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CAMILA CRISTINA IANONI MATIUSSO

PHYSICAL EXERCISE IN DIFFERENT PHASES CAN IMPROVE GLUCOSE HOMEOSTASIS IN ADULT MALE RATS

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Tese apresentada ao Programa de Pós-graduação em Ciências Biológicas (Área de concentração - Biologia Celular e Molecular) da Universidade Estadual de Maringá, para obtenção do grau de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. Paulo Cezar de Freitas Mathias

Coorientador: Dr. Douglas Lopes de Almeida

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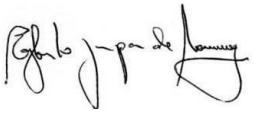

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Camila Cristina Ianoni Matiusso nasceu em 21/07/1993 em Maringá/PR. Possui graduação em Ciências Biológicas pela Universidade Estadual de Maringá (UEM) (2015). Concluiu o mestrado em ciências biológicas no ano de 2018, na Universidade Estadual de Maringá, com a dissertação intitulada "Bloqueio colinérgico durante a lactação atenua o desenvolvimento da obesidade em ratos adultos". Atualmente é doutoranda no Programa de Pós-graduação em Ciências Biológicas da Universidade Estadual de Maringá e professora de biologia celular, bioquímica e fisiologia na Universidade Cesumar (UNICESUMAR). Tem experiência na área de biologia celular e fisiologia, atuando principalmente nos seguintes temas: desnutrição, exercício físico, secreção de insulina e homeostase da glicose.

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Apresentação

Esta dissertação é composta de dois artigos científicos, sendo o primeiro uma revisão intitulada **"Effects of perinatal maternal exercise on glucose homeostasis in adult male offspring – what is trending in recent experimental studies?"**, e o segundo um trabalho experimental intitulado **"Moderate intensity and low frequency exercise improves glycemic homeostasis in adult wistar rats undernourished during lactation"**. Os trabalhos abordam sobre a plasticidade fisiológica do metabolismo, tema de estudo inserido no conceito DOHaD (*Developmental Origins of Health and Disease*). Neste sentido, a revisão discute os efeitos da atividade física feita pela mãe durante o período perinatal na homeostase glicêmica da prole na vida adulta. Adicionalmente, o trabalho experimental apresenta os benefícios na homeostase da glicose de uma atividade física de moderada intensidade e baixa frequência iniciada após o desmame na prole adulta de animais desnutridos durante a lactação por meio de uma dieta de baixa proteína oferecida a mãe.

Em consonância com as regras do programa de pós-graduação em ciências biológicas, o artigo de revisão foi redigido de acordo com as normas da revista *Journal of Developmental Origins of Health and Disease*, com atual fator de impacto 3.034. Enquanto o trabalho experimental foi redigido de acordo com as normas da revista *Frontiers In Physiology*, com atual fator de impacto 4.566.

O PRESENTE TRABALHO FOI REALIZADO COM APOIO DO CNPQ, CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTÍFICO E TECNOLÓGICO - BRASIL.

Resumo geral:

Introdução: O conceito DOHaD (Developmental Origins of Health and Disease) tem por objetivo entender o efeito de diversos estresses em fases sensíveis do desenvolvimento, ou em 'janelas' nas quais há maior susceptibilidade de modulação no metabolismo, e a relação com o desenvolvimento de saúde ou doenças a longo prazo. Dessa forma a gestação e a lactação são fases bem estabelecidas de susceptibilidade para o desenvolvimento metabólico da prole. Estresses durante essas fases podem modular órgãos e sistemas de forma que tais consequências possam ser observadas na vida adulta da prole. O exercício físico é um tipo de intervenção amplamente usada tanto como fator profilático, quanto para reverter quadros metabólicos adversos. Este trabalho busca avaliar os efeitos do exercício físico materno na prole macho adulta, bem como o efeito do exercício físico no metabolismo de animais desnutridos durante a lactação por meio de uma dieta pobre em proteína oferecida a mãe.

Metodologia: A fim de entender os efeitos na homeostase glicêmica do exercício materno na prole macho, uma revisão foi realizada após uma busca eletrônica na base *PubMed*. Os artigos publicados em inglês entre 2012 e 2022 foram identificados e isolados com base na avaliação de prole macho adultos de mães exercitadas. Ao final da busca foram analisados 9 trabalhos. A fim de entender os efeitos do exercício físico no metabolismo da prole de mães que receberam dieta pobre em proteína durante a lactação, ratos *Wistar* fêmeas de 70 dias e machos de 80 dias foram colocados para acasalamento. Após o nascimento foi ofertado a mãe uma ração normoproteica (21%) (NP) durante toda a lactação ou pobre em proteína (4%) (LP) durante os primeiros 14 dias da lactação. Aos 21 dias os ratos serão desmamados. Dos 30 aos 90 dias de vida os animais dos grupos LP e NP foram submetidos ao treinamento aeróbico. Peso corporal e consumo foram avaliados durante a vida do animal e aos 90 dias de vida, foram feitas análises *in vivo* e *ex vivo.*

Resultados: Parte da literatura analisada na revisão utilizou o exercício em mães obesas e a outra parte utilizou o exercício em mães não obesas. Os resultados mais expressivos de redução do peso corporal, gordura corporal, tolerância à glicose e melhora da sensibilidade à insulina foram encontrados nos filhos de mães obesas. Os filhos de mães exercitadas não obesas não apresentaram diferenças significativas nos parâmetros analisados na maioria dos estudos. Na prole desnutrida, o exercício moderado também foi capaz de diminuir o peso corporal e as reservas de gordura em ambos os grupos (NP-EX e LP-EX), em comparação com os sedentários. Os animais LP-SD apresentaram

intolerância à glicose e alta sensibilidade à insulina, porém os animais LP-EX apresentaram melhora nestes parâmetros, embora tais melhoras não tenham sido observadas na área das ilhotas pancreáticas. Além disso, foi possível observar que o exercício físico melhora os parâmetros bioquímicos do estresse oxidativo.

Conclusão: Os trabalhos analisados apresentam que o exercício materno durante os períodos de preconcepção e gravidez, gravidez ou gravidez e lactação melhora a homeostase da glicose na prole masculina adulta. No entanto, existem algumas limitações, como o uso de dieta rica em gordura pelas mães e à intensidade do exercício não apresentada, que impedem o isolamento do fator exercício nesses benefícios. Nos animais desnutridos durante a lactação concluímos que o exercício aeróbico de intensidade moderada foi capaz de melhorar a homeostase da glicose, tais melhoras podem estar relacionadas a alterações fisiológicas na musculatura esquelética proporcionadas pelo exercício físico, além de menores níveis de SOD e LOOH em o pâncreas.

General abstract

Introduction: The DOHaD (Developmental Origins of Health and Disease) concept aims to understand the effect of several factors applied in sensitive stages of development, also called 'windows', in which there is greater susceptibility to modulation in metabolism and the relationship with long-term health or diseases. Thus, as pregnancy and lactation are well-established phases of susceptibility to the offspring's metabolic formation, stresses during these phases can modulate organs and systems so that consequences can be observed in the offspring's adult life. Physical exercise is a type of intervention widely used both as a protective factor and to reverse adverse metabolic conditions. This work seeks to evaluate the effects of maternal physical exercise on adult male offspring, as well as the effect of physical exercise on the metabolism of malnourished animals during lactation through a low-protein diet offered to the mother.

Methods: In order to understand the effects on glycemic homeostasis of maternal exercise in male offspring, a review was performed following an electronic data base PubMed search. Articles published in English between 2012 and 2022 were identified and isolated based on the assessment of adult male offspring from exercised mothers. Atthe end of the search, 9 works were analyzed. In order to understand the effects of physicalexercise on the metabolism of the offspring of mothers who received a low-protein diet during lactation, 70-day-old female and 80-day-old male Wistar rats were placed for mating. After birth, dams were offered a normal protein diet (21%) (NP) during the entirelactation period or a low protein diet (4%) (LP) during the first 14 days of lactation. At 21 days the litters were weaned. From 30 to 90 days of life, the animals in the LP and NPgroups were submitted to aerobic training. Body weight and consumption were evaluatedduring the life of the animal and at 90 days of life, in vivo and ex vivo analyzes were performed.

Results: Part of the literature analyzed in the review used exercise in obese mothers and the other part used exercise in non-obese mothers. The most expressive results of reduction in body weight, body fat, glucose tolerance and improvement in insulin sensitivity were found in the children of obese mothers. The children of non-obese exercised mothers showed no significant differences in the parameters analyzed in most studies. In malnourished offspring, moderate exercise was also able to decrease body weight and fat stores in both groups (NP-EX and LP-EX), compared with sedentary ones. The LP-SD animals showed glucose intolerance and high insulin sensitivity, but the LP-EX animals showed improvement in these parameters, although such improvements were

not observed in the area of the pancreatic islets. In addition, it was possible to observe that physical exercise improves the biochemical parameters of oxidative stress.

Conclusion: The analyzed works show that maternal exercise during preconception and pregnancy, pregnancy or pregnancy and lactation periods improves glucose homeostasis in adult male offspring. However, there are some limitations, such as the use of a high-fat diet by mothers and the intensity of exercise not presented, which prevent the isolation of the exercise factor in these benefits. In malnourished animals during lactation, we concluded that moderate-intensity aerobic exercise was able to improve glucose homeostasis, such improvements may be related to physiological changes in skeletal muscle provided by physical exercise, in addition to lower levels of SOD and LOOH in the pancreas.

EFFECTS OF PERINATAL MATERNAL EXERCISE ON GLUCOSE HOMEOSTASIS IN ADULT MALE OFFSPRING – WHAT IS TRENDING IN RECENT EXPERIMENTAL STUDIES?

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Abstract

The Developmental Origins of Health and Disease (DOHaD) concept studies the long-term effects of interventions made at sensitive stages of development, in this sense, pregnancy is a stage in which the entire nervous and metabolic system is being formed in the offspring, given that interventions made during this stage can generate consequences later for the offspring. physical exercise is well known for its beneficial effects on those who practice it, however it is not well established in the literature what the effects of this intervention are in a sensitive phase of development such as pregnancy, or the periorus that surrounds it. Thus, the objective of this review was to evaluate the effects of maternal exercise on adult male offspring. A review was performed after an electronic search on PubMed. Articles published in English between 2012 and 2022 were identified and isolated based on the assessment of adult male offspring from exercised mothers. Part of the analyzed literature used the exercise in obese mothers and the other part used non-obese mothers. The most expressive results of reduction in body weight, fat mass, glucose tolerance and improvement in insulin sensitivity were found in the offspring of obese mothers. The offspring of non-obese exercised mothers did not show significant differences in the parameters analyzed in most studies.Current evidence confirms that maternal exercise during the periods of preconception and pregnancy, pregnancy alone or pregnancy and lactation improves glucose homeostasis in adult male offspring. However, there are some limitations, such as the use of an obesogenic diet by mothers concomitantly with exercise and exercise intensity which prevent the isolation of the exercise factor in these benefits.

Key-words: DOHaD; Exercise mother, Offspring; Glucose homeostasis; Programming windows

1. Introduction

In 2019 the worldwide prevalence of type 2 diabetes and glycemic disorders was estimated to be 9.3% or 463 million people, and this number is expected to increase [1]. Obesity is a major risk factor for type 2 diabetes, with the two diseases sharing many overlap points in both, causes and outcomes. Although these metabolic diseases are often considered preventable, in reality they are complex and arise from a combination of genetic susceptibility and environmental factors [2]. In recent years it has become well established that risk patterns for obesity, disturbed glycemic metabolism and type 2 diabetes can originate from alterations in growth and metabolism during critical windows of development [3]. The Developmental Origins of Health and Disease (DOHaD) concept [4] describes through experimental, clinical and epidemiological data the impact of maternal lifestyle, among other factors, in the development of physiological and neuronal circuits, and their maturation, in the offspring [5].

Healthy lifestyle habits spanning from preconception to postpartum are considered as a major safeguard for achieving successful pregnancies and to prevent, or at least attenuate, mother and offspring diseases. Among the priorities established by the World Health Organization (WHO) are healthy diet and nutrition, weight management, physical activity, planned pregnancy and physical, mental and psychosocial health. Most studies covering the topic of healthy pregnancies focus on maternal diet, as well as moderate physical activity throughout pregnancy [6].

It has long been recognized that exercise has important health benefits for individuals with metabolic disorders, and that regular physical exercise can delay or prevent the onset of obesity and other metabolism related diseases [7, 8]. In humans, maternal physical activity has been shown to influence perinatal outcomes. Studies investigating diet and physical exercise in humans during pregnancy have shown that exercise reduces gestational weight, decreases the risk for caesarean surgery and, regarding offspring, results in small but significant reductions in birth weight. Maternal exercise has also been associated with lower BMI in offspring at 8 years-old[9].

As the onset of type 2 diabetes and most of metabolic diseases typically occur in adult life, studies designed to capture the full extent of maternal exercise's effects on offspring health have largely utilized rodent models [10]. Murine dams, exercised during

72 developmental stages of life, has been used to investigate the long-term effects of
73 maternal physical activity in the offspring metabolism [10-12]. In the present study, we
74 review the recent literature in order to understand the effects of maternal physical
75 exercise, associated or not with different diets, during preconception and/or pregnancy on
76 glycemic homeostasis in adult male rats.
77

78 ## 2. Methods

79 A systematic review of the experimental evidence was performed on the effects
80 of maternal physical exercise during the gestation period on adult male offspring. Articles
81 published in English were evaluated using the PubMed search engine, using the key terms
82 'Exercise Gestational and rodent' (506 results) and 'Maternal Exercise and rodent' (415
83 results). The primary research studies included for comparison involve animal models
84 only. All materials published in the last 10 years were included in the review.
85 Studies were initially selected based on title; Duplicate articles and articles that
86 do not publish original research were excluded. Studies were then selected by a review of
87 abstracts that met the appropriate inclusion criteria, as follows: Maternal exercise during
88 pregnancy (work that used before pregnancy and during lactation was also included,
89 provided that the exercise comprised the gestation window), analyzes in the adult male
90 offspring, assessments on the glucose metabolism of the offspring. If abstracts met the
91 criteria, a full analysis of the full text was performed using similar inclusion criteria. A
92 total of 85 studies were included for review and, after exclusion, 15 studies remained for
93 further analysis, of which 9 were finally selected for review (Figure 1).
94

95 ## 3. Results and Discussion

96 The effects of exercise depend on the type of exercise, intensity, duration and
97 frequency. This review used some criteria to select the works to be evaluated, such as:
98 being an author work, having been published between 2012-2022, physical exercise
99 having been done on mothers (the exercise could include other phases as long as the
100 pregnancy was also used), the evaluations were performed on adult offspring (equal to or
101 greater than 12 weeks), we only used the evaluations performed on male offspring. At the
102 end of screening 9 studies were selected to this review. In general, in these studies the
103 dams were submitted to aerobic exercise protocols during preconception (PC), gestation
104 (G) or both and gestation and lactation (L), 5 or 6 times per week, through 4 weeks during

PC to all days during G or during G and L (table 1). Despite not having an established criterion, none of the 9 selected studies used high intensity during the training protocol.

In this review, we seek to the recent findings on the effects of physical exercise during pre-conception and perinatal phases on glucose homeostasis in the adult male offspring. Studies that performed dietary intervention, but also had control groups (no diet use), were analyzed in this review, however, in these studies only comparisons between control diet groups were used. Most of the studies addressing maternal lifestyle and the consequent influence on the life of adult offspring associates a set of interventions during preconception and pregnancy, only pregnancy or pregnancy and lactation.

These studies show that the greatest benefits of exercise during CP, G and/or L found on offspring metabolism, were when an obesogenic intervention was offered to the mother along with exercise. Studies such as Quiclet, *et al.*, (2017), Quiclet, *et al.*, (2016), Stanford, *et al.*, (2014) and Fidalgo *et al.*, (2013), who among their groups evaluated the trained control diet group vs the exercised control diet group, found subtle differences or no significant difference in the parameters evaluated by this review, as can be seen in works 5, 7, 8 and 9 from table 1. In the next sessions we will present and discuss the findings of the selected works.

3.1 Maternal Exercise effects on offspring body weight and fat accumulation

Most of the studies included in the present review show that the association of diet and training, or just training, does not significantly affect the body weight of the offspring at weaning [13-17]. The counterpoint to this data was observed in Quiclet, et al., (2017), in which the offspring of trained dams presented lower body weight at weaning [18].

Similarly, the body weight of adult offspring from trained dams was observed to be significantly reduced in different studies [11, 18, 19], while other data showed no significant change in the final body weight of adult offspring [13-17, 20]. In general, the studies that shows reduced body weight attribute this effect to a lower offspring food intake after lactation [21]. Besides, there research shows changes in the milk composition of exercised mothers, which seems to play a protective role in offspring body weight gain [19]. Additionally, another study found in the literature, from exercised mothers during PC and G and evaluating adult offspring, observes an increase in the expression of neuropeptide Y (NPY) and suggests that this may explain the reduction in offspring body weight, through lower food intake. One of the limitations for the evaluation of this work is that the offspring receive a high-fat diet from 12 to 28 weeks of age, however, lower

consumption was observed during these 12 weeks, demonstrating that maternal exercise can have an influence even in the absence of a obesogenic diet [21]. Others authors also pointed to changes in the milk composition of mothers exercised, as increase the concentration of the oligosaccharide 3'-sialyllactose (discussed ahead), as it might exert a protective effect in offspring body mass [19].

Furthermore, it is important to note that when the exercise is performed during PC and G, there is a decrease in body fat, which help to explain the lower body weight of these animals [11, 19]. Although there are reports in the literature of decreased fat mass in the offspring of exercised mothers, no changes were found in plasma levels of triglycerides in this offspring.

3.2 Maternal exercise and offspring long-term glycemic homeostasis

In order to better understand the effects of maternal exercise on offspring long-term glucose homeostasis, we considered the studies findings on fasting blood glucose and fasting insulinemia, glucose tolerance and insulin sensitivity.

An improvement in the glycemic response was observed during the glucose tolerance test in five of the studies evaluated, [13, 14, 16, 19, 20]. with the others showing no significant difference in this parameter [11, 15, 17, 18]. It is worth of note that the studies that shows improved glucose tolerance and insulin sensitivity were those using models that received an obesogenic diet and training in the mother before and during pregnancy. Therefore, there are some theories to explain this improvement observed in the offspring: (1) changes in skeletal muscle; (2) changes in the composition of the milk produced by the mother; (3) changes in the microbiota.

It was observed that training in obese mothers can lead to muscle increase in the offspring of factors that activate mitochondrial gene expression, such as PGC1-alpha. Leading to increase in mitochondrial proteins key for muscle glucose and lipid oxidation, thus increasing muscle utilization of these macronutrients [13, 22].

Furthermore, it was observed that maternal exercise before and/or during pregnancy may increase the concentration of the oligosaccharide 3'-sialyllactose (3'SL) which is associated with benefits in glycemic metabolism in male rats, besides amelioration of the cardiac function in female offspring [19]. The insulin content was also shown to be increased in the milk of exercised mothers during pregnancy and lactation, which was associated with modulation of thermogenesis and energy regulation pathways in the offspring that could facilitate glucose clearance [15]. In addition, Zhou *et al*., 2020,

demonstrates that exercise before and during pregnancy in obese mothers can modulate the fetal microbiota for the appearance of beneficial microorganisms and the decrease of bacteria harmful to the metabolism [20].

Improvement in insulin sensitivity was observed in the offspring of exercised mothers. The hypotheses found to explain the better insulin sensitivity are linked to the better glucose tolerance observed in some studies. Zheng *et al*., 2020, observed that offspring of obese and exercised mothers during preconception and pregnancy have reduced pancreatic beta cell size and fasting insulin secretion that likely respond to improved glucose tolerance, noting the improvement in sensitivity the insulin [14]. In addition, previous studies show that there is a decrease in the pPKB/PKB ratio, which confirms the decrease in insulin and the increase in sensitivity because this decrease in plasma insulin does not alter glucose tolerance [16]. Furthermore, it is known that decreased body adiposity and levels of inflammatory cytokines such as IL-6 are also linked to improved insulin sensitivity and such parameters have been observed in offspring under these conditions [18]. Finally, Wasinski *et al*., 2015, shows that the improvement in sensitivity may also be related to the muscle increase in adiponectin and the decrease in leptin [21]. Such factors may contribute to glucose homeostasis in these offspring.

In addition to the parameters evaluated by this review, the literature is comprehensive when it comes to the intervention of physical activity in preconception and perinatal phases. Thus, it is necessary to highlight that studies that evaluated the difference in the response of maternal training in female and male offspring, reached the conclusion that the response of metabolic improvement for males was more accentuated [13, 19]. Furthermore, it has been shown that the benefits of perinatal exercise are more pronounced when it is performed by both the mother and the father [14, 23].

3.3 Final remarks and future perspectives

The developmental origins of health and disease (DOHaD) hypothesis arose initially out of human cohort studies showing that people of lower birth weight had a higher risk of adult metabolic syndrome. Animal experiments have shown that manipulation in mothers during pregnancy can lead to widespread and permanent changes in tissue structure, body composition, endocrine responses and metabolism in the offspring. In this sense, we seek to evaluate the effects of maternal physical exercise on adult male offspring.

We observed that exercise in obese mothers is able to decrease the body weight of the offspring or not change it (non-obese mothers) and improve the metabolic glucose homeostasis evaluated by parameters such as: glucose and fasting insulin, glucose tolerance, insulin sensitivity, fat mass and plasma triglyceride levels. These no improvements in glucose metabolism occurred mainly in offspring of obese mothers, demonstrating the protective effect of exercise during CP and G for young/adult offspring, in contrast to most other findings in non-obese mothers demonstrating that exercise during pregnancy PC, G and L are not able to negatively modulate the offspring's glycemic homeostasis. Among the analyzed studies, only Quiclet et al. (2016) observed an increase in glucose tolerance in the adult offspring of mothers trained during PC and G. This divergence demonstrates some of the weaknesses that studies on maternal exercise have, since it is already.

It is well established in the literature that the effect of exercise is dependent on the intensity and time of exercise, type of exercise and age and type of animal. there is no unanimity among these factors in the works evaluated here. In addition, pregnancy is a sensitive programming window, while even interventions such as physical activity can become harmful when performed at high intensity and the intensity is not well established in most of the studies evaluated. And still there is a limitation in the present review, as we did not assess the intensity or equipment in which the exercise was performed, nor did it exclude studies that associated exercise with dietary interventions from the screening.

In this way, we conclude that physical exercise during CP/G or G/L in obese mothers can promote positive metabolic changes in the glucose metabolism of male offspring and, also, exercise when performed by non-obese mothers during these phases, seems to have no effect. great effects or still not negatively affect adult male offspring

In view of the above, it is important to carry out further research on the topic of physical exercise during pregnancy that pays attention to the age, type and intensity of exercise to clearly present the effects on the offspring. Furthermore, it is suggested that transgenerational assessments be carried out in order to assess the epigenetic effects of exercise during pregnancy.

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5. Conflict of interest:

The authors declare no competing interests.

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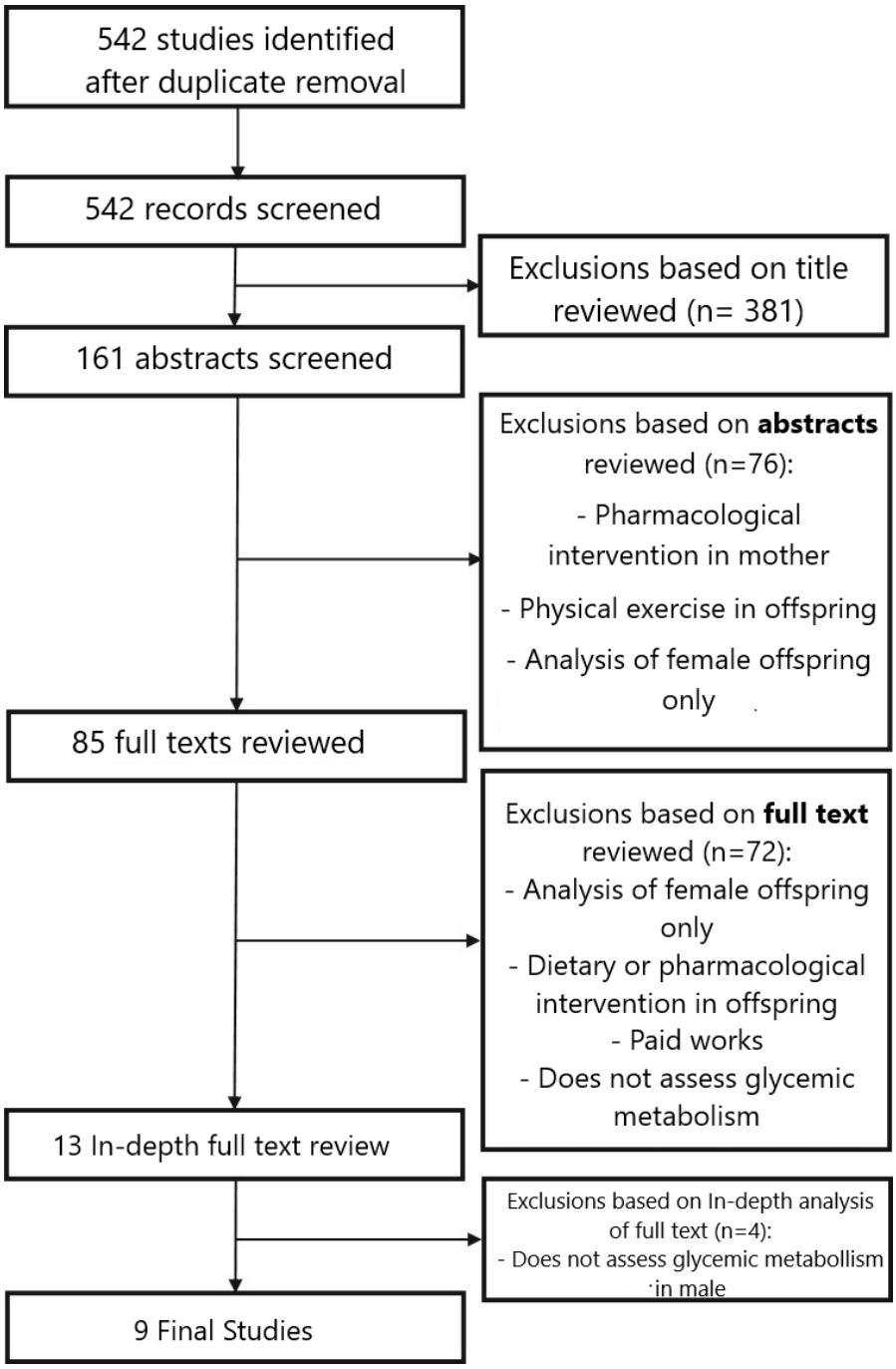
7. Figure legends

Figure 1. Systematic analysis layout for literature inclusion. The PubMed was used to identify all studies published between 2012 and 2022 in which adult male offspring were used to assess the effects of maternal exercise during preconception and pregnancy, pregnancy alone or pregnancy and lactation on glycemic homeostasis. A total of 542 articles published in English were selected; 85 were eligible for critical review resulting in a total of 9 articles to be included in this review.

8. Figure

Figure 1



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326 9. Table

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328 Table 1. Compiled literature on the effect of physical exercise performed by the
329 mother on adult male offspring.

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	Reference	Animal Model and DI	Exercise Frequency and intervention period	Offspring Evaluation	Main Results	
1	**Laker; Altıntaş; Lillard, *et al.*, 2021**	**C57BL/6 HFD**	**Daily – G Running wheels**	**±40 weeks**	**BW 21 day**	**=**
					BW 40 weeks	**=**
					FG	**-**
					FI	**-**
					Glucose tolerance	**-**
					Insulin Sensitivity	+
					Fat mass	**NE**
					Triglycerides	**NE**
2	**Zhou; Xiao; Li, *et al.*, 2020**	**C57BL/6 HFD**	**3 weeks – PC Every day – G Running wheels**	**24 weeks**	**BW 21 day**	**NE**
					BW 24 weeks	**=**
					FG	**NE**
					FI	**-**
					Glucose tolerance	**-**
					Insulin Sensitivity	+
					Fat mass	**NE**
					Triglycerides	**=**
3	**Harris; Pinckard; Wright; *et al.*, 2020**	**C57BL/6 HFD**	**2weeks: 5day/week – PC 2weeks: 5day/week – G Treadmill**	**52 weeks**	**BW 21 day**	**NE**
					BW 52 weeks	**-**
					FG	**NE**
					FI	**-**
					Glucose tolerance	**-**
					Insulin Sensitivity	**NE**
					Fat mass	**-**
					Triglycerides	**NE**

4	**Zheng; Alves-Wagner; Stanford *et al.*, 2020**	**C57BL/6 HFD**	**2 weeks – PC 15-17 days - G Running wheels**	**52 weeks**	**BW 21 day**	**=**
					BW 52 weeks	**=**
					FG	**-**
					FI	**=**
					Glucose tolerance	**-**
					Insulin Sensitivity	+
					Fat mass	**NE**
					Triglycerides	**NE**
5	**Quiclet; Dubouchaud; Berthon; *et al.*, 2017**	**Wistar**	**4 weeks: 5day/week – PC 18 days – G Treadmill**	**10 weeks**	**BW 21 day**	**-**
					BW 10 weeks	**-**
					FG	**NE**
					FI	**=**
					Glucose tolerance	**=**
					Insulin Sensitivity	**=**
					Fat mass	**NE**
					Triglycerides	**NE**
6	**Ribeiro; Tófolo; Martins, *et al.*, 2017**	**Wistar**	**3 day/week – G 3 day/week – L Treadmill**	**12 weeks**	**BW 21 day**	**=**
					BW 12 weeks	**=**
					FG	**-**
					FI	**=**
					Glucose tolerance	**=**
					Insulin Sensitivity	+
					Fat mass	**=**
					Triglycerides	**NE**
7	**Quiclet; Siti; Dubouchaud, *et al.*, 2016**	**Wistar**	**4 weeks: 5 day/week – PC 18 days: 5 day/week – G Treadmill**	**±28 weeks**	**BW 21 day**	**=**
					BW 28 weeks	**=**
					FG	**=**
					FI	**=**
					Glucose tolerance	**+**
					Insulin Sensitivity	**=**
					Fat mass	**=**

					Triglycerides	**NE**
8	**Stanford; Min-Young; Getchell, *et al.*, 2014**	**C57BL/6**	**2 weeks – PC** **Daily – G** **Running wheels**	**52 weeks**	**BW 21 day**	**NE**
					BW 52 weeks	**-**
					FG	**NE**
					FI	**=**
					Glucose tolerance	**=**
					Insulin Sensitivity	**NE**
					Fat mass	**=**
					Triglycerides	**NE**
9	**Fidalgo; Falcão-Tebas; Bento-Santos, *et al.*, 2013**	**Wistar**	**4 weeks: 5 day/week – PC** **4 weeks: 5 day/week – G** **Treadmill**	**± 21 weeks**	**BW 21 day**	**=**
					BW 21 weeks	**=**
					FG	**=**
					FI	**NE**
					Glucose tolerance	**=**
					Insulin Sensitivity	**=**
					Fat mass	**NE**
					Triglycerides	**NE**

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Notes: In the literature, nine studies were observed. the signs indicate whether the evaluated parameters increased (+), decreased (-) or there was no significant difference (=). The results are from the comparison of adult male offspring from sedentary vs exercised mothers. If the study investigated the offspring of mothers with and without dietary intervention, for this review only the results of the group derived from non-obese mothers are presented. Other intervention (OI): high-fat, high-sucrose diet (HFHS); high fat diet (HFD). Intervention period: PC (pre-conception); G (gestation) and L (lactation); Main: FG (fasting glucose); FI (fasting insulin); + (increased); - (decreased); = (no significant difference) and NE (Not Evaluated)

MODERATE INTENSITY AND LOW FREQUENCY EXERCISE IMPROVES GLYCEMIC HOMEOSTASIS IN ADULT WISTAR RATS UNDERNOURISHED DURING LACTATION.

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Abstract

Maternal protein restriction during lactation can lead the offspring to metabolic dysfunctions in adult life, especially related to glycemic homeostasis. On the other hand, physical exercise is a non-pharmacological method that can prevent, attenuate or treat metabolic diseases. We investigated the effects of moderate-intensity, low-frequency aerobic exercise in adult male rats from dams that received a low-protein diet during the lactation period. On postnatal-day 0 (PN0) the *Wistar* rats dams from a diet with normal protein content (20%) throughout lactation to the NP group or LP group were offered a diet containing low protein content (4%) during the first 14 days of lactation. At PN30, all animals performed a maximal effort test and a part of the animals from the NP and LP groups started the moderate aerobic exercise protocol (NP-EX and LP-EX) and another set did not perform the training (NP-SD and LP-SD). At 90 days, effort test was performed on all animals, as well as *in vivo* and *ex vivo* experimental procedures. Malnutrition during lactation caused low weight in animals at PN21 and PN90, as well as a decrease in body fat stores. Moderate exercise also was able to decrease body weight and fat stores. The LP-SD animals showed glucose intolerance and high insulin sensitivity, however the LP-EX showed improvement in these parameters and such improvements were also observed in the area of the pancreatic islets. Besides, it was possible to observe that physical exercise improves the biochemical parameters of oxidative stress. Thus, we conclude that moderate-intensity aerobic exercise was able to improve glucose homeostasis in malnourished animals during part of lactation, such improvements may be related to physiological changes in skeletal muscle provided by physical exercise, in addition to lower levels of SOD and LOOH in the pancreas.

Keywords: Lactation, Aerobic exercise, Metabolic programming, Malnutrition, DOHaD.

1. Introduction

According to the World Health Organization, child malnutrition remains a serious public health problem, with a multifactorial cause. An environment of nutritional imbalance in early life is associated with an increased risk of developing metabolic diseases in adulthood (1), such events stem from plasticity during development and can be explained by the Developmental Origins of Health and Disease (DOHaD) concept describes, through scientific data, the impact of stressful insults within sensitive development windows, on the physiology and maturation of neuronal circuits in neonates (2). This is because periods such as pregnancy, lactation, childhood and puberty are forming neuronal connections in the individual's nervous system (3). In rodents, specifically, much of neurogenesis begins *in utero* and goes through the first weeks of life. Thus, the maternal diet during the lactation period is important, as several regulatory mechanisms are not yet fully formed at birth, undergoing rapid maturation in most organs and systems during this phase (4).

The administration of a low-protein (LP) diet in rodents is a well-established model to investigate the link between early malnutrition and adult metabolic disorders. Previous studies have shown that female rats fed a LP diet during pregnancy and/or lactation give rise to offspring that show metabolic changes in adulthood. Additionally, when this protein restriction is done only during lactation, it can lead the offspring to manifest impaired glucose metabolism (3, 5, 6).

The literature has shown that these metabolic disturbances caused by nutritional stresses in perinatal life can be reversed or alleviated when the environment exerts a new positive stimulus, such as regular physical exercise (7). Physical exercise can be understood as any prescribed or oriented body movement that results from muscle contraction, resulting in an increase in basal and resting energy expenditure (8). Physical activity induces positive organic adaptations, such as: muscle hypertrophy, maturation of the nervous system, specially related to the motor control, adaptations in the cardiovascular and respiratory system and protection against the onset and aggravation of metabolic diseases (9).

Studies in rodents have shown that moderate-intensity aerobic exercise is capable of promoting maintenance of glucose homeostasis and improving the activity of the Autonomic Nervous System (ANS) (10). Furthermore, exercise is able to attenuate or revert metabolic disorders resulting from nutritional stresses in this and other stages of life (10-12).

Thus, considering the importance of the lactation period as a plasticity window for long-term metabolism and the beneficial effects associated to the aerobic physical exercise, the aim of the present study was to evaluate whether a moderate-intensity and low-frequency aerobic exercise program, performed in male Wistar rats, offspring from malnourished mothers during lactation by a low-protein diet, may improve glycemic homeostasis altered by perinatal malnutrition.

2. Materials and Methods

2.1 Ethical approval

All experiments were conducted according to the ARRIVE guidelines (13) and with Brazilian Association for Animal Experimentation (COBEA) standards. Protocols were approved by the Ethics Committee in Animal Research of the State University of Maringa (protocol number 1376270418).

2.2 Experimental design and diets

After one week of adaptation, female and male Wistar rats (70 and 80 days of age, respectively) were mated in a ratio of three females to each male, and the pregnant females were transferred to individual cages and fed a standard diet. At birth (postnatal-day 0 (PN30)), the litters were standardized to eight pups per dam, preferentially male, and divided into two experimental groups. The control dams (n = 15) received a normal-protein diet (20.5% protein; Nuvital®; Curitiba/PR, Brazil; NP group) throughout lactation, while the other group of mothers was fed a low-protein diet (n = 15, 4% protein; LP group) for the first 14 days of lactation. The composition of low-protein diet has been previously described (14). At 21 days the animals of all groups were weaned and 5 males were left per litter. On PN30, animals were divided into four groups according to their physical training: normal-protein diet sedentary (NP-SD), low-protein diet sedentary (LP-SD), normal-protein diet and submitted to physical training NP-EX), and lowprotein diet and submitted to physical training (LP-EX). Trained rats ran in a treadmill over a period of 8 weeks (3 days/week-1, 44 min/day-1, at 55-65% VO2max). The experimental procedures were conducted at ninety days of age. Throughout the experimental period, the animals were kept under controlled temperature (23 ± 2°C) and photoperiod (7:00 a.m. to 7:00 p.m., light cycle) conditions. The animals received water and food *ad libitum*.

2.3 Effort test

At PN30, 45, 60, 75 and 90, the animals in the exercised group (NP-EX and LP-EX) were submitted to the effort test to determine VO2max with the aid of a Havard Aparatus® gas analyzer and individual treadmill appropriate for rodents (Panlab®), while the animals from the sedentary groups (NP-SD and LP-SD) performed the stress test only at 30 and 90 days of life. The test consisted of a 5-minute warm-up at an intensity of 10 cm/s, with a 0° inclination with an increase of 9 cm/s every 3 minutes, until the animal was exhausted. At the end of the running pen, a stainless steel grid emitted electrical stimuli (0.25 mA) to keep the animal moving, as previously explained (15). It was considered as exhaustion parameter the animal not being able to keep pace in the race. In this case, the animal ran only with its front paws, raising its hind paws on the shock grid (no longer responding to the electrical stimulus of the treadmill). The VO2max. was

considered as the value reached when, even in the face of an increase in load, there was no increase in O_2 consumption of ± 5% (16).

2.4 Protocol of physical training

A protocol of physical training was performed according to Almeida et al. (11) The intensities used to prescribe the moderate training protocol were between 55 and 65% of the final speed in the maximum effort test. On PN30, the effort test was performed. On the 32nd, the training of the exercised groups began. This same procedure was followed on all other test dates. The training sessions lasted 44 minutes, with a 2-minute warm-up at 16 cm/s, training at intensities between 55-65% of the maximum speed obtained in the effort test and a 2-minute cool-down at the end at 16 cm/s. The training protocol was performed in the morning (approximately 9 am), 3 times a week, for 8 weeks. The training sessions were carried out on a special treadmill for rodents (Panlab, Harvard Apparatus®, Cornellà - Barcelona - Spain).

2.5 Body weight gain, caloric intake and fat pad store measurements

Body weight and food intake were determined every two days from weaning until 90 days of age. Food intake was calculated as the difference between the amount of diet remaining (Df) and the amount presented previously (Di), divided by the number of animals in the cage and by the number of days: [FI(g) = (Df –Di)/2/3]. The area under the curve (AUC) was calculated. At 90 days of age, the rats were anaesthetized with thiopental (45 mg/kg of body weight), decapitated and laparotomized to remove their retroperitoneal, periepididymal and mesenteric fat pad stores. The weight of fat pads was expressed in relation to the body weight of each animal (g/100g) of body weight

2.6 Intravenous glucose tolerance test (ivGTT)

At 90 days of age, a batch of animals (n = 4 litters per group) were subjected to a surgical procedure to perform the ivGTT, as previously described (3). After a 12-hour fast, blood samples were removed before the injection of glucose (1 g/kg of body weight) (0 min) and 5, 15, 30 and 45 min afterward. Glucose concentration was measured by the glucose oxidase method using a commercial kit (GoldAnalisa®; Belo Horizonte, MG, Brazil). The glucose response during the test was calculated by AUC.

2.7 Intraperitoneal insulin tolerance test (ipITT)

Another batch of animals (n = 4 litters per group) were cannulated, and the ipITT was performed after a 6-hour fast. They received an injection of insulin (1 U/kg of body weight), and blood samples were collected, as previously reported [21]. Glucose concentration was measured by the glucose oxidase method using a commercial kit (GoldAnalisa®; Belo Horizonte, MG, Brazil). Subsequently, the rate of glucose tissue uptake or the rate constant for plasma glucose disappearance (Kitt) was calculated (17).

2.8 Immunohistochemical evaluation of endocrine pancreas and brown fat

At 90 days of euthanasia, the animals had pancreas and brown fat samples removed (n=6-8 per group), placed in 4% paraformaldehyde, fixed for 24 hours and then embedded in paraffin as previously described (18). Five µm sections for each 30 µm interval were made using a microtome and placed on glass slides. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy (5 x 40x optical zones per slice). ImageJ for Windows (Open Source) was used in the analysis.

2.9 Analysis of oxidative stress parameters in Pancreas and Soleus

2.9.1 Pancreas and Muscle Sample preparation

Muscle and pancreatic tissue samples will be homogenized in 200 mM potassium phosphate buffer (pH 6.5). A part of the homogenate will be used for the quantification of glutathione (GSH) and the rest will be centrifuged for 20 minutes at 9,000rpm. With the supernatant will be performed techniques for measuring the enzymatic activity of superoxide dismutase (SOD), Catalase (CAT), glutathione S-transferase (GST) and the measurement of levels of lipid hydroperoxide (LOOH).

2.9.2 Measurement of SOD enzyme activity

The supernatant of the samples will be homogenized in tris-HCl buffer (200 mM) and EDTA (2 mM; pH 6.5) and 1 mM pyrogallol will be added. The solution will be incubated at room temperature for 20 minutes and the reaction will be stopped with 1N HCl. The solution will be centrifuged for 4 minutes at 14,000rpm and the supernatant will be pipetted into microplates for reading in a spectrophotometer at 405 nm. The results will be expressed in SOD Unit/mg protein

2.9.3 Measurement of CAT enzyme activity

The supernatant of the samples will be diluted in potassium phosphate buffer (0.2 M; pH 6.5) in the proportion of 1:10. In a 96-well plate, the sample will be homogenized in a solution containing tris-HCl-EDTA buffer (0.1M; pH 8.5), distilled water and hydrogen peroxide (H2O2). The reading will be taken at 240 nm. Results will be expressed in mmol/min/mg protein

2.9.4 Measurement of GST enzyme activity

Samples will be diluted in potassium phosphate buffer (0.1M; pH 6.5) and pipetted into a 96-well plate. The reaction will be initiated by the addition of a solution with potassium phosphate buffer (0.1 M; pH 6.5), 1-chloro-2,4-dinitrobenzene (CDNB) and GSH. The reading will be done in a spectrophotometer at 340 nm using the extinction coefficient of 9.6 mM/cm. Results will be expressed in mmol/min/mg protein

2.9.5 Quantification of GSH

12% trichloroacetic acid will be added to the homogenate, which will be homogenized and centrifuged for 15 minutes at 9,700g. Tris buffer (0.4 M; pH 8.9) will be added to the 96-well microplate and the reaction will start with the addition of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB; 1 mM). In a spectrophotometer (415 nm) the reading will be carried out in up to 5 minutes, and the values obtained will be interpolated in a standard curve of GSH. Results will be expressed as µg GSH/g tissue.

2.9.6 Measurement of the LOOH level

The supernatant of the samples will be homogenized in methanol (1:4) and centrifuged for 20 minutes at 10,000g (4ºC). The supernatant (60 µl) and 240 µl of the reactive medium containing xylenol orange, sulfuric acid (25 mM), butylated hydroxytoluene (BHT; 4 mM) and FeSO4NH4 (250 mM) will be added in 96-well plates and incubated in the dark for 30 minutes, at room temperature. The reading will be done in a spectrophotometer at 560 nm. The concentration of LOOH will be determined from the extinction coefficient of 4.3 mM 1.cm-1. Results will be expressed in mmol/mg tissue

2.10 Statistical analysis

The results are presented as the mean with the standard error (SEM). Statistical analysis was performed using Student's *t*-test or two-way ANOVA (analysis of variance) followed by Tukey's post hoc test. A Pvalue < 0.05 was considered statistically significant for the effects of a low-protein diet (D), a physical exercise (E) or the interaction (I) of low protein and high-fat. Analyses were conducted in GraphPad Prism version 6.01 for Windows (GraphPad Software, Inc. San Diego, CA, USA).

3. Results

3.1 Body weight and food intake

As shown in figure 1A, the animals that received a low-protein diet during the first 14 days of lactation (LP) showed a significant reduction in body weight at 21 days of life compared to the NP group (P<0.0001). The area under curve of the evolution of body weight during the all life of the animal after lactation (21 to 90 days), in Figure 1B, demonstrates the influence of the diet on the groups (P<0.0001), and also, the post-test shows a significant decrease in body weight both in animals belonging to the LP-SD group, in relation to the NP-SD group (P<0.0001), and in the LP-EX animals, when compared to the NP-EX group (P <0.0001). Similarly, figure 1C shows the weight at 90 days of the 4 groups, which shows the action of both diet and exercise (P<0.0001; P<0.01, respectively), since it is possible to observe in the post-test significant decrease in the final weight of the NP-EX animals in relation to the NP-SD group (P<0.05).

On the other hand, the food intake evaluated between 21 and 90 days (figure 1D) of the animals from mothers that consumed a low-protein diet during the first 14 days of lactation - LP-SD and LP-EX (P<0.001) showed a significant increase in relation to the NP-SD and NP-EX groups, demonstrating the effect of the diet on the groups (P<0.0001).

3.2 Fat accumulation in brown and white adipose tissue

It is observed in figures 2 A, B and C the effect of the diet on the accumulation of fat (P<0.0001), in this context, it is observed that the animals of the LP-SD group significantly reduced the stores of retroperitoneal, periepididymal and mesenteric fat, when compared to animals in the NP-SD group (P<0.0001). Furthermore, it is possible to observe the effect of exercise in the groups in the retroperitoneal, periepididymal and mesenteric fat stores (P<0.0001; P<0.05 and P<0.0001, respectively). The post-test shows a significant decrease in these fat pads among animals in the NP-EX and NP-SD groups

(P<0.0001; P<0.05 and P<0.0001, respectively). The analysis of the results (Figures 2 A and C) demonstrates the interaction between the Diet and Exercise factors on the groups (P<0.05), and a decrease in retroperitoneal fat stores is observed in the post-test (LP-EX group vs LP-SD, P<0.05 Figure 2A). Additionally, a significant decrease in the retroperitoneal and periepididymal stores was observed in the LP-EX group when compared to the NP-EX animals (P<0.01,). No significant differences were observed in brown fat stores between groups (figure 2D).

3.3 Morphometric and Biochemistry analysis of skeletal muscle

Figures 3A and B demonstrate that there were no significant differences in the weight of the gastrocnemius and soleus muscle among the groups evaluated.

Biochemical analyzes of oxidative stress in muscle showed no significant difference between groups (Figure 3 C, E and F). However, it was possible to observe an interaction between the factors low-protein diet and moderate-intensity aerobic exercise in GSH content (p<0,05) (figure 3D).

3.4 Glucose homeostasis during the glucose (ivGTT) and insulin tolerance tests (ipITT)

The results demonstrated through the area under the curve during the ivGTT, figure 4 A, show that there was an effect of the low-protein diet (P<0.0001), as well as an interaction of diet and exercise factors (P<0.001). In the post-test, a significant increase in blood glucose was observed during the test in the LP-SD group when compared to the NP-SD group (P<0.01). On the other hand, there was a decrease in glycemia in the animals in the LP-EX group, in relation to the LP-SD group (P<0.0001).

These changes in blood glucose were accompanied by changes in insulin sensitivity as shown in Figure 4B. The result of the kITT demonstrates that there was a significant increase in insulin sensitivity in LP-SD animals when compared to NP-SD animals (P<0.05), while the animals in the LP-EX group showed a decrease in sensitivity compared to the LP-SD group (P<0.05). Thus, it is observed that there was an effect of both the low-protein diet (P<0.0001) and exercise (P<0.05).

3.5 Final speed during effort test (30 and 90 days)

According to figure 5 A, the animals in the LP group, at 30 days of age, showed significantly better performance during the maximal effort test when compared to the

294 animals in the NP group (p<0.05). This increase in final velocity in the group of
295 malnourished animals during lactation was, similarly, observed at 90 days among the
296 exercised groups, as shown in figure 5 B.

297 The effect of diet (P<0.0001) and exercise P<0.001) was observed on the final
298 speed in the effort test at 90 days of age. Both LP groups presented significantly higher
299 final velocity during the effort test than the animals of both NP groups (LP-SD x NP-SD
300 P<0.0001 and LP-EX x NP-EX P<0.01). As expected, the animals in the exercised groups
301 also showed better performance during the test, when compared to the animals in the
302 sedentary groups (NP-SD x NP-EX P< 0.05 and LP-SD x LP-EX P< 0.05).

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304 *3.6 Morphometric and biochemistry analysis of the pancreas*

305 According to figure 6, the area of the pancreatic islets was affected by both the
306 low-protein diet (P<0.0001) and moderate aerobic exercise (P<0.05). Thus, it is observed
307 that the animals in the LP group, both sedentary and exercised, had a smaller islet area
308 when compared to the NP (SD and EX) groups (NP-SD x LP-SD P<0.05; NP-EX x LP-
309 ED P<0.01). There was no significant difference between sedentary and exercised within
310 their groups.

311 No difference was demonstrated in pancreas GSH content and CAT activity
312 (figure 6 B and D). However, exercise was effect in SOD activity (p<0,05) and LOOH
313 content (p<0,05), even as diet was effect in SOD (p<0,01) and GST (p<0,05) activity.
314 There was no interaction between diet and exercise factors for the biochemical parameters
315 analyzed.

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317 *3.7 Morphometric analysis of brown adipose tissue*

318 Figure 7B shows the influence of moderate aerobic training on the number of
319 brown fat adipocytes (P<0.05). There was no significant difference for the other
320 parameters or in the analysis between groups.

321 It can be seen in figure 7B the influence of moderate aerobic exercise on the area
322 of brown fat adipocytes, as well as the interaction between the factors (P<0.05). Also, it
323 is observed in the figure that the animals of the NP-EX group had a higher area of
324 adipocytes when compared to the animals of the LP-EX group (P<0.05) and also of the
325 NP-SD group (P<0.01).

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4. Discussion

Several factors are involved in the increased incidence of child malnutrition (1). Studies have shown that both the prenatal and postnatal environments have a long-term influence on an individual's growth and development. In particular, the neonatal and infant developmental environment is determinant for metabolic health later in life (19, 20). Consumption of a low-protein diet (4%) during critical stages of development, such as lactation, is responsible for disrupting normal physiological development, directing the body to disturbances in metabolism (21, 22). The present study sought to evaluate the effect of moderate-intensity, low-frequency aerobic physical activity on the offspring male rats of malnourished mother during lactation and showed, for the first time, that such an intervention was able to improve glycemic homeostasis in these animals.

The literature shows that the beneficial effects of physical exercise on the body depend directly on the intensity, duration, frequency and stage of life in which it is performed (23). In this sense, we observed in our work that the exercise of moderate intensity and low frequency, performed for 60 days, was able to modulate the organism for a better performance during the maximum effort test in NP-EX animals at 90 days of age. These data were previously observed in studies that used a similar protocol of aerobic exercise (11, 16). Interestingly, the animals in the LP group showed higher final speed during the maximal effort test both at 30 days and at 90 days. Some hypotheses are found in the literature to explain this better performance of animals that were malnourished during lactation. Among which, de Brito Alvez, et al. (2017) observed that animals that underwent perinatal malnutrition showed increased expression of genes key to the oxidation of glucose and fatty acids in skeletal muscle, and also, Gosby, et al. (2003) showed in their work that maternal protein restriction was able to increase liver glycogen stores (24, 25).

In this study, we did not observe any statistical difference in the weight of the soleus and gastrocnemius muscles (figure 3). Acute and chronic contractile activity triggers a plethora of signals that induce beneficial metabolic and biochemical adaptations to enhance muscle health and performance. For example, the moderate increases in ROS produced by exercise are able to repair and strengthen the oxidative capacity of the cell (26). Despite physical exercise being an inducer of mitochondrial oxidative increase and consequent large producer of ROS, long-term exercise is an adaptive state in which this pathway of production is attenuated with each exercise session, including the reduction of ROS production, in this regard, our results showed that there is an interaction between the effects of diet and exercise in the reduction of glutathione in the LP-EX group,

however, there was no significant difference between the groups in this, nor in the other biochemical analyzes of oxidative stress in soleus muscle (26, 27). The moderate-intensity, low-frequency exercise used as an intervention in the present study is described in the literature as a promoter of improvement in the metabolism of unhealthy animals (7, 28), however this methodology has not been demonstrated as capable of promoting significant muscle hypertrophy or changes in muscle oxidative stress biomarkers.

The literature suggests that aerobic exercise is capable of efficiently mobilizing white adipose tissue, during and after exercise, as a source of energy substrate (29). In this perspective, the NP-EX exercised animals, compared to the NP-SD, showed a decrease in their retroperitoneal, periepididymal and mesenteric fat stores at 90 days of age. The reduction in the three fat stores was reflected in the final body weight of these animals, which were also reduced. Achten, et al. (2003) showed that exercise of moderate intensity and for more than 30 minutes per session is able to promote high levels of mobilization of fat stores and other important peripheral adaptations that contribute to fat loss (30). The animals that suffered malnutrition during lactation (LP) also showed a decrease in fat stores, as well as a lower body weight both at 21 days and at 90 days of age. Other studies carried out with the same model corroborate the effects of diet on body mass and composition found in the present study (31, 32). Low body weight can be attributed to the lower consumption of milk by animals, since milk production by mothers is reduced (33), and the consumption of a low-protein diet by mothers during lactation leads to changes in macronutrients in the breastmilk (34).

On the other hand, the animals in the LP group, both sedentary and exercised, showed an increase in food consumption after weaning. Previous work has observed similar results in malnourished animals in perinatal life (3, 21, 35). Martins, et al. (2018) demonstrates that this increase is due to a consumption peak that happens shortly after lactation. The increase in food consumption by these animals had no effect on body weight or fat stores, this may be happening because perinatal malnutrition leads to changes in pathways in the central nervous system, which governs food intake and energy expenditure, preventing food gain. weight even with increased consumption (32, 36, 37).

Differently from what was found for skeletal muscle, where no effects of diet or exercise were observed, in the present study glycemic homeostasis seems susceptible to modulation by both factors. Our results present LP animals with glucose intolerance during ivGTT and high insulin sensitivity, demonstrated by kITT (figure 4). Active adipogenesis in rodents occurs at the end of pregnancy and continues into weaning (38).

The hormones leptin and insulin act by directly regulating adiposity via the central nervous system, thus, changes in the levels of these hormones due to early nutritional imbalance can modulate energy regulatory circuits (39). Previous work has shown that consumption of a low-protein diet by the mother during lactation causes low levels of fasting insulin (21, 31) and leptin, as well as changes in the glycemic curve during the glucose and insulin tolerance test (3, 21).

Glucose intolerance can be explained by the low release of insulin by the isolated pancreatic islets, as observed in the work by Oliveira, et al. (2014) (40). In order to seek physiological homeostasis, these animals seem to have increased glucose uptake by muscles and adipose tissue, since they have high expression of the GLUT-4 receptor in these tissues, which partly explains the high sensitivity to insulin during ipITT.

Additionally, in our work, aerobic physical exercise was efficient in improving glucose intolerance in lactating malnourished (LP) animals (figure 4 A). Studies show that moderate-intensity aerobic exercise is effective in protecting metabolism against the development or worsening of metabolic diseases (11, 41), this may be related to the insulin-independent translocation of GLUT-4 to the membrane that occurs in muscle during physical activity (42). Furthermore, our results show that the increased insulin sensitivity in LP-SD animals is normalized when these animals were submitted to aerobic training (Figure 4B), once intolerance was normalized.

Ingestion of a low protein diet during lactation by the mother is capable of altering the morphology of pancreatic islets in the offspring. The beneficial action of physical exercise on glucose homeostasis in this model was accompanied by a positive effect in the area of the pancreatic islets (figure 6). Despite the exercise effect in the islets area, our results demonstrate that malnourished animals, both sedentary and exercised, have a smaller pancreatic islet area compared to animals in the same exercise protocol. This decrease in the islet area had already been reported in the literature (43). Furthermore, other studies point out that these offspring that are malnourished during lactation have less pancreatic proliferation and vascularization (44, 45). In the other hand, acute and chronic muscle contractile activity triggers a plethora of signals that induce beneficial metabolic and biochemical adaptations to enhance muscle health and performance. For example, the moderate increases in ROS produced by exercise are able to repair and strengthen the oxidative capacity of the cell (26). The result of chronic exercise is a heightened adaptive state in which the signaling response to each exercise bout is attenuated, including reduced ROS production. In this sense, we observed in our work

that such adaptations are also observed in the pancreas, since exercise was a significant factor in reducing SOD activity and also LOOH content.

Thus, we conclude that moderate-intensity, low-frequency aerobic exercise was able to improve glycemic homeostasis in male rats of mothers who consumed a low-protein diet during part of lactation, and this improvement may be associated with lower levels of markers of oxidative stress in the pancreas, as well as the effect of exercise on the pancreatic islet area. More studies are needed to elucidate the findings of this work; however, we observed that neonatal malnutrition programmed organs and systems involved in glycemic control, reaffirming the importance of lactation in metabolic and physiological development. Furthermore, we can suggest that moderate-intensity aerobic exercise is an important non-pharmacological intervention to restore glucose homeostasis.

5. Acknowledgments: We thank Ms Maroly Pinto and Ms Marli Licero for helping to care for the rats in the animal facility. This work was supported by the Co-ordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq).

6. Conflict of interest: The authors declare no competing interests.

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5. Figures legends

Figure 1: Body weight gain and food intake. Body weight from 1 to 21 days (A) and 21 to 90 days of life (B) final weight at 90 days (B) and food intake from 21 to 90 days (D). Values expressed as mean ± SEM of 20 to 25 rats from 4 different litters. The bar graph in figure A represents the area under curve (AUC). ****P<0.0001 for the difference assessed by the t-Student test. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # # #P< 0.001 # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and **P<0.01, ***P<0.001, * ***P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 2 – Accumulation of white adipose tissue. Accumulation of retroperitoneal (A), periepididymal (B) mesenteric (C) fat. Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # #P<0.01 # # #P< 0.001, # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and *P<0.05, * *P<0.01, ***P<0.001, ****P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 3 – Accumulation and Biochemistry parameters of skeletal muscle. Accumulation of soleus (A), Gastrocnemius (B) muscle, LOOH (C), GSH (D), SOD (E) and GST (F). Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # #P<0.01 # # #P< 0.001, # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and *P<0.05, * *P<0.01, ***P<0.001, ****P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

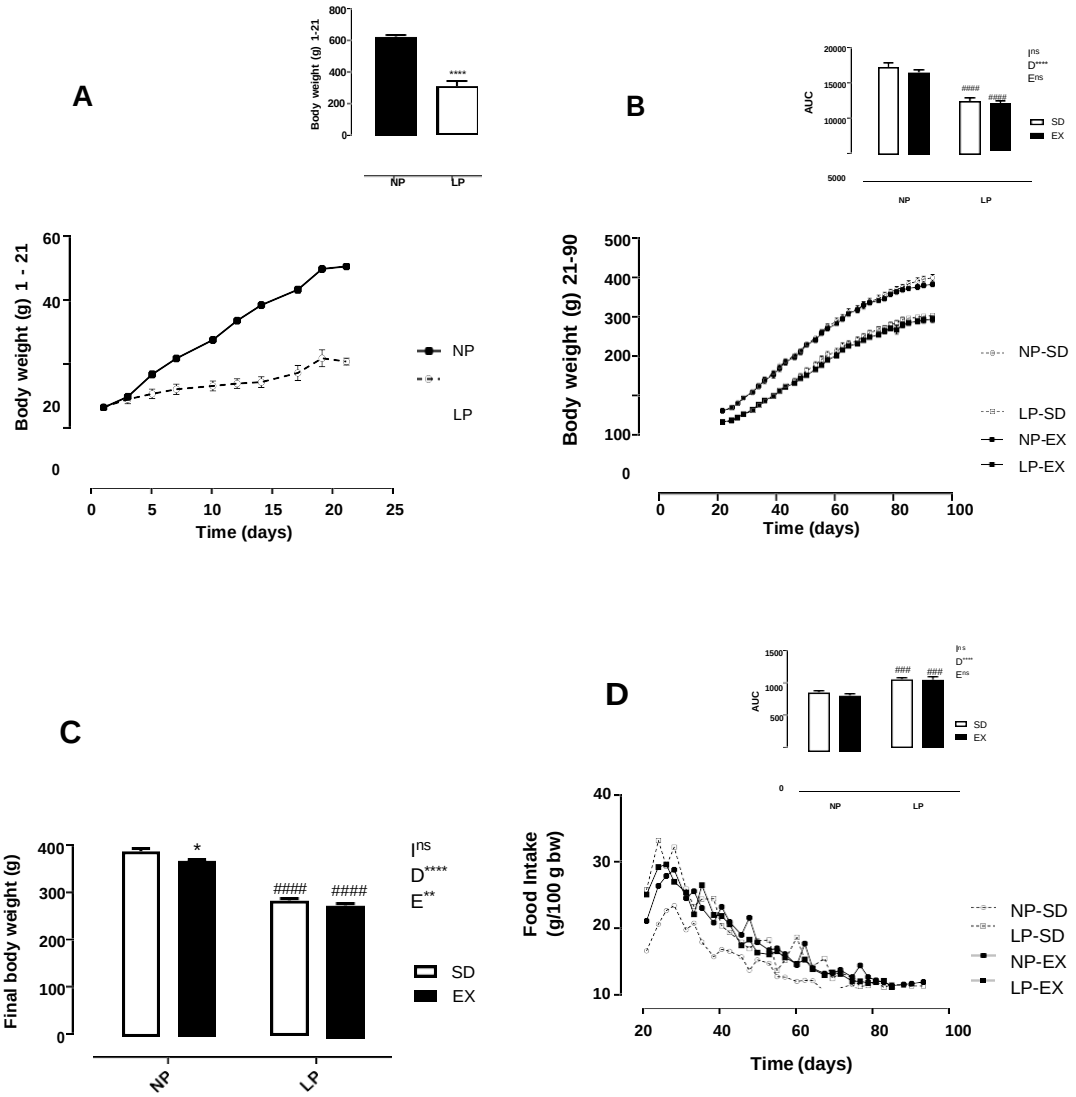
Figure 4 - Plasma glucose concentration during ivGTT and ipITT. Intravenous Glucose Tolerance Test, ivGTT (A), Intraperitoneal Insulin Tolerance Test ipITT and Blood Glucose Decay Rate Constant kITT (B). Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # #P<0.01 # # #P< 0.001, # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and *P<0.05, * *P<0.01, ***P<0.001, ****P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 5 – Speed at PN30 and PN90 during the maximal effort test. Speed during the effort test at 30 days (A) and speed during effort test at 90 days (B). In figure A, *P<0.05 for difference assessed by Student's t test. Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # #P<0.01 # # #P< 0.001, # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and *P<0.05, * *P<0.01, ***P<0.001, ****P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 6 – Morphometric and Biochemistry analysis of the pancreas. Pancreatic islet area (A). Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, # #P<0.01 # # #P< 0.001, # # # #P<0.0001 for analysis between NP vs LP groups under the same conditions and *P<0.05, * *P<0.01, ***P<0.001, ****P<0.0001 for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 7 – Morphometric analysis of brown adipose tissue. Number of adipocytes (A). Adipocyte area (B). Values expressed as mean ± SEM of 20 to 25 rats from 4 to 5 different litters. Data were submitted to two-way ANOVA analysis of variance considering the factors: (D) Low-protein diet during the first 14 days of lactation, (E) Aerobic exercise from 30 to 90 days and (I) interaction between factors D and E. # P<0.05, for analysis between NP vs LP groups under the same conditions and **P<0.01, for analysis between SD vs EX under the same conditions analyzed by Tukey's post-test. NP: normoproteic diet group, LP: low-protein diet group, SD: sedentary group, EX: exercised group. ns: non-significant result.

Figure 1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676 **Figure 2**

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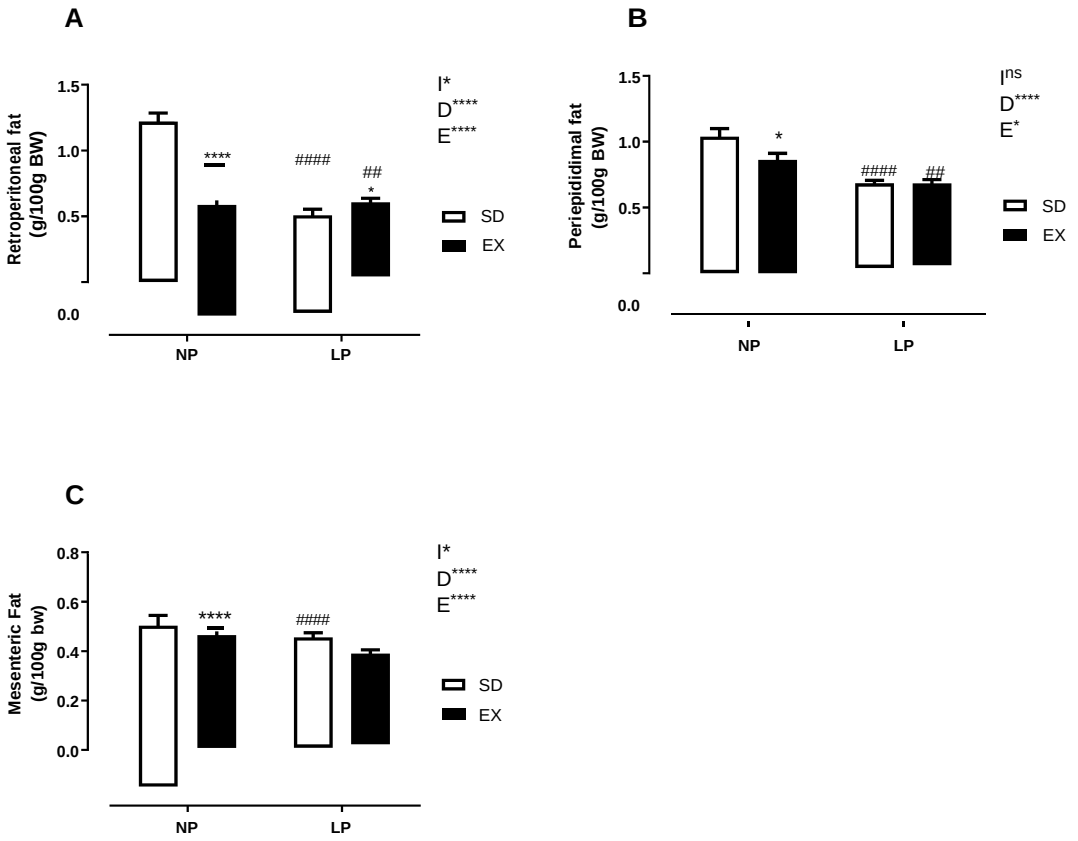

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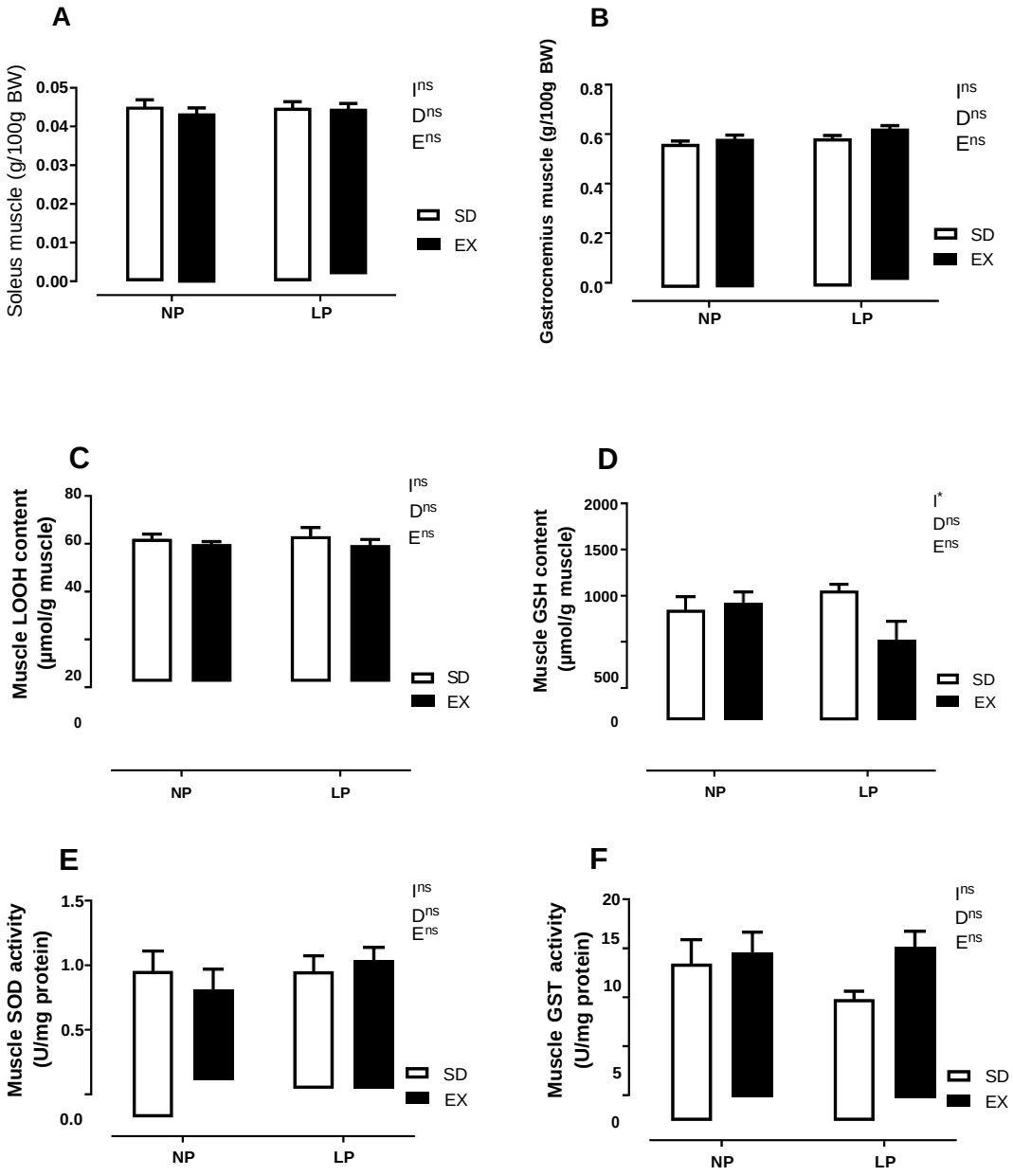

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Figure 4

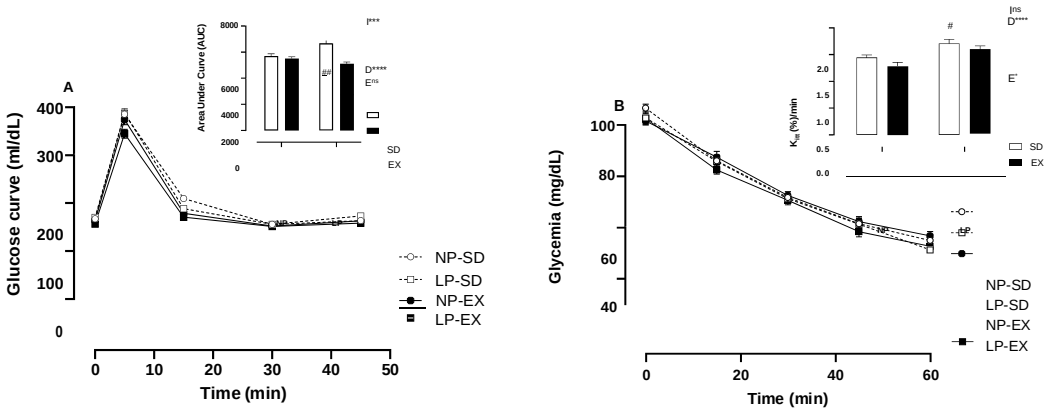
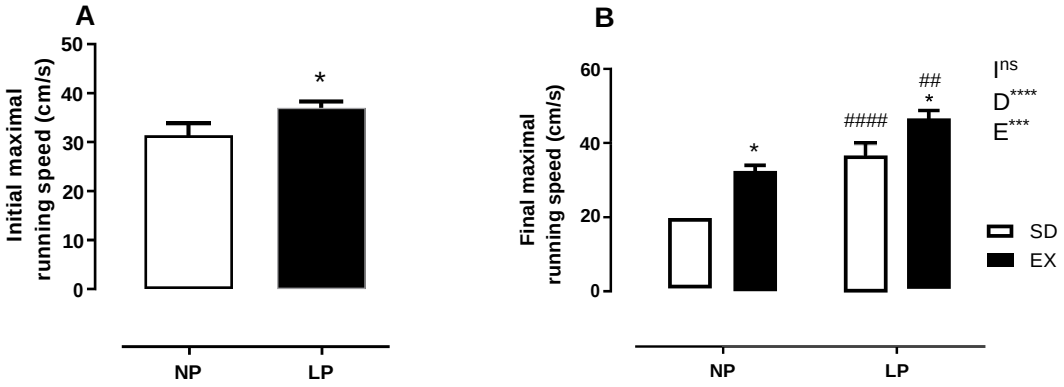


Figura 5



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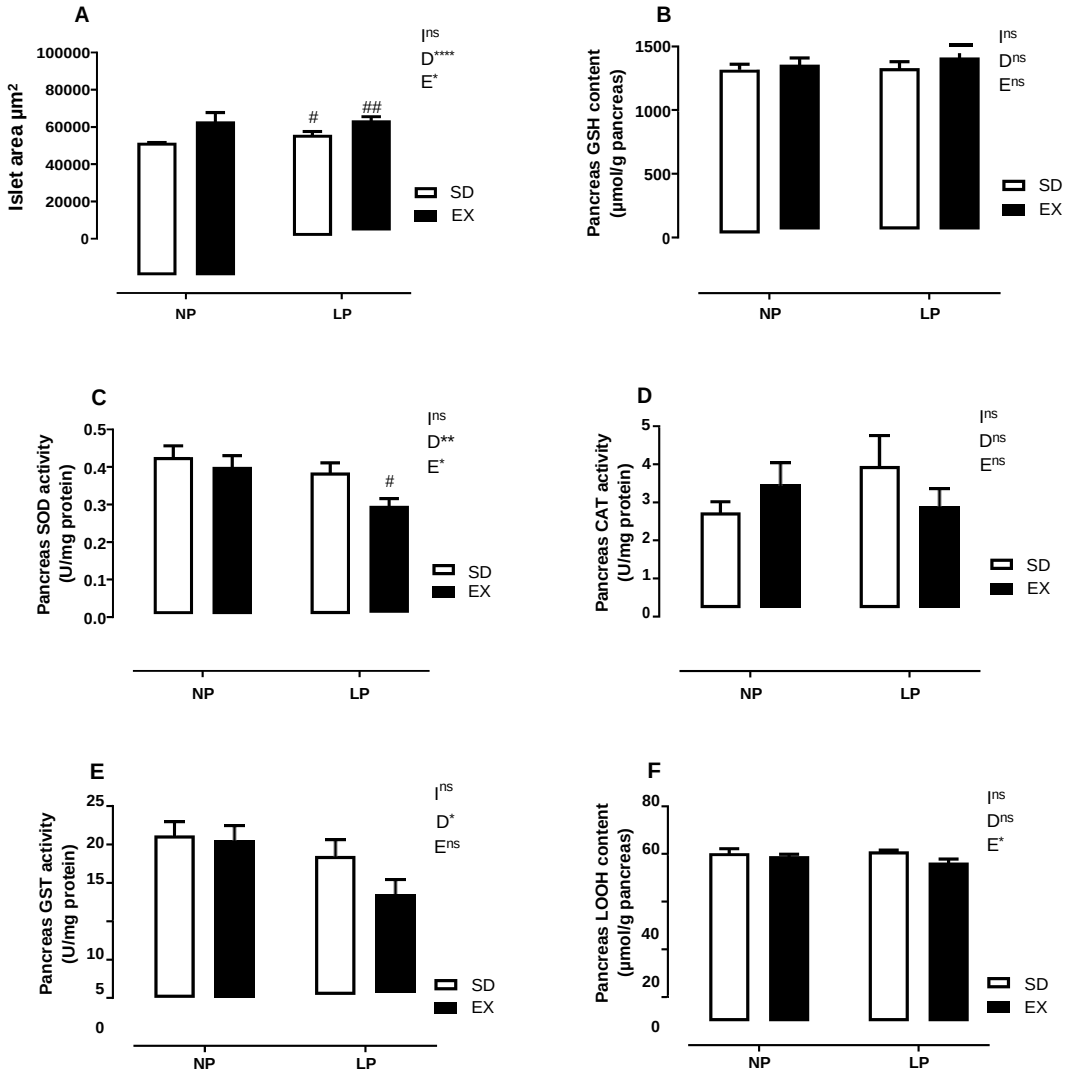

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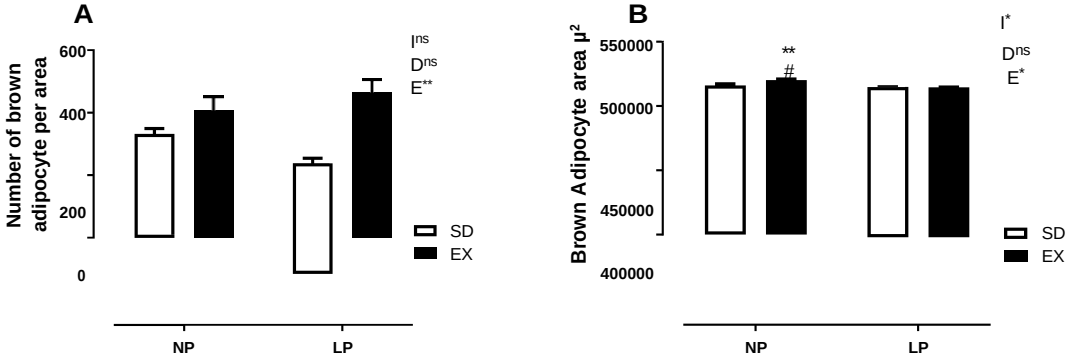
700 **Figure 7**



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