, for most protocols utilized, the energy entering via food intake was balanced with the energy expended by the low-to-moderate physical activity, which resulted in a non-differentiation in body weight gain.

In clinical and animal studies, exercise has shown improvements to glucose and insulin homeostasis, and lipidic profile (Vega, Reyes-Castro et al. 2015; Mishra and Kishore 2018). This corroborates with the results presented by some of the selected studies. Adipose tissue and skeletal muscle play a decisive role in metabolism homeostasis and both are impacted by the physical activity. It includes anti-inflammatory effects and upregulation of the glucose transporter type 4 (GLUT4) expression in the skeletal muscle and adipose tissue, which causes a higher tissue responsiveness to insulin (Ren, Semenkovich et al. 1994; Hussey, McGee et al. 2011).

In the present review variable effects of maternal exercise on the pup's birth weight have been found, with most of them reporting a lower body weight. It is important to recognize that some of these studies also utilized the undernutrition, and the gestational diabetes induction on the rats, which is related to the occurrence of lower birth weight of the pups (Seghieri, Anichini et al. 2002; Bayol, Jones et al. 2004). One of the selected studies also found a reduced number of pups per litter, however the method chosen in the study was to utilize a protocol of physical exercise in submaximal intensity, which might not only cause a lower birth weight, but also a number of foetal resorption. It happens because in response to a

high intense physical activity stimulation, a great redistribution of blood flow to the skeletal muscle occurs, causing a temporary decrease in disponible oxygen and nutrient to the developing foetus, compromising its growth (Thomas, Clapp et al. 2008).

Voluntary maternal physical activity has shown to increase glucose tolerance and insulin sensibility in adult mice offspring (Carter, Lewis et al. 2012). Similarly, the same result was found in some of the studies analysed in this review, which also includes findings of improvements on the lipidic profile and expression of GLUT 4, Myod1, and PGC1α (Vanheest and Rodgers 1997; Falcão-Tebas, Tobias et al. 2012; Carter, Qi et al. 2013; Raipuria, Bahari et al. 2015; Ribeiro, Tofolo et al. 2017). These are important results since Myod1 is a marker related to myogenesis and muscle differentiation, while PGC1α increases mitochondrial biogenesis and GLUT4 enhances glucose tolerance, leading to a better metabolic homeostasis overall (Ren, Semenkovich et al. 1994; Eisele and Handschin 2014). These responses might be associated with epigenetic mechanisms supported by a higher maternal aerobic condition, body lean, placental volume, and oxygen and nutrient supply to the foetus (Bateson, Barker et al. 2004; Clapp, Schmidt et al. 2004; Haakstad, Voldner et al. 2007).

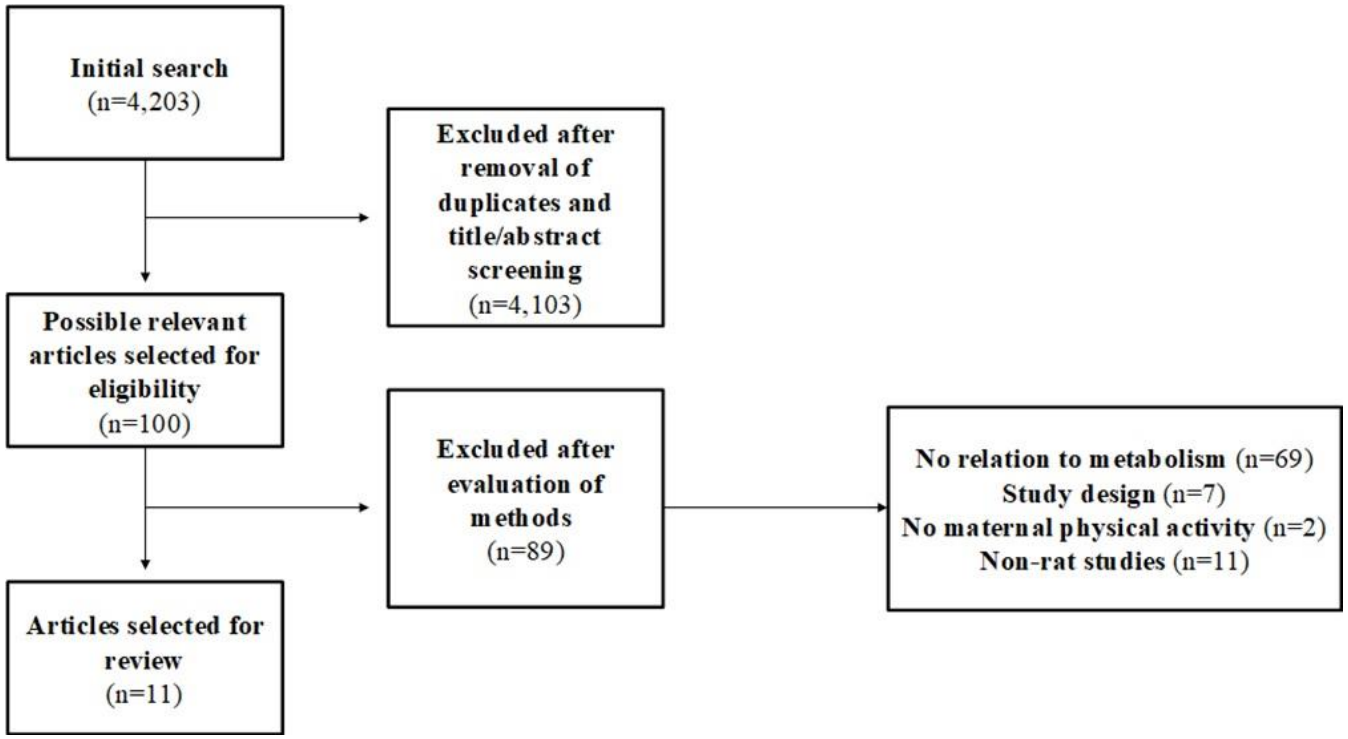


**Figure 1.** Flow diagram of each step taken for the search and selection of the articles.

**Table 2.** Methodological interventions described by the selected studies

| Reference | Strain | Age (days) or body weight (g) | Protocol of maternal physical activity | Additional interventions |
|---|---|---|---|---|
| Uriu-Hare et al., 1989 | Wistar rats | 260-280g | *Treadmill* (adaptation of 20 days prior gestation at 15min/day, 5days/week, 16m/min gradually increased to 45min/day, 5 days/week, 16m/min. The same program was maintained throughout pregnancy up to day 19) | Diabetes induction prior gestation (Streptozotocin intravenous administration) |
| Denadai et al., 1994 | Wistar rats | 60-day-old | *Treadmill* (adaptation of 30 days at 5min/day, 5days/week, 9m/min. During gestation trained group underwent a program for 30min/day, 5days/week, 90% VO2max. Pregnancy was interrupted at the 7th, 14th, and 20th day for 3 different groups) | — |
| VanHeest et al., 1997 | Sprague-Dawley rats | 150-175g | *Treadmill* (adaptation of 14 days at 15min/day, 5days/week, 10m/min up to 60min/day, 5days/week, 20m/min for 8 weeks. Same program continued during gestation) | Diabetes induction prior gestation (Streptozotocin intravenous administration) |
| Falcão-Tebas et al., 2012 | Wistar rats | 60-day-old | *Treadmill* (adaptation of 4 weeks prior gestation, 60min/day, 5days/week, 65%$VO_{2max}$; during gestation there was a progressive decrease in intensity and duration down to 20min/day, 30%$VO_{2max}$) | Undernutrition (animals fed with low-protein diet) |
| Damasceno et al., 2012 | Wistar rats | 200g | *Swimming* (adaptation of 1 month prior gestation on a cage with 10cm of water; the program continued 6days/week throughout pregnancy on a cage with 40cm water with a gradual increase on duration up to 60min of activity) | Diabetes induction prior gestation (Streptozotocin intravenous administration) |
| Carter et al., 2013 | Sprague-Dawley rats | 85-day-old | *Running wheel* (access to a running wheel for voluntary physical activity prior to gestation, during mating and gestation, and up to the postnatal day 12) | — |
| Corvino et al., 2015 | Wistar rats | 200g | *Swimming* (adaptation of 5 days prior gestation on a cage with 10cm of water; 3days/week, on a | Diabetes induction prior gestation (Streptozotocin |

| | | | | |
|---|---|---|---|---|
| | | | cage with 40cm water for 15min, followed by 15min of rest and a second 15min of activity during gestation) | intravenous administration) |
| Raipuria et al., 2015 | Sprague-Dawley rats | 160-170g | *Running wheel* (10 days prior gestation the rats were housed in a cage with a running wheel for voluntary physical activity where they remained until the delivery of the pups) | Obesity induction prior gestation (animals fed with high-fat diet) |
| Quiclet et al., 2016 | Wistar rats | 100-day-old | *Treadmill* (started 4 weeks prior gestation, 5days/week, speed and duration gradually increased to reach 25m/min and 60min; program was maintained until 18 days of gestation) | — |
| Corvino et al., 2016 | Wistar rats | 200g | *Swimming* (adaptation of 5 days prior gestation on a cage with 10cm of water; 3days/week, on a cage with 40cm water for 15min, followed by 15min of rest and a second 15min of activity during gestation) | Diabetes induction prior gestation (Streptozotocin intravenous administration) |
| Ribeiro et al.,2017 | Wistar rats | 70-day-old | *Treadmill* (adaptation of 1-2 weeks prior gestation, 10min/day, 3days/week, 10cm/s; and 30min/day, 3days/week, 30%$VO_{2max}$ during gestation) | Offspring overnutrition (number of pups reduced to 3 male per litter) |

**Table 3.** Analysis of the physical activity outcomes on the mothers and the offspring

| Reference | Mothers | Offspring |
|---|---|---|
| Uriu-Hare et al., 1989 | No effects on body weight during pregnancy; No differences on glucose blood concentration; lower insulin levels | Increased fetal weight of pups from diabetic dams; no effects on glucose blood concentration and fewer number of calcified skeletal sites at birth. |
| Denedai et al., 1994 | Reduced body weight gain during pregnancy and lower food intake. No differences in protein, glucose, cholesterol, and triglycerides plasmatic concentrations | Reduced number of pups at birth and lower body weight at the 7th, 14th, and 20th day of life. |
| VanHeest et al., 1997 | No differences in body weight gain, food intake, glycaemia, and insulinemia. Lower cholesterol blood concentrations | No differences in size and number of litters at birth; no significant effect on body mass, reduced glucose plasma concentration, and increased insulin plasma concentration at day 28 of age. |
| Falcão-Tebas et al., 2012 | Lower body weight gain | Lower body weight from 60th to 270th day; normalization of glycaemia and cholesterol in comparison to undernourished pups at 270 days of life. |
| Damasceno et al., 2012 | Reduced body weight gain during pregnancy; no difference in glycaemia; increased total proteins and muscular glycogen in exercised diabetic dams | Reduced fetal weight. |
| Carter et al., 2013 | — | Improved glycemia, glucose tolerance and insulin sensitivity in mature offspring (15 to 17 month old rats). |
| Corvino et al., 2015 | Lower triglyceride concentrations and improved insulin sensibility | Reduced body weight at birth. |
| Raipuria et al., 2015 | Reduced triglyceride concentrations, lower blood glucose | Lower body weight at birth; reduced visceral fat mass; reduced plasma triglyceride, blood glucose, and insulin |

| | | |
|---|---|---|
| | and insulin concentration with no lasting effect. | concentrations; normalization of mRNA expression of PGC1α, GLUT4 and MyoD1 in comparison to pups from obese dams at 19 days old. |
| Quiclet et al., 2016 | Reduction of fat depots, no effect in body weight | At 3 to 4 weeks old, there was no effects in glycemia and insulinemia, glucose tolerance and insulin sensitivity; it was observed lower pPKB/PKB ratio in the muscle with no difference in the liver, and lower islet insulin secretion in low-glucose and high-glucose conditions. |
| Corvino et al., 2016 | No differences in body weight and glycemia; improved glucose tolerance and insulin sensibility | Reduced body weight at birth. |
| Ribeiro et al., 2017 | Lower mesenteric fat pad stores and fasting glucose; improved glucose tolerance, insulin sensibility and $VO_{2max}$ | Lower fat tissue accretion; improved glucose tolerance, improved insulin tolerance, insulinemia and glycaemia in adult offspring (90 days old). |

GLUT4 (glucose transporter type 4) mRNA (messenger ribonucleic acid); MyoD1 (myoblast determination protein 1); PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha); PKB (protein kinase B); $VO_{2max}$ (maximal oxygen consumption)

## Conclusion

This systematic review provides a summary of studies that examined the different protocols of physical activity during pregnancy and its association to the metabolic responses on the mothers and/or offspring. In this context, low-to-moderate physical activity before and during gestation leads to a better body composition and metabolic homeostasis on the mother and offspring. However, in combination with gestational diabetes it is often associated to a lower birth weight, which is connected to metabolic disorders in adult life. Therefore, additional studies evaluating the further effects of physical activity in diabetic mother's offspring are needed to determine whether it is or not recommended in those situations.

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|---|---|

| type | |
|---|---|
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