UNIVERSIDADE FEDERAL DO PAMPA

NATHALIE SAVEDRA GOMES

EXPOSIÇÃO PRÉ-NATAL E PERINATAL À DIETA HIPERLIPÍDICA INDUZ COMPORTAMENTO TIPO-ANSIOSO, ALTERAÇÕES METABÓLICAS NA PROLE DE CAMUNDONGOS

Itaqui
2017

NATHALIE SAVEDRA GOMES

PRENATAL AND PERINATAL EXPOSURE TO HIGH-FAT DIET INDUCED METABOLIC ALTERATIONS AND ANXIETY-LIKE BEHAVIOR IN MICE OFFSPRING

Trabalho de Conclusão de Curso apresentado ao Curso de Nutrição da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Nutrição.

Orientador: Cristiano Ricardo Jesse

Itaqui
2017

NATHALIE SAVEDRA GOMES

PRENATAL AND PERINATAL EXPOSURE TO HIGH-FAT DIET IMPACT ANXIETY BEHAVIOR, METABOLIC SYNDROME AND INFLAMMATION IN OFFSPRING IN MICE

Trabalho de Conclusão de Curso apresentado na Faculdade de Nutrição da Universidade Federal do Pampa como requisito básico para a conclusão do curso de Nutrição.

Trabalho de conclusão defendido e aprovado em: 12 de junho de 2017.
Banca examinadora:

Prof. Dr. Cristiano Ricardo Jesse
Orientador
Curso de Nutrição – UNIPAMPA

Prof. Drª. Carla Pohl Sehn
Banca
Curso de Nutrição – UNIPAMPA

Prof. Dr. Leandro Cattelan Souza
Banca
Curso de Nutrição – UNIPAMPA

AGRADECIMENTOS

Agradeço primeiramente a Deus, pois se não fosse sua misericórdia não chegaria até aqui, obrigada Jesus!

Agradeço aos meus pais Cibeli e Silvio, e ao meu padrasto Erodi os quais confiaram em mim e me deram a oportunidade de concretizar e encerrar mais uma caminhada da minha vida. Sei que não mediram esforços para que este sonho se realizasse, sem a compreensão, ajuda e confiança de vocês nada disso seria possível, amo vocês!

Ao meu Avô Urias Savedra, por sempre em suas orações interceder por mim, pois se hoje estou concluindo mais uma etapa na minha vida foi pelas tuas orações ao meu favor, te amo!

Aos meus irmãos Lauren, Matheus e Eliézer que sempre me incentivaram a nunca desistir, obrigada por tudo.

Agradeço ao meu cunhado Cristiano Streck pelo incentivo a cursar Nutrição, muito obrigada pelo apoio.

Agradeço aos meus sogros Osório e Adelaide Chaves pela confiança e carinho depositados em mim.

Ao meu esposo Jeferson, obrigada pela paciência, compreensão as minhas ausências, incentivo, e por me ajudar muitas vezes como um ombro amigo, serei eternamente grata por ter você ao meu lado, te amo!

Agradeço em especial ao Prof. Dr. Cristiano Jesse pela orientação, por todo aprendizado durante esse período de iniciação científica, muito obrigada!

Agradeço também a Prof. Dr. Carla Pohl Sehn pelo carinho, paciência e por compartilhar comigo seu conhecimento.

A todos os professores os quais contribuíram de alguma forma para minha formação, obrigada!

Agradeço a todos os colegas de curso, em especial minhas amigas Bianca Bertolazi e Eveline Bálsamo, pelas horas de estudo e por sempre estarem ao meu lado, enfim gurias conseguimos!

“Porque quando estou fraco então sou forte. ”

2 Coríntios 12:10

RESUMO

A obesidade induz um estado inflamatório de baixo grau e tem sido associado com alterações comportamentais e de ansiedade. Estudos anteriores indicaram que o consumo crônico de uma dieta rica em gordura leva à inflamação sistêmica e alterações no metabolismo em humanos e em modelos animais. Aqui, foi investigamos o impacto da dieta materna hiperlipídica (HFD) durante a gravidez e na lactação no comportamento da prole (comportamento tipo a ansiedade), e nos marcadores bioquímicos (triglicerídeos, colesterol, glicose e corticosterona), e inflamatórios (fator de necrose tumoral alpha (TNFα) interleucina-1β (IL-1β) e interleucina 6 (IL-6)) no plasma de camundongos. Observamos que a HFD materna aumentou o comportamento ansiogênico e o ganho de peso em descendentes do sexo feminino. Além disso, a descendência (feminina e masculina) dos camundongos fêmeas alimentadas com HFD também apresentaram aumento nos níveis de triglicerídeos, colesterol, glicose e de corticosterona, inflamatórios e nos níveis plasmáticos TNFα, IL-1β e IL-6. Esses dados sugerem que a HFD materna age de um insulto pré-natal/perinatal que impacta significativamente o comportamento da prole, os parâmetros metabólicos e inflamatórios. Em conclusão, esses resultados sugerem que a assistência pré-natal e perinatal em um dos períodos mais sensíveis, pode contribuir para o futuro dos indivíduos e os distúrbios em um período crítico no desenvolvimento podendo comprometer sua saúde. Consequentemente, a assistência nutricional na gestação pode dar acesso a conhecimentos úteis sobre o risco de distúrbios metabólicos e na saúde mental.

Palavras-chave

Obesidade, Dieta materna hiperlipídica, Inflamação, Alteração metabólica.

ABSTRACT

Obesity is related to chronic inflammatory state and has been associated with behavioral and anxiety alterations. Previous study have indicated that chronic high fat consumption leads to systemic inflammation and alterations in metabolism in humans and animal models. Here, we investigated the impact of maternal high fat diet (HFD) during pregnancy and lactation on offspring behavior (anxiety-like behavior), and biochemical (triglycerides, cholesterol, glucose and corticosterone) and inflammatory (tumor necrosis factorα (TNFα), interleukin-1β (IL-1β) and 6 (IL-6)) plasmatic markers in mice. We found that maternal HFD increased weight gain and anxiety in female offspring. Additionally, offspring (female and male) from HFD-fed dams also exhibited increased biochemical (triglycerides, cholesterol, glucose and corticosterone) and inflammatory (TNFα, IL-1β and IL-6) plasmatic levels. These data indicate that maternal HFD acts as a prenatal/perinatal insult that significantly impacts offspring behavior, metabolism and inflammation. In conclusion, these results suggest that prenatal and perinatal care is one of the most sensitive periods, which may contribute to the future of individuals and disorders in a critical period in development and may compromise their health. Consequently, nutritional assistance in gestation can provide access to useful knowledge about the risk of metabolic disorders and mental health.

Keywords

Obesity, maternal high-fat diet, Inflammation, Metabolic alteration.

Este trabalho está na forma de artigo científico seguindo as normas da revista

Revista de nutrição/ Brazilian Journal of Nutrition

PRENATAL AND PERINATAL EXPOSURE TO HIGH-FAT DIET INDUCED METABOLIC ALTERATIONS AND ANXIETY-LIKE BEHAVIOR IN MICE OFFSPRING

Categoria
Bioquímica Nutricional

Autores
Nathalie Savedra Gomes, Cristiano R. Jesse*

Laboratory of Pharmacological and Toxicological Evaluations Applied to Bioactive Molecules, Federal University of Pampa, Itaqui, CEP 97650-000, RS, Brazil.

*Correspondence should be sent to:

Cristiano R. Jesse

Phone and FAX number: +55-55-34321853

E-mail: cristianoricardojesse@yahoo.com.br

Sumário

1. Introduction

The prevalence of obesity and overweight have increased substantially over the past three decades, with variations across countries in levels and trends [1]. Currently about one-third of pregnant women are obese [2]. Thus, maternal obesity may be one of the main complications in children's brain development during the pre and post-natal period. Epidemiological studies have shown that maternal obesity has harmful effects on children's brain development, which can result in cognitive disorders associated with depression, anxiety, attention deficit and Alzheimer's disease [2,3]. Maternal obesity also predisposes children to metabolic disorders, such as hypertension, diabetes, dyslipidemias, and metabolic syndrome [4].

One study showed that the offspring of obese rats show abnormalities in the development of neural circuits [5]. Maternal obesity may alter hypothalamic sensitivity to leptin, which is the satiety hormone, and increase the expression of hypothalamic peptides by regulating the offspring's appetite. It is suggested that a high fat diet (HFD) may have harmful consequences on the cognitive ability in pups [6]. Similarly, animal studies indicated that obese mice kept on an HFD diet showed deficiency in memory and cognitive function. These deficits may be related to the effects of these diets on regulation of brain-derived neurotrophic factor (BDNF) and the increase of preinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1β and interleukin-6 in the hippocampus [7]. These results suggest that the reduction in BDNF resulting from a long-term ingestion of a HFD can impair learning and memory by interfering with hippocampal function [8]. Recent studies indicate that HFD consumption may lead to an increase in free radicals. In addition, the diet induced in genetic models of obesity, increased oxidative stress in the brain of rodents [9]. Maternal HFD consumption during pregnancy can also cause dysregulation in the metabolism of triglycerides and adipose tissue, leading offspring to a metabolic syndrome [10].

Despite this, it is unclear how HFD-induced obesity can cause anxious behavior and metabolic abnormalities in offspring, thus the aim of the study was to analyze anxious behavior and metabolic abnormalities in the offspring of mice treated with a HFD.

2. Materials and methods

2.1 Animals

Experiments were performed using C57BJ/6 mice (3 months old, weighing 30–40 g). Animals were maintained at 22-25ºC with free access to water and food, under a 12:12h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out during light phase on the day. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources (#001/2015) and with the approval of Ethical Committee for Animal Use of Federal University of Pampa, Brazil.

2.2. Maternal High fat diet (HFD) exposure

For the purpose of maternal HFD exposure (60% energy from fat), female mice sere exposure to HFD 3 weeks during gestation and 3 weeks during lactation. Control dams were exposed to the normal laboratory chow (control diet; 10% energy from fat, Puro Trato, RS).

All offsprings were weaned and sexed on postnataly day (PND) 21. Littermates of the same sex caged separately. All animals were maintained under and libitum food (laboratory chow) and water, and were kept in a temperature and humidity controlled animal vivarium under a 12:12 h reversed light-dark cycle. Offspring from eight independent litters (8 HFD and 8 control) were randomly selected for the behavioral and molecular test. Both male and female offsprings were include in all the tests described below to assess potential sex-dependent effects of the maternal HFD exposure. A first cohort of HFD and control offspring was subject to behavioral testing in the following test order: (1) open field test, (2) elevated pluz maze, and (3) light-dark test. An independent cohort of HFD and control was use for body weight, composition (fat body) and plasmatic determinations. All behavioral test were carried out when the offsprings reached adult stage of development (post day 90 (PND 90)). Total body weight was measured using a standard top-loading laboratory balance. Body composition was measured post-mortem for adult offspring. Adiposity is expressed as a percentage of fat relative to total body weight.

2.3. Open field test (OFT)

The OFT was carried out to evaluate if the diet produced effects on locomotor activity. The animals were submitted individually for a period of 5 min to an OFT apparatus (Insight model EP 154C). The total distance (unit: mm) was computed [11].

2.4. Elevated plus maze test (EPMT)

This test has been widely validated to measure anxiety in rodents [12]. The apparatus consists of two elevated (26cm high) and open arms (16 × 5cm) positioned opposite to one another and separated by a central platform (5 × 5cm) and two arms of the same dimension, but enclosed by walls (16 × 5 × 10cm) forming a cross. The maze is lit by a dim light placed above the central platform. During a 5min test period, the number of entries either the open and enclosed arms, plus the time spent in the open arms was recorded. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: (1) time spent in the open arms relative to the total time spent in the plus-maze (300s), expressed as percentage; (2) number of entries into the open arms relative to the total number of entries into both open and closed arms, expressed as percentage. The anxiolytic effectiveness of a drug is illustrated by a significant statistical augmentation of parameters in open arms (time and/or entries).

2.5. Light-dark test (LDT)

The light–dark box is a sensitive model to detect activity in disorders related to generalize anxiety [13]. The test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior in response to novel environment and light. The apparatus consisted of two compartments: an open topped rectangular box (46 × 27 × 30 cm high), is divided into a small (18 × 27 cm) area and a large (27 × 27 cm) area with an opening door (7.5 × 7.5 cm) located in the center of the partition at floor level. The smaller compartment was painted black and covered with a roof. The other compartment had no roof and was brightly illuminated by a 60W bulb located 25cm above the box. Each animal was placed at the center of the illuminated compartment, facing one of the dark areas, and total number of transitions between the two compartments, latency to enter the dark and the time spent in the light compartment was recorded during 5min. Anxiolytic activity could be

evaluated by time spent in the illuminated compartment and the number of transitions.

2.6. Plasma determinations

After behavioral tests, mice were euthanized with barbiturate overdose (pentobarbital sodium 150 mg/kg; i.p. route) and blood was collected by cardiac puncture into tubes containing heparin (1 UI/ll) and EDTA.

2.6.1. Measurement of metabolites

The plasma glucose concentration was measured by using a glucose reagent strip and a glucometer (Glucotouch, Lifescan, Milpitas, CA). Fasting plasma lipids (ie, triglycerides and total cholesterol) were measured in plasma samples by using an automated system (Konelab 20; Thermo Electron Corporation).

2.6.2. Cytokine and corticosterone levels

Levels of tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and corticosterone in plasma were measured using sample aliquots of 10 µL and mouse cytokine ELISA DuoSet Kits from R&D Systems (Minneapolis, MN, USA), according to the manufacturer's instructions (protein range of 31.25–2,000 pg). The level of cytokine was estimated by interpolation from a standard curve by colorimetric measurements at 450 nm (correction wavelength 540 nm) on an ELISA plate reader (Berthold Technologies-Apollo 8-LB 912, KG, Germany). Results are shown as pg/mg.

2.7. Statistical analysis

The data distribution was verified by applying the Kolmogorov-Smirnov test. Results are presented as the mean ± standard error of the mean (SEM). Comparisons between the experimental and the control groups were performed by two-way ANOVA (sex X diet treatment = independent variables) followed by Bonferroni post hoc test, when appropriate. A value of $P < 0.05$ was considered to be statistically significant. All tests were carried out using the GraphPad software 5.0 (San Diego, California, USA).

3. Results

3.1. Effects caused by sex and diet on behavioral tests in nice

Statistical analysis of the % open arm entries yielded significant effect of sex ($F_{1, 24} = 6.04$, $p < 0.02$), diet ($F_{1, 24} = 4.57$, $p < 0.04$), but not sex × diet interaction ($F_{1, 24} = 3.17$, $p < 0.09$). Post hoc comparisons showed that HFD

significantly decreased the % open arm entries of female mice when compared to control/female and to HFD/male ($p < 0.01$) (**Fig. 1A**).

Two-way ANOVA of the % time in open arms demonstrated a significant effect of sex × diet interaction ($F_{1, 24} = 4.24$, $p < 0.05$), nor main effect of sex ($F_{1, 24} = 0.68$, $p < 0.42$) and diet ($F_{1, 24} = 1.59$, $p < 0.22$). Post hoc comparisons showed that HFD significantly decreased the % time in open arms of female mice when compared to control/female and to HFD/male ($p < 0.01$) (**Fig. 1B**).

Figure 1

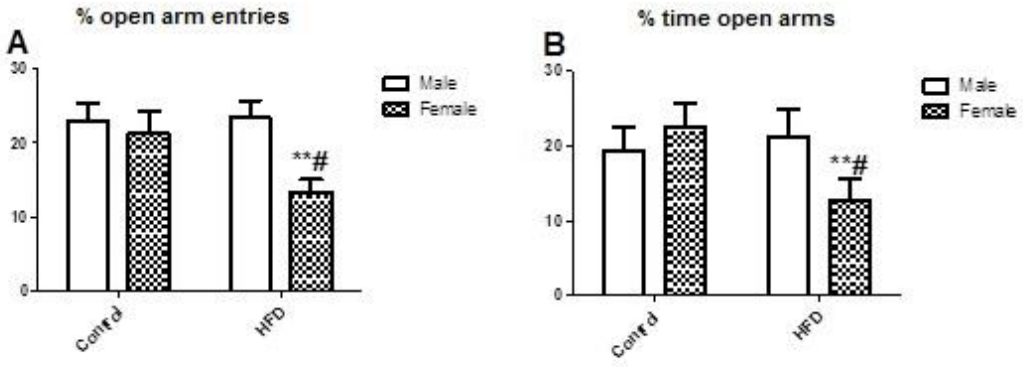

Fig. 1. (A) Effect of high fat diet exposure on on the % open arm entries and **(B)** on the % time on open arms the elevated pluz maze. Values are mean ± S.E.M. (n=8 per group). **: p<0.01 when compared with control group (male or female); #: p<0.01 when compared with male groups (control or HFD) (Two-way ANOVA and Bonferroni comparison test).

Statistical analysis of latency Light to dark side (L-D) did not demonstrated effect of sex ($F_{1, 24} = 0.89$, $p < 0.35$), diet ($F_{1, 24} = 0.05$, $p < 0.96$) and sex × diet interaction ($F_{1, 24} = 1.62$, $p < 0.22$) (**Fig. 2A**).

Two-way ANOVA of the number of transitions did not shown effect of sex ($F_{1, 24} = 0.05$, $p < 0.82$), diet ($F_{1, 24} = 1.26$, $p < 0.27$) and sex × diet interaction ($F_{1, 24} = 1.70$, $p < 0.21$) (**Fig. 2B**).

Statistical analysis of the time in light demonstrated a significant effect of sex × diet interaction ($F_{1, 24} = 4.44$, $p < 0.05$), nor main effect of sex ($F_{1, 24} = 2.18$, $p < 0.15$) and diet ($F_{1, 24} = 2.49$, $p < 0.12$). Post hoc comparisons showed that HFD significantly decreased the time in light of female mice when compared to control/female and to HFD/male ($p < 0.01$) (**Fig. 2C**).

Figure 2

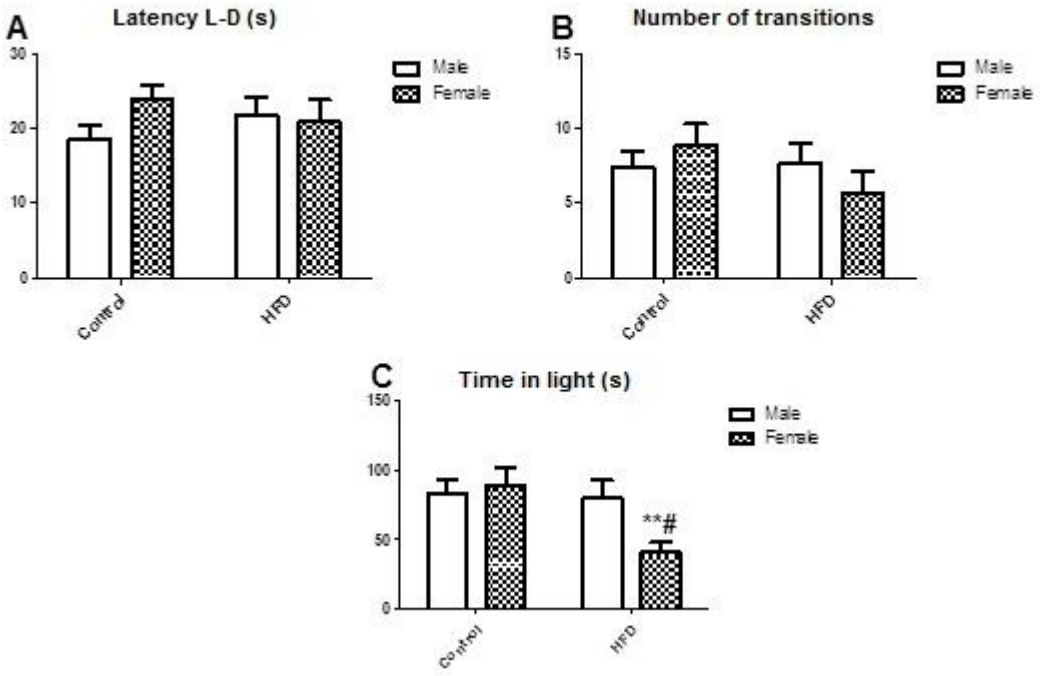

Fig. 2. (A) Effect of high fat diet exposure the latency Light-dark (L-D) **(B)** number of transitions and **(C)** on time of light (seconds- s) in the LDT. Values are mean ± S.E.M. (n=8 per group). **: p<0.01 when compared with control group (male or female); #: p<0.01 when compared with male groups (control or HFD) (Two-way ANOVA and Bonferroni multiple comparison test).

Two-way ANOVA of the locomotor activity in the open field test did not demonstrated effect of sex ($F_{1, 24} = 0.39$, $p < 0.54$), diet ($F_{1, 24} = 0.05$, $p < 0.93$) and sex × diet interaction ($F_{1, 24} = 0.05$, $p < 0.93$) (**Fig. 3A**).

Statistical analysis of body weight at 8º day demonstrated a significant effect of sex ($F_{1, 24} = 10.26$, $p < 0.005$), diet ($F_{1, 24} = 4.56$, $p < 0.04$) and sex × diet interaction ($F_{1, 24} = 4.70$, $p < 0.04$). Post hoc comparisons showed that HFD significantly increased the body weight at 8º day of female mice when compared to control/female ($p < 0.01$) (**Fig. 3B**).

Two-way ANOVA of body weight at 90º day demonstrated a significant effect of sex ($F_{1, 24} = 13.05$, $p < 0.002$), diet ($F_{1, 24} = 11.01$, $p < 0.004$) and sex × diet interaction ($F_{1, 24} = 4.57$, $p < 0.04$). Post hoc comparisons showed that HFD significantly increased the body weight at 90º day of female mice when compared to control/female ($p < 0.01$) (**Fig. 3C**). In addition, the body weight

was significantly lower of the control-female when compared to control/male ($p < 0.01$) (**Fig. 3C**).

Statistical analysis of total body fat demonstrated a significant effect of diet ($F_{1, 24} = 17.65$, $p < 0.001$), sex × diet interaction ($F_{1, 24} = 10.23$, $p < 0.005$), but nor main effect of sex ($F_{1, 24} = 0.34$, $p < 0.56$). Post hoc comparisons showed that HFD significantly increased the total body fat of female mice when compared to control/female and male/HFD ($p < 0.01$) (**Fig. 3D**). Moreover, the total body fat of the control/female group was significantly decreased when compared to control/male ($p < 0.01$).

Figure 3

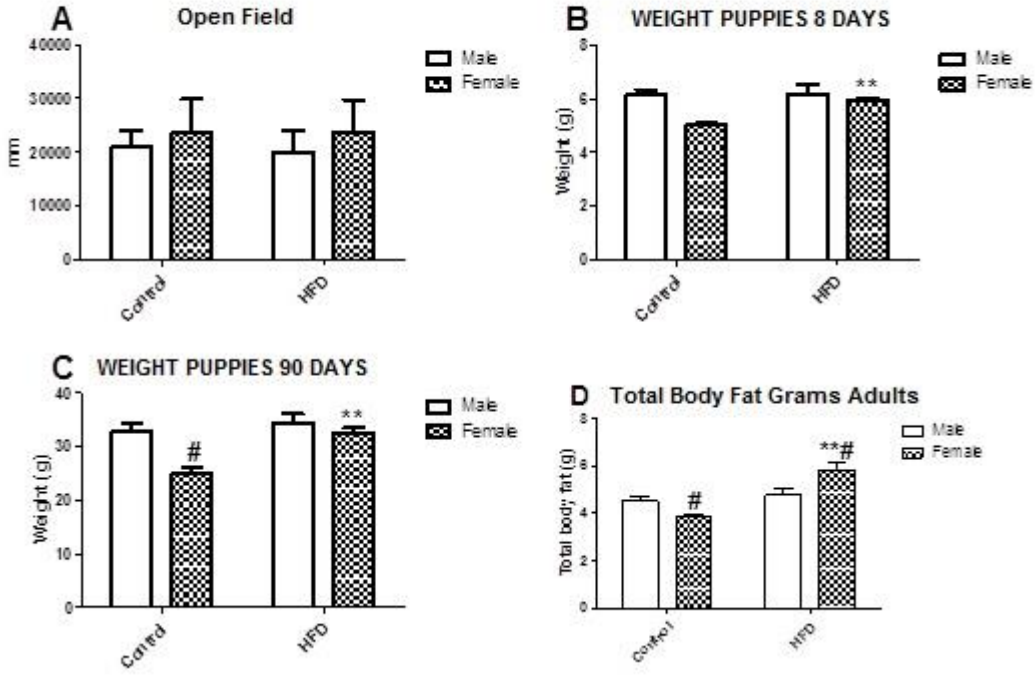

Fig. 3. (A) Effect of high fat diet exposure on the locomotor activity in the Open-field test (OFT) (L-D), **(B)** on the body weight at 8º day, **(C)** on the body weight at 90º days and **(D)** total body fat (grams – g). Values are mean ± S.E.M. (n=8 per group). **: p<0.01 when compared with control group (male or female); #: p<0.01 when compared with male groups (control or HFD) (Two-way ANOVA and post hoc Bonferroni multiple comparison test).

3.2. Effects caused by sex and diet on plasmatic determinations in mice

Two-way ANOVA of the triglycerides levels yielded a significant effect of diet ($F_{1, 24} = 18.51$, $p < 0.001$), but nor main effect of sex ($F_{1, 24} = 0.29$, $p < 0.59$) and sex × diet interaction ($F_{1, 24} = 0.34$, $p < 0.56$). Post hoc comparisons showed

that HFD significantly increased the of triglycerides levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4A**).

Statistical analysis of the total cholesterol levels demonstrated a significant effect of diet ($F_{1, 24} = 16.99$, $p < 0.001$), but nor main effect of sex ($F_{1, 24} = 2.24$, $p < 0.16$) and sex × diet interaction ($F_{1, 24} = 0.60$, $p < 0.45$). Post hoc comparisons showed that HFD significantly increased the of triglycerides levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4B**).

Two-way ANOVA of the glucose levels showed a significant effect of diet ($F_{1, 24} = 16.82$, $p < 0.001$), but nor main effect of sex ($F_{1, 24} = 1.58$, $p < 0.23$) and sex × diet interaction ($F_{1, 24} = 2.04$, $p < 0.17$). Post hoc comparisons showed that HFD significantly increased the of glucose levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4C**).

Statistical analysis of the total TNFα levels yield a significant effect of diet ($F_{1, 24} = 18.22$, $p < 0.001$), but nor main effect of sex ($F_{1, 24} = 0.13$, $p < 0.72$) and sex × diet interaction ($F_{1, 24} = 1.01$, $p < 0.33$). Post hoc comparisons showed that HFD significantly increased the of TNFα levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4D**).

Two-way ANOVA of the IL-1β levels demonstrated a significant main effect of sex ($F_{1, 24} = 8.19$, $p < 0.01$) and diet ($F_{1, 24} = 22.40$, $p < 0.001$), but not sex × diet interaction ($F_{1, 24} = 0.08$, $p < 0.78$). Post hoc comparisons showed that HFD significantly increased the of IL-1β levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4E**). In addition, the IL-1β levels were significantly higher in the female/HFD group when compared to male/HFD group ($p < 0.01$) (**Fig. 4E**).

Statistical analysis of the IL-6 levels yield a significant main effect of sex ($F_{1, 24} = 6.73$, $p < 0.02$) and diet ($F_{1, 24} = 78.41$, $p < 0.001$), but not sex × diet interaction ($F_{1, 24} = 0.07$, $p < 0.79$). Post hoc comparisons demonstrated that HFD significantly increased the of IL-6 levels in mice when compared to control diet in both sexes ($p < 0.01$) (**Fig. 4F**). In addition, the IL-6 levels were significantly increased in the female/HFD group compared to male/HFD group ($p < 0.01$) (**Fig. 4F**).

Two-way ANOVA of the corticosterone levels showed a significant main effect of sex ($F_{1, 24} = 20.68$, $p < 0.001$) and diet ($F_{1, 24} = 31.61$, $p < 0.001$), but not sex × diet interaction ($F_{1, 24} = 0.14$, $p < 0.71$). Post hoc comparisons

demonstrated that HFD significantly increased the of corticosterone levels in mice when compared to control diet in both sexes (p < 0.01) (**Fig. 4G**). In addition, the corticosterone levels were significantly higher in the female groups compared to male groups (p < 0.01) (**Fig. 4G**).

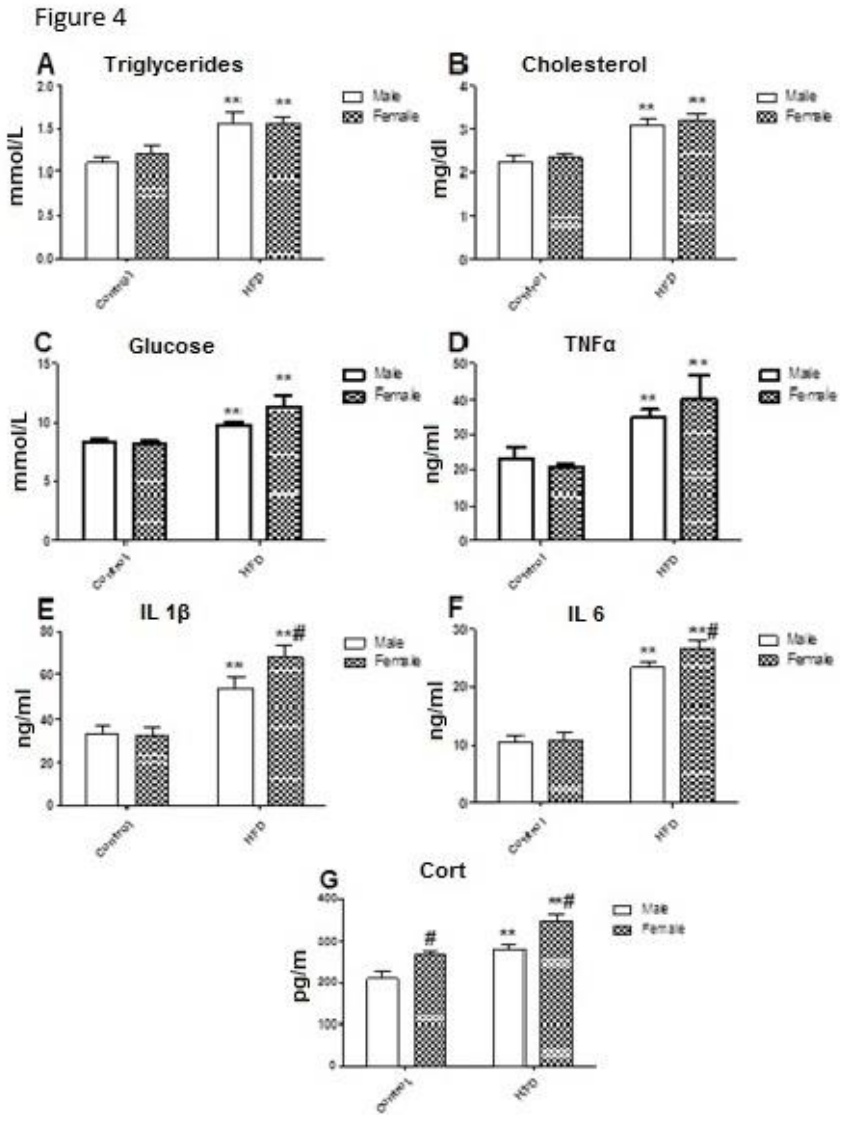

Fig. 4. Effects of high fat diet exposure in triglycerides **(A)**, cholesterol **(B)**, glucose **(C),** TNFα **(D),** IL1β **(E),** IL-6 **(F)** and corticosterone **(G)** levels in plasma of mice. Values are mean ± S.E.M. (n=6 per group). **: p<0.01 when compared with control group (male or female); #: p<0.01 when compared with male groups (control or HFD) (Two-way ANOVA and post hoc Bonferroni multiple comparison test).

4. Discussion

Obesity has become a worldwide epidemic and we have not yet fully understand the effects of maternal diet on offspring health [14]. In developed

countries, approximately 30% of all pregnancies are now complicated by maternal obesity [15]. HFD exposure during development may lead to multiple deleterious health outcomes, including anxiety-related mental disorders. The mechanisms by which prenatal events impact offspring behavioral, metabolic, and brain outcomes are usually complex, though poorly defined, and may involve several physiological pathways, as well as changes in maternal-offspring interactions after birth [16,17]. The present study demonstrated that maternal high-fat diet for 6 weeks during gestation and lactation contributed to the exaggerated weight gain, anxiety-related behavior and inflammatory markers in the adult offspring. These alterations in anxiety behavior are associated with changes in body weight gain and biochemical (triglycerides, cholesterol, glucose and corticosterone) and inflammatory (TNFα, IL-1β and IL-6) levels in plasma of adult offspring, indicating a dysregulation on inflammatory response.

In the EPMT and LDT, offspring of dams exposure to HFD showed enhanced anxiety-like behavior. Our data are in line with previous finding that demonstrate increased levels of anxiety in offspring born in maternal HFD [16]. In humans, maternal obesity has been linked with child obesity, increased inflammations and anxiety disorders [18]. In this way, a maternal HFD may disrupt anxiety responses via neural inflammation with involvement of glucocorticoid receptor and neurotrophin signaling pathways [16,17]. Several previous studies have indicated that behaviors characteristic of anxiety in adulthood are also consistent with impulsive/risk-taking exploratory behaviors in adolescent animals [19,20]. For example, in a study by Colorado et al. [21] adolescent offspring that experienced maternal separation, known to increase anxiety behavior in adulthood, spent an increased amount of time in the center of an Open Field (both a novel Open Field and a familiar Open Field) and in the lighted portion of the Light–dark transition box. Likewise, high levels of exploration of the open arms of the Elevated Plus Maze has also been suggested to be an indicator of impulsive behavior [22].

Another important finding of the study present was that the sex differences in anxiety-like behavior. These data are consistent with previous studies demonstrating that females are more vulnerable to the HFD exposure (EFERE). In the current study, HFD exposure did not affect behavioral changes

in anxiety tests in male offspring. The data obtained in our study did not justify the behavioral alterations only in female, thus the biochemical and inflammatory markers analyzed was altered in both sexes. One hypothesis for the anxiety-like behavior in female are the effects of HFD exposure are due to the inflammatory or endocrine-disrupting effects of this obesogen during sensitive periods of brain development [16-19].

Considering the plasmatic parameters measured, our results are coherent with a resistance to the development of metabolic syndrome, as stated in the medical definition of this syndrome (metabolic syndrome corresponds to elevated plasmatic glucose, triglycerides and cholesterol). Importantly, we also showed that, once adult, these mice demonstrated a body weight and total body fat higher to control diet group, suggesting that our findings are attributable to HFD exposure. In adult animals, chronic HFD exposure is well known to enhance the activation of the HPA axis due to increased secretion of ACTH and corticosterone [23]. Thus, the differences in body weight were found between HFD and control mice, it remains possible that metabolic parameters and body composition, as a result of HFD exposure may have contributed to the differences in anxiety behavior observed in this study.

In humans, obese mothers have increased TNFα, IL-1β, IL-6 in plasma and breast milk and increased IL-6 gene expression in placental macrophages [24-26]. In rodents, increased fetal IL-6 levels from high fat/sugar-fed dams are also observed [26]. These results demonstrate brain effects of perinatal HFD exposure, and suggest that changes in inflammatory pathway genes may be relevant for anxiety behavior, at least in the present experimental context. Currently, it is unknown if mice have similar sex differences in microglial colonization; however, increased IL-1β, TNFα and IL6 gene expression has been shown in female brains [26], suggesting that during early mouse development, female brains have a more pro-inflammatory contex compared to males. This may be important since elevated CNS pro-inflammatory cytokines, including IL-1β and/or TNFα, have been associated with increased anxiety, decreased cognition, and altered social behaviors [26]. Additionally, increased hippocampal IL-1 β has been observed in offspring of both sexes with altered behavior [27]. Therefore, regardless of differences in the impacted sex between studies, maternal obesity results in increased offspring inflammation that is

associated with behavioral abnormalities. Thus, the alterations in cytokines, which occurred in a sexually dimorphic manner, may underlie distinct behavioral phenotypes in adult male and female offspring.

In summary, these data demonstrate that HFD exposure during pregnancy and lactation periods results in altered anxiety in offspring. Inflammation, reflected by elevated IL-1 β, TNF α and IL-6 affected female offspring, may be a mechanism for the enduring behavioral alterations as a result of pre- and postnatal exposure to maternal HFD. Importantly, our data highlight dietary intervention as a potential therapeutic strategy to offset the deleterious effects of maternal obesity on offspring behavior and inflammation.

5. References

1. NG, MARIE ET AL. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, v. 384, n. 9945, p. 766-781, 2014.

2. VAN LIESHOUT, R. J.; TAYLOR, V. H.; BOYLE, M. H. Pre-pregnancy and pregnancy obesity and neurodevelopmental outcomes in offspring: a systematic review. Obesity reviews, v. 12, n. 5, p. e548-e559, 2011.

3. ALLEN, Karina L. et al. Risk factors for full-and partial-syndrome early adolescent eating disorders: a population-based pregnancy cohort study. Journal of the American Academy of Child & Adolescent Psychiatry, v. 48, n. 8, p. 800-809, 2009.

4. ROFEY, Dana L. et al. A longitudinal study of childhood depression and anxiety in relation to weight gain. Child psychiatry and human development, v. 40, n. 4, p. 517-526, 2009.

5. WILLIAMS, Lyda et al. Animal models of in utero exposure to a high fat diet: a review. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, v. 1842, n. 3, p. 507-519, 2014.

6. D O'BRIEN, Phillipe et al. Neurological consequences of obesity. The Lancet Neurology, v. 16, n. 6, p. 465-477, 2017.

7. WU, Ting et al. Maternal obesity caused by overnutrition exposure leads to reversal learning deficits and striatal disturbance in rats. PloS one, v. 8, n. 11, p. e78876, 2013.

8. HATANAKA, Yusuke; KABUTA, Tomohiro; WADA, Keiji. Disturbance in Maternal Environment Leads to Abnormal Synaptic Instability during Neuronal Circuitry Development. Frontiers in neuroscience, v. 11, 2017.

9. FLEGAL, Katherine M. et al. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. Jama, v. 307, n. 5, p. 491-497, 2012.

10. GUH, Daphne P. et al. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC public health, v. 9, n. 1, p. 88, 2009.

11. WALSH R.N., CUMMINS, R.A. The open-field test: a critical Review. Psychol. Bull. V.83, p.482-504, 1976.

12. PELLOW, S., CHOPIN, P., FILO, S.E., BRILEY, M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods v. 14, p. 149-167, 1985.

13. COSTALL, B., JONES, B.J., KELLY, M.E., NAYLOR, R.J., TOMKINS, D.M. Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol Biochem Behav v. 32, p. 777-85, 1989.

14. GODFREY KM, GLUCKMAN PD, HANSON MA. Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol Metab v. 2, p.199–205, 2010.

15. CATALANO PM. Management of obesity in pregnancy. Obstet Gynecol v. 109, p. 419–433, 2007.

16. BOLTON JL, AUTEN RL, BILBO SD. Prenatal air pollution exposure induces sexually dimorphic fetal programming of metabolic and neuroinflammatory outcomes in adult offspring. Brain Behav Immun v. 37, p.30-44, 2014.

17. BOLTON JL, HUFF NC, SMITH SH, MASON SN, FOSTER WM, AUTEN RL, BILBO SD. Maternal stress and effects of prenatal air pollution on offspring mental health outcomes in mice. Environ Health Perspect v. 121, p. 1075-82, 2013.

18. FARAH N, HOGAN AE, O ’ CONNOR N, KENNELLY MM, O ’ SHEA D, TURNER MJ. Correlation between maternal inflammatory markers and fetomaternal adiposity. Cytokine v. 60, p. 96 – 99, 2005.

19. JACOBSON-PICK S, AUDET M-C, NATHOO N, ANISMAN H. Stressor experiences during the juvenile period increase stressor responsivity in adulthood: Transmission of stressor experiences. Behav Brain Res v.216, p.365–374, 2011.

20. MCCORMICK CM, GREEN MR. From the stressed adolescent to the anxious and depressed adult: investigations in rodent models. Neuroscience v.249, p.242–257, 2013.

21. COLORADO RA, SHUMAKE J, CONEJO NM, GONZALEZ-PARDO H, GONZALEZ- LIMA F. Effects of maternal separation, early handling, and standard facility rearing on orienting and impulsive behavior of adolescent rats. Behav Process v.71, p.51–58, 2006.

22. ALMEIDA SS, TONKISS J, GALLER JR. Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus- maze test. Physiol Behav v.60, p.675–680, 1996.

23. TANNENBAUM BM, BRINDLEY DN, TANNENBAUM GS, DALLMAN MF, MCARTHUR MD, MEANEY MJ. High-fat feeding alters both basal and stress-induced hypothalamic–pituitary–adrenal activity in the rat. Am J Physiol v.273, p.1168–E1177, 1997.

24. COLLADO MC, LAITINEN K, SALMINEN S, ISOLAURI E. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. Pediatr Res v.72, p.77 – 85, 2002.

25. DESAI N, ROMAN A, ROCHELSON B, GUPTA M, XUE X, CHATTERJEE PK, TAM TAM H, METZ CN. Maternal metformin treatment decreases fetal inflammation in a rat model of obesity and metabolic syndrome. Am J Obstet Gynecol v.209, p.1-9, 2003.

26. CRAIN JM, NIKODEMOVA M, WATTERS JJ: Microglia express distinct M1 and M2 phenotypic markers in the postnatal and adult central nervous system in male and female mice. J Neurosci Res v.91, p.1143 – 1151, 2003.

27. BILBO SD, TSANG V: Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J Off Publ Fed Am Soc Exp Biol v.24, p.2104 – 2115, 2010.