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EFFECTS OF MATERNAL SUPPLEMENTATION WITH CHIA OIL ON HEPATIC AND METABOLIC PARAMETERS IN A POSTNATAL OVERNUTRITION MODEL

EFEITOS DA SUPLEMENTAÇÃO MATERNA COM ÓLEO DE CHIA SOBRE PARÂMETROS HEPÁTICOS E METABÓLICOS EM UM MODELO DE HIPERALIMENTAÇÃO PÓS-NATAL

EFECTOS DE LA SUPLEMENTACIÓN MATERNA CON ACEITE DE CHÍA SOBRE PARÁMETROS HEPÁTICOS Y METABÓLICOS EN UN MODELO DE HIPERALIMENTACIÓN PÓS-NATAL

Danyela Almeida Barreto
Mestra em Nutrição e Saúde, Universidade Federal de Lavras, Brasil
E-mail: danyelaalmeida6@gmail.com

João Paulo Lima de Oliveira
Doutor em Plantas Medicinais, Aromáticas e Condimentares, Universidade Federal de Lavras, Brasil
Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Brasil
E-mail: joaopaulolimanut@gmail.com

Helena Mariano Coelho Costa Monteiro
Graduada em Nutrição Bacharelado, Universidade Federal de Lavras, Brasil
E-mail: hmccmonteiro@gmail.com

Geraldo de Sousa Cândido
Doutor em Agroquímica, Universidade Federal de Lavras, Brasil
E-mail: geraldo.candido@ufla.br

Isaac Filipe Moreira Konig
Doutor em Agroquímica, Universidade Federal de Lavras, Brasil
Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Brasil.
E-mail: isaac.konig@ufrgs.br

Mary Suzan Varaschin
Doutora em Medicina Veterinária, Universidade Estadual Paulista Júlio de Mesquita Filho, Brasil
Departamento de Medicina Veterinária, Universidade Federal de Lavras, Brasil
E-mail: msvaraschin@ufla.br

Laura Cristina Jardim Pôrto Pimenta
Doutorado em Ciências Biológicas (Fisiologia e Farmacologia), Universidade Federal de Minas Gerais, Brasil

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Departamento de Nutrição, Universidade Federal de Lavras, Brasil
E-mail: laurap@ufla.br

Isabela Coelho de Castro
Doutora em Biologia Celular e Molecular, Universidade Federal do Paraná, Brasil
Departamento de Nutrição, Universidade Federal de Lavras, Brasil
E-mail: isabela.castro@ufla.br

Abstract

Early-life nutritional disturbances can induce long-term metabolic alterations through developmental programming. Chia oil (ChO), a rich source of α-linolenic acid and other bioactive compounds, has been associated with beneficial effects on glucose metabolism and oxidative stress. This study investigated whether maternal ChO supplementation attenuates metabolic and hepatic oxidative alterations in offspring subjected to postnatal hyperalimentation in a mouse model induced by postnatal litter-size reduction. Female BALB/c mice received ChO (5 g/kg body weight), corn oil (5 g/kg body weight), or water from mating through lactation. After birth, litters were adjusted to standard size (8–10 pups) or reduced size (3–4 pups) to induce postnatal hyperalimentation, generating six experimental groups. Male offspring were evaluated at 120 days of age for body weight, adiposity, glucose tolerance, insulin sensitivity, plasma biochemical parameters, hepatic lipid content, and oxidative stress markers. Postnatal hyperalimentation increased body weight at 21 days, with a persistent difference only between HChO and CChO at 120 days. No significant differences were observed in the Lee index, tissue weights, fasting biochemical parameters, or oxidative stress markers. CCO showed a lower OGTT AUC than C and HCO, while HChO showed a lower IST AUC than CChO. Maternal ChO supplementation had limited metabolic effects in male BALB/c offspring exposed to postnatal overnutrition. Although it modulated insulin sensitivity, no consistent changes were observed in other metabolic or oxidative stress parameters. In conclusion, the hyperfeeding model was not entirely effective in inducing metabolic changes in BALB/C mice and maternal ChO supplementation exerted limited effects on metabolic parameters in offspring exposed to postnatal hyperalimentation.

Keywords: Early-life nutrition; Alpha-linolenic acid; Insulin sensitivity; Glucose homeostasis; Oxidative stress.

Resumo

Distúrbios nutricionais no início da vida podem induzir alterações metabólicas de longo prazo por meio da programação metabólica. O óleo de chia (OC), uma fonte rica em ácido α-linolênico e outros compostos bioativos, tem sido associado a efeitos benéficos sobre o metabolismo da glicose e o estresse oxidativo. Este estudo investigou se a suplementação materna com OC atenua alterações metabólicas e hepáticas relacionadas ao estresse oxidativo na prole submetida à hiperalimentação pós-natal, em um modelo murino induzido pela redução do tamanho da ninhada. Fêmeas BALB/c receberam OC (5 g/kg de peso corporal), óleo de milho (5 g/kg de peso corporal) ou água, desde o acasalamento até o período de lactação. Após o nascimento, as ninhadas foram ajustadas para o tamanho padrão (8–10 filhotes) ou reduzidas (3–4 filhotes) para induzir a hiperalimentação pós-natal, resultando em seis grupos experimentais. Os descendentes machos foram avaliados aos 120 dias de idade quanto ao peso corporal, adiposidade, tolerância à glicose, sensibilidade à insulina, parâmetros bioquímicos plasmáticos, conteúdo lipídico hepático e marcadores de estresse oxidativo. A hiperalimentação pós-natal aumentou o peso corporal aos 21 dias, com uma diferença persistente apenas entre os grupos HChO e CChO aos 120 dias. Não se observaram diferenças significativas no índice de Lee, nos pesos dos tecidos, nos parâmetros bioquímicos em jejum ou nos marcadores de estresse oxidativo. O grupo CCO apresentou uma AUC do OGTT inferior à dos grupos C e HCO, enquanto o grupo HChO apresentou uma AUC do IST inferior à do grupo CChO. A suplementação materna com ChO teve efeitos metabólicos limitados na prole masculina BALB/c exposta à hiperalimentação pós-natal. Embora tenha modulado a sensibilidade à insulina, não se observaram

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alterações consistentes em outros parâmetros metabólicos ou de estresse oxidativo. Em conclusão, o modelo de hiperalimentação não foi totalmente eficaz na indução de alterações metabólicas em camundongos BALB/C e a suplementação materna com OC exerceu efeitos limitados sobre os parâmetros metabólicos da prole exposta à hiperalimentação pós-natal.

Palavras-chave: Nutrição no início da vida; Ácido α-linolênico; Sensibilidade à insulina; Homeostase da glicose; Estresse oxidativo.

Resumen

Las alteraciones nutricionales durante las primeras etapas de la vida pueden inducir cambios metabólicos a largo plazo mediante la programación del desarrollo. El aceite de chía (AC), una fuente rica en ácido α-linolénico y otros compuestos bioactivos, se ha asociado con efectos beneficiosos sobre el metabolismo de la glucosa y el estrés oxidativo. Este estudio investigó si la suplementación materna con AC atenúa las alteraciones metabólicas y hepáticas relacionadas con el estrés oxidativo en la descendencia sometida a hiperalimentación posnatal, en un modelo murino inducido mediante la reducción del tamaño de la camada. Las hembras BALB/c recibieron AC (5 g/kg de peso corporal), aceite de maíz (5 g/kg de peso corporal) o agua desde el apareamiento hasta el período de lactancia. Después del nacimiento, las camadas se ajustaron a un tamaño estándar (8–10 crías) o se redujeron (3–4 crías) para inducir hiperalimentación posnatal, generando seis grupos experimentales. Las crías macho fueron evaluadas a los 120 días de edad en cuanto al peso corporal, adiposidad, tolerancia a la glucosa, sensibilidad a la insulina, parámetros bioquímicos plasmáticos, contenido lipídico hepático y marcadores de estrés oxidativo. La hiperalimentación posnatal aumentó el peso corporal a los 21 días, con una diferencia persistente únicamente entre los grupos HChO y CChO a los 120 días. No se observaron diferencias significativas en el índice de Lee, los pesos tisulares, los parámetros bioquímicos en ayunas ni los marcadores de estrés oxidativo. El grupo CCO presentó un área bajo la curva (AUC) de la prueba de tolerancia a la glucosa oral (OGTT) inferior a la de los grupos C y HCO, mientras que el grupo HChO presentó un AUC de la prueba de tolerancia a la insulina (IST) inferior a la del grupo CChO. La suplementación materna con ChO tuvo efectos metabólicos limitados en la descendencia masculina de BALB/c expuesta a una sobrealimentación posnatal. Aunque moduló la sensibilidad a la insulina, no se observaron cambios consistentes en otros parámetros metabólicos o de estrés oxidativo. En conclusión, el modelo de sobrealimentación no resultó del todo eficaz para inducir cambios metabólicos en ratones BALB/C y la suplementación materna con AC ejerció efectos limitados sobre los parámetros metabólicos de la descendencia expuesta a hiperalimentación posnatal.

Palabras clave: Nutrición en las primeras etapas de la vida; Ácido α-linolénico; Sensibilidad a la insulina; Homeostasis de la glucosa; Estrés oxidativo.

1. Introduction

Experimental evidence from rodent models has demonstrated that postnatal overnutrition induced by litter-size reduction promotes persistent alterations in energy homeostasis, increasing susceptibility to obesity and insulin resistance later in life (Donato, 2023). Consistent with these observations, postnatal overfeeding induces an early increase in body weight at weaning (20–30%), which may persist throughout growth and into adulthood (15–20%), and is associated with metabolic

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disturbances, particularly impaired glucose regulation and central adiposity (Rigal *et al.*, 2024). Furthermore, rodents overfed during lactation develop excessive weight gain, hepatic insulin resistance, and disruptions in glucose and lipid metabolism at weaning, with some of these alterations persisting into adulthood (Zheng *et al.*, 2025). Collectively, these findings highlight the postnatal period as a critical developmental window during which nutritional excess can program long-term metabolic outcomes (Donato, 2023).

Essential fatty acids are vital for growth, development, cellular homeostasis, and various physiological processes but cannot be synthesized endogenously, making dietary intake essential. Among them, omega-3 polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), have been extensively studied due to their health-promoting effects (Bae; Lim; Lim, 2023; Shahidi; Ambigaipalan, 2018).

During pregnancy, omega-3 PUFA supplementation may reduce placental and cerebral inflammation, oxidative stress, mitochondrial damage, and adverse pregnancy outcomes (Sun; Zhang, 2022). An experimental study in mice demonstrated that maternal omega-3 supplementation attenuated early excessive weight gain, modulated inflammatory and oxidative stress markers in offspring, and mitigated metabolic dysfunction associated with neonatal overnutrition, reinforcing the role of maternal nutritional interventions in developmental programming (Lopes *et al.*, 2025). These findings support the concept that maternal nutritional interventions during critical developmental periods may represent a promising strategy to modulate offspring health and reduce susceptibility to metabolic disorders.

In this context, chia oil (ChO) constitutes a rich source of ALA, the metabolic precursor of the omega-3 PUFAs EPA and DHA, which represents 52.3% of its total fatty acid composition in ChO (Leão *et al.*, 2025). Compared with marine-derived oils, ChO also provides bioactive compounds such as tocopherols, phytosterols, carotenoids, and phenolic compounds, while representing a sustainable and renewable alternative for dietary supplementation (Balakrishnan *et al.*, 2025;

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Ghafoor *et al.*, 2020; Marineli *et al.*, 2015; Martínez-Cruz; Paredes-López, 2014). The effects of ChO supplementation on lipid metabolism remain controversial and appear to depend on the experimental model. Indeed, a meta-analysis assessing the effects of chia supplementation on the lipid profile in patients with metabolic disorders found no significant effects on triglycerides, total cholesterol, HDL, or LDL (Run *et al.*, 2024). However, evidence from animal studies suggests that ChO may improve lipid profiles (increasing HDL-c and reducing total cholesterol) and glycemic homeostasis (improving glucose tolerance and insulin sensitivity), while enhancing the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Leão *et al.*, 2024). Therefore, ChO exhibits antioxidant and anti-inflammatory properties and may be beneficial against metabolic disorders (Ahmed *et al.*, 2021; Cavalli *et al.*, 2021; Moreira *et al.*, 2022), although these effects require further investigation.

In a previous study by our research group, maternal chia oil (ChO) supplementation was investigated in a mouse model of postnatal undernutrition. Offspring from supplemented dams exhibited improved glucose tolerance, reduced compensatory weight gain, lower oxidative stress, and decreased hepatic lipid accumulation compared with unsupplemented animals (Leão *et al.*, 2025). However, whether maternal ChO supplementation can also mitigate the adverse metabolic consequences of postnatal overnutrition remains unknown. Given the growing interest in maternal nutritional interventions as strategies for developmental programming, investigating the effects of maternal ChO supplementation in offspring exposed to postnatal overnutrition may provide new insights into preventive approaches against metabolic dysfunction. Based on the antioxidant and metabolic properties of chia oil (Leão *et al.*, 2024; Marineli *et al.*, 2015), we hypothesized that maternal ChO supplementation during pregnancy and lactation would attenuate the adverse metabolic effects induced by postnatal overnutrition in the offspring. We further hypothesized that these effects would be associated with improved glucose and lipid metabolism and reduced hepatic oxidative stress, thereby contributing to beneficial developmental programming.

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Therefore, this study aimed to evaluate the effects of maternal supplementation from ChO on metabolic markers, including glucose and lipid profiles, as well as hepatic oxidative stress, in BALB/c mice adult offspring subjected to postnatal overnutrition, as a model of metabolic programming.

2. Materials and Methods

2.1. Fatty acid composition of chia and corn oils by GC-FID

The chia and corn oil (CO) used in this study were purchased from a local market (Chia Oil - Pazze, 05071-6, Panambi/RS, Brazil; Corn oil, Liza®, lot L07C). Fatty acid composition of chia and corn oils was conducted at the Central Laboratory for Chemical Analysis and Prospecting of the Federal University of Lavras. Fatty acid samples were analyzed using gas chromatography (GC 2010-Shimadzu) equipped with a flame ionization detector and split injector at a split ratio of 1:50. A 100m long, 0.25mm diameter, and 0.2μm thick Supelco film-fused silica capillary column (SP-2560; Bellefonte, PA, USA) was used. The chromatographic conditions consisted of an initial column temperature of 140°C held for 5 min, followed by a temperature ramp of 4 ◦C/min until reaching 240°C, which was maintained for 30 min, resulting in a total run time of 60 min. Both the injector and detector were set at 260°C. Helium was used as the carrier gas. Fatty acids were identified by comparing their retention times with those of the chromatographic standards (Supelco 37 standard FAME Mix, Supelco Inc., USA). The results were expressed as the percentage of total fatty acids detected.

2.2. Animals

This study was approved by the Ethics Committee on the Use of Animals (CEUA/UFLA) (protocol no. 050/2019; approved on April 23, 2020) and was conducted in the Animal Facility of the Department of Nutrition (DNU) at the Federal University of Lavras (UFLA). The experimental period lasted approximately 180

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days. BALB/c mice were used because they are a well-established inbred strain for metabolic research, providing a suitable model for investigating the effects of dietary interventions on metabolic and immune outcomes (Boi *et al.*, 2016). The number of animals was based on a previous study that employed a similar methodological approach (Leão *et al.*, 2025). For the study, 30 female and 10 male BALB/c mice, aged between 45-55 days and with an average weight of 30 g, were obtained from the Central Animal Facility of UFLA for mating and formation of hyperfed and control groups. Throughout the experimental period, the animals were maintained in a 12-hour light/dark cycle, with a controlled temperature of 22±2 °C and consumption of standard food (Nuvilab® CR-1, Quimtia S/A, Colombo, PR, Brasil) and water ad libitum. The F1 generation obtained from these matings consisted of 74 male offspring, which were included in the experimental analyses.

2.3. Maternal supplementation

From mating until offspring weaning (21 days after birth), females were randomly allocated to one of three maternal treatment groups. All treatments were administered once daily by oral gavage. The ChO group received chia seed oil (5 g/kg body weight; Pazze®, lot 05071-6), the corn oil group received commercial corn oil (5 g/kg body weight; Liza®, lot L07C), which served as an oil comparator because of its high omega-6 fatty acid content, and the unsupplemented control group received an equivalent volume of water by oral gavage .

The supplementation dose was based on previous findings by Leão *et al.* (2025), who reported beneficial metabolic effects of ChO at this concentration in a model of postnatal undernutrition. Supplementation was administered daily throughout the mating, gestation, and lactation periods, totaling approximately 56 days. All animals had ad libitum access to a standard normocaloric diet in pellet format (Nuvilab® CR-1, Quimtia S/A, Colombo, PR, Brazil). Detailed information regarding the nutritional composition of this diet can be found in Leão *et al.* (2025). The experimental groups were summarized in Figure 1.

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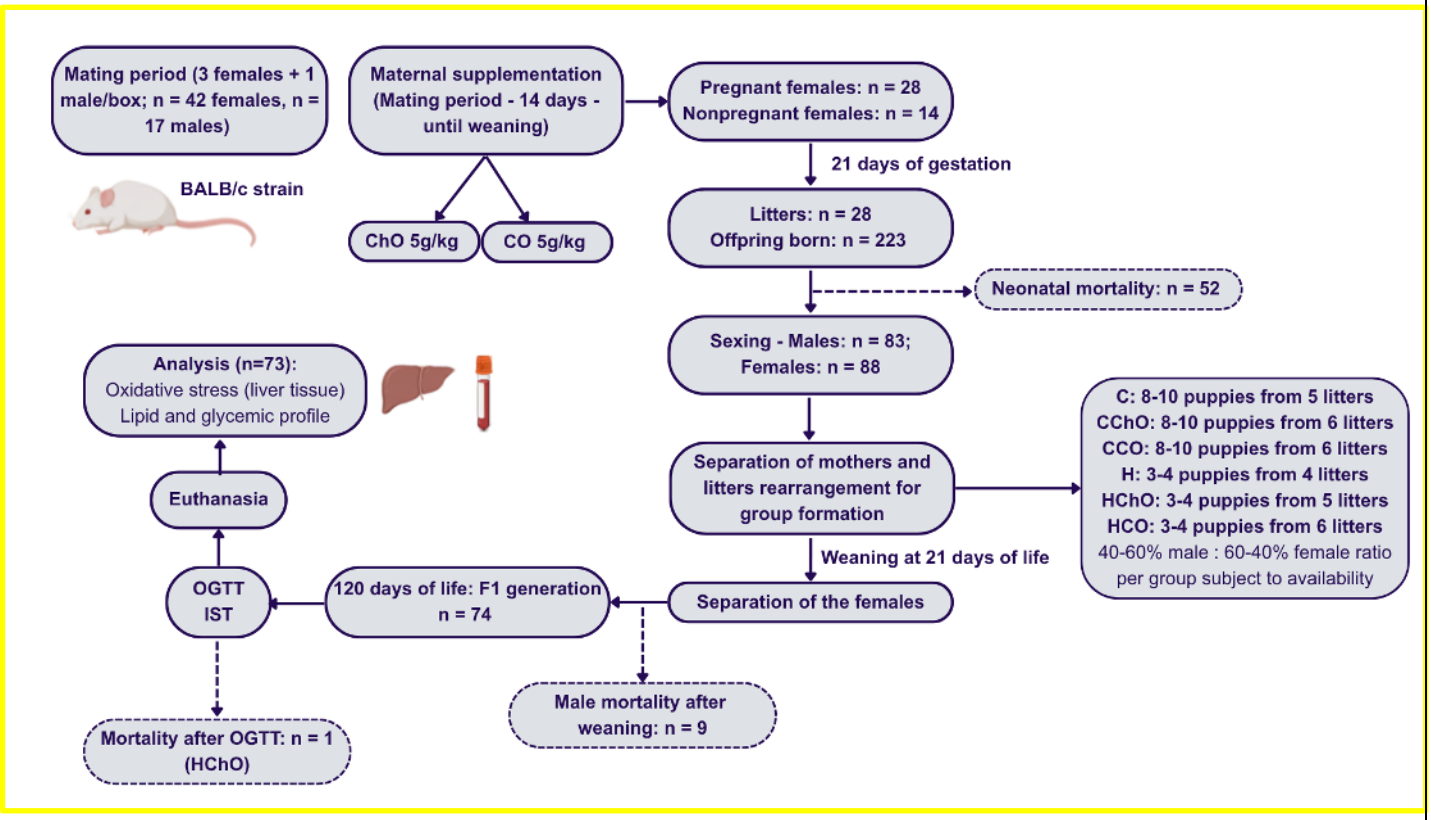

Figure 1. Experimental Design. ChO: chia oil, CO: corn oil, C: control group, CChO: control chia oil group, CCO: control corn oil group, H: hyperfed group, HChO: hyperfed chia oil group, HCO: hyperfed corn oil group, OGTT: oral glucose tolerance test, IST: insulin sensitivity test.

2.4. Experimental design

For mating, one male was housed with three females for 14 days. Afterward, the male was removed, and pregnancy was monitored through maternal body weight gain until parturition. Postnatal hyperalimentation was induced by litter-size reduction during lactation, a well-established model that decreases competition for maternal milk and results in enhanced early-life weight gain, accompanied by long-term hormonal and metabolic alterations when compared with standard litter conditions (Habbout *et al.*, 2013; Plagemann, 2008). Two days after birth, litter size was adjusted according to the experimental design. Within each maternal treatment group, litters were randomly allocated to either the hyperfed condition, with litter

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size reduced to 3–4 pups (both males and females), or the standard condition, with 8–10 pups per litter.

This design generated six experimental groups: Control (C) (n = 14), Control supplemented with chia oil (CChO) (n = 11), Control supplemented with corn oil (CCO) (n = 13), Hyperfed (H) (n = 11), Hyperfed supplemented with chia oil (HChO) (n = 11), and Hyperfed supplemented with corn oil (HCO) (n = 14). One animal from the HChO group died after the oral glucose tolerance test (OGTT). Therefore, the HChO group comprised 10 animals for all subsequent analyses (Figure 1). Throughout the study, dams continued to receive their assigned daily supplementation until offspring weaning. Only male offspring were included because the experimental model was standardized for male animals and to avoid the potential confounding effects of hormonal fluctuations during the estrous cycle, as they can influence metabolic and inflammatory responses. Since the experimental unit consisted of F1 offspring, group sizes varied according to the natural distribution of male pups among litters.

Body weight (BW) was recorded weekly using an electronic scale throughout the experimental period. The Lee index, used as an indirect indicator of adiposity in rodents, was calculated as the cube root of body weight (g) divided by the naso–anal length (mm), measured from the tip of the nose to the anus (Rogers; Webb, 1980).

Blinding was not performed during outcome assessment or data analysis, as investigators were aware of the experimental group allocation. This was necessary to ensure that animals from all experimental groups were adequately represented in the different analyses. Within each experimental group, animals were randomly selected to minimise potential selection bias.

2.5. Oral glucose tolerance test and insulin sensitivity test

At 120 days of age, an oral glucose tolerance test (OGTT) was performed. After a 6-h fasting period, the animals received a D-glucose solution (2 g/kg body weight) by gavage. Blood glucose concentrations were measured from tail blood

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samples at 0, 15, 30, 60, and 120 min using an Accu-Chek Active® glucometer. Glucose values were expressed as mg/dL, and the area under the curve (AUC) was calculated to evaluate glucose tolerance.

Twenty-four hours after the OGTT, insulin sensitivity was assessed in fed animals through an insulin sensitivity test (IST). Mice received an intraperitoneal injection of regular insulin (0.75 U/kg body weight), and blood glucose concentrations were determined from tail blood samples at 0, 15, 30, and 60 min after injection. Glucose values were expressed as mg/dL, and the AUC was calculated to evaluate the overall glycemic response to insulin administration.

2.6. Euthanasia

Two days after reaching 120 days of age, following a 12-h fasting period, the animals were anesthetized intraperitoneally with a mixture of Ketamine (200 mg/kg), Xylazine (30mg/kg), and cotton moistened with isoflurane placed in the nasal region and, subsequently, euthanized by cardiac exsanguination. The collected blood was centrifuged to obtain plasma. The liver and epididymal and retroperitoneal adipose tissues (AT) were removed and weighed. The plasma and liver were stored in a -80°C freezer for subsequent analyses.

2.7. Glycemic and lipidemic profile

Fasting plasma concentrations of glucose, triacylglycerol (TAG), total cholesterol, and HDL-cholesterol were determined using colorimetric enzymatic methods, according to the commercial Liquiform® systems (Labtest, Lagoa Santa, Brasil). Samples were prepared, and analyses were performed according to the manufacturer's instructions. Values were presented in mg/dL.

2.8. Serum insulin concentration

The concentration of insulin in the plasma was quantified using the ELISA kit

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(Enzyme-Linked Immunosorbent Assay) specific for mice Ins1 (Ref. RAB0817, Merck KGaA, Darmstadt, Germany). The procedure followed the protocol according to the manufacturer. The result was expressed in µIU/mL.

2.9. Lipid content of liver tissue

Lipids were extracted by organic solvents according to the methodology of (Folch; Lees; Sloane Stanley, 1957). The extracted samples remained in the oven for drying at 37 °C for around three days to obtain total lipid levels.

2.10. Oxidative stress of liver tissue

Liver tissue samples (100 mg) were homogenized in 1.0 mL of phosphate-buffered saline (PBS). The homogenates were centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatants were stored at −20 °C for subsequent determination of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (ROOH), and antioxidant enzyme activities, superoxide dismutase (SOD) and catalase (CAT). Total protein concentration was determined using the Bradford method (Bradford, 1976). Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Buege & Aust (1978). Briefly, 1 mL of TBARS reagent containing trichloroacetic acid (TCA, 15%), thiobarbituric acid (TBA, 0.0375%), and hydrochloric acid (HCl, 0.25 N) was added to the samples. The tubes were incubated in a boiling water bath (100 °C) for 15 min and subsequently cooled under running water. After cooling, 0.75 mL of n-butanol was added, followed by centrifugation. Absorbance was measured at 535 nm using a spectrophotometer. TBARS levels were expressed as nmol of malondialdehyde (MDA) per mg of protein.

The concentration of ROOH was determined using the ferrous oxidation–xylenol orange (FOX) assay (Nelson; Kiesow, 1972; Nouroozzadeh; Tajaddinisarmadi; Wolff, 1994). This method is based on the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) by hydroperoxides under acidic conditions. The ferric

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ions subsequently bind to the indicator xylenol orange, forming a blue-purple chromophore. Absorbance was measured at 560 nm, and the results were expressed as μmol/mg of protein.

CAT activity was determined based on the decrease in hydrogen peroxide (H_2O_2) absorbance at 240 nm resulting from its decomposition by catalase ($2H_2O_2 \rightarrow 2H_2O + O_2$) (Aebi, 1984). The reaction mixture contained 2 mL of 50 mM phosphate buffer, 50 μL of H_2O_2 solution (0.3 M), and 100 μL of tissue homogenate. Absorbance was recorded at 240 nm at 0 and 60 s using a spectrophotometer. Catalase activity was calculated according to the following equation: ($Abs_0s - Abs_{60}s$)/0.1 × dilution factor/mg of protein. Results were expressed as ΔE/min/mg of protein.

SOD activity was determined based on its ability to scavenge superoxide radicals ($O_2\bullet^-$), thereby reducing the auto-oxidation rate of pyrogallol, according to a method adapted from Dieterich *et al.* (2007). The reaction mixture consisted of 30 μL of tissue homogenate, 99 μL of phosphate buffer, 6 μL of MTT (dimethylthiazole diphenyltetrazolium bromide), and 15 μL of pyrogallol. The samples were incubated at 37 °C for 5 min, after which the reaction was stopped by the addition of 150 μL of DMSO (dimethyl sulfoxide). Absorbance was measured at 570 nm using a spectrophotometer. SOD activity was expressed as U SOD/mg protein.

2.11. Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. Normally distributed variables were analyzed using parametric tests and are expressed as mean±standard deviation (SD). Variables that did not meet the normality assumption were log-transformed before parametric analysis, when appropriate.

One-way or two-way analysis of variance (ANOVA) was performed as appropriate. For two-way ANOVA, the experimental factors and their interaction were included in the analysis. When significant effects or interactions were detected, Šídák's multiple-comparisons test was used for post hoc comparisons. The area under the curve (AUC) for glycemia during the OGTT and IST was analyzed using

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one-way ANOVA followed by Tukey's multiple-comparisons test.

The glycemic response over time during the OGTT and IST was analyzed using two-way ANOVA, considering experimental group and time as factors, as well as their interaction. When significant effects or interactions were detected, Šídák's multiple-comparisons test was used to assess differences between groups at individual time points.

Oxidative stress–related variables (SOD, CAT, TBARS, and ROOH) that did not meet the normality assumption were log-transformed and subsequently analyzed using two-way ANOVA.

Data presented in tables are expressed as mean±standard deviation (SD), whereas data presented in figures are expressed as mean±standard error of the mean (SEM). Statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism software, version 9.0.2 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Fatty acid composition of oils

Fatty acid composition of chia and corn oils was detailed in Table 1. Commercial ChO presented the following lipid composition: omega-3 (52,3%), omega-6 (23,9%), omega-9 (14,3%), and other fatty acids (9%). Commercial corn oil contained omega-6 (47,6%), omega-9 (37,4%), and other fatty acids (15%).

Table 1 - Fatty acid composition of chia and corn oils

Fatty acid composition	Chia oil (%)	Corn oil (%)
Butyric Acid Methyl Ester (C4:0)	n.d.	0.01
Lauric Acid Methyl Ester (C12:0)	0.03	0.02
Pentadecanoic Acid Methyl Ester (C15:0)	0.02	0.04
Palmitic Acid Methyl Ester (C16:0)	7.20	12.01

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Palmitoleic Acid Methyl Ester (C16:1)	n.d.	0.10
Heptadecanoic Acid Methyl Ester (C17:0)	0.11	0.06
Stearic Acid Methyl Ester (C18:0)	1.86	2.11
Oleic Acid Methyl Ester (C18:1n9c)	14.43	36.35
Linoleic Acid Methyl Ester (C18:2n6c)	23.73	47.42
Arachidic Acid Methyl Ester (C20:0)	n.d.	0.45
cis-11-Eicosenoic Acid Methyl Ester (C20:1)	n.d.	0.95
Linolenic Acid Methyl Ester (C18:3n3)	52.43	0.18
cis-8,11,14 Ecosatrienoic Acid Methyl Ester (C20:3n6)	0.16	0.13
cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3)	n.d.	0.16

n.d. = not detected.

3.2. Body and tissue weight

Body weight at 21 was significantly affected by the postnatal nutritional model (C x H, $p<0.01$; CChO x HChO, $p<0.01$ and CCO x HCO $p=0.0001$), with no significant effects of supplementation. However, body weight at 120 days showed a difference only between the HChO and its control group (CChO, $p<0.001$); and no other significant differences were observed in the other groups. No changes were found in the Lee index data (Table 2).

Table 2 - Body weight at 21 and 120 days of life and Lee Index

	C n=14	**CChO n=11**	**CCO n=13**	**H n=11**	**HChO n=10**	**HCO n=14**
Weight 21 days (g)	10.29±0.75	10.39±1.6	9.95±2.69	12.98±1.39*	13.41±1.42#	13.51±2.52 &
Weight 120 days (g)	35.89±1,84	34.63±1,93	35.84±3,0	38.27±1,66	39.69±1,94#	37,56±3,49
Lee Index	0,0345±0,0 0076	0,0343±0,0 0095	0,0339±0,0 0097	0,0348±0,0 0095	0,0348±0,0 0134	0,0347±0,0 0123

C: control group, CChO: control chia oil group, CCO: control corn oil group, H: hyperfed group, HChO: hyperfed chia oil group, HCO: hyperfed corn oil group.

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Data are presented as mean±standard deviation. Statistical analysis was performed using two-way ANOVA. Post hoc comparisons were performed by Šídák's multiple comparisons test. *p<0.01 vs C; #p<0.01 vs CChO; &p=0.0001 vs CCO.

No significant differences were observed in liver weight and retroperitoneal or epididymal adipose tissue weights (Table 3).

Table 3. Weight of the liver, retroperitoneal and epididymal adipose tissue (AT) normalized by body weight (BW in mg).

Tissue weight	C n= 14	CChO n= 11	CCO n= 13	H n= 11	HChO n= 10	HCO n= 14
Liver (mg/g BW)	45.1±3.2	47.7±3.9	43.3±4.8	44.8±5.1	49.5±6.4	45.6±4.3
Retroperitoneal AT (mg/g BW)	2.20±1.3	2.2±1.0	4.1±1.9	2.9±2.1	2.8±1.4	2.8±2.2
Epididymal AT (mg/g de BW)	9.98±4.8	11.8±3.1	10.6±3.9	12.1±3.2	12.8±4.6	11.2±6.7

Note: C: control group, CChO: control chia oil group, CCO: control corn oil group, H: hyperfed group, HChO: hyperfed chia oil group, HCO: hyperfed corn oil group. Data are presented as mean±standard deviation. Statistical analysis was performed using two-way ANOVA. Post hoc comparisons were performed by Šídák's multiple comparisons test.

3.3. Biochemical parameters

Table 4 presents fasting blood glucose, insulinemia, and lipid profile in the experimental groups. Blood glucose, insulinemia, total cholesterol, triacylglycerols, HDL-cholesterol, and liver total lipids did not differ significantly among the groups.

Table 4. Fasting blood glucose, insulinemia and lipid profile of the experimental groups

Variables	C n=5	CChO n=5	CCO n=5	H n=5	HChO n=5	HCO n=5
Blood glucose (mg/dL)	68.37±29.21	103.60±32.32	48.91±58.38	68.91±55.01	133.00±26.92	108.20±45.03

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Insulinemia (µIU/mL)	147.60±64.36	177.60±106.50	81.81±25.30	94.54±52.57	225.00±161.10	107.90±86.87
Total cholesterol (mg/dL)	77.60±28.24	62.32±36.83	46.14±18.85	48.70±28.89	84.49±10,14	65.79±25.02
HDL-cholesterol (mg/dL)	11.20±4.97	9.29±2.03	7.80±2.42	11.33±8.47	10.43±2.55	7. 53±2.14
Triacylglycerols (mg/dL)	52.01±10.75	69.35±23.31	29.57±1.31	51.63±28.65	61.58±13.67	39.71±7.07
Liver lipids:						
Total lipids (mg/g of liver)	270.60±67.12	320.20±95.57	344.80±93.53	309.60±68.67	324.30±174	297.80±50.94

Note: C: control group, CChO: control chia oil group, CCO: control corn oil group, H: hyperfed group, HChO: hyperfed chia oil group, HCO: hyperfed corn oil group. Values are expressed as mean±standard deviation. Statistical analysis was performed using two-way ANOVA. Post hoc comparisons were performed by Šídák's multiple comparisons test.

3.4. Metabolic markers

During the OGTT, the glycemic response differed significantly among the experimental groups over the 0–120 min period (Figure 2A). In the 0 min, the control group (C) exhibited a higher mean glycemic level compared with the CCO group ($p<0.05$). Statistically significant differences at baseline were also observed between the CCO and HCO groups ($p<0.05$), with the CCO group presenting higher mean glucose levels compared with the HCO groups (Figure 2A). Further comparisons between the control group (C) and the CCO group, as well as between the CCO and HCO groups, also revealed significant differences at 120 min ($p<0.05$), with higher mean glycemic levels observed in the C group compared with the CCO group and in the CCO group compared with the HCO group (Figure 2A). The results related to the AUC of the OGTT showed that the glycemic response over time differed among the groups (group effect and group × time interaction, $p<0.05$). In the multiple comparison analysis at each time point, the CCO group presented significantly lower glycemic

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values compared with the control group (C) at baseline (0 min; $p<0.01$) and at 120 min when compared with the HCO group ($p<0.05$), which led to a reduction in AUC by the CCO group (versus C, $p<0.01$, and HCO groups, $p<0.05$) (Figure 2B). No statistically significant differences were observed among the other groups (Figure 2B).

Related to IST, significant differences in glycemic levels among the experimental groups were observed at 15 min. At this time point, the CChO group exhibited significantly higher glycemic levels compared with the HChO group ($p < 0.05$) (Figure 2C). However, no statistically significant differences were observed at the other time points between 0 and 60 minutes (Figure 2C).

Regarding the AUC of the IST, the HChO group also showed a reduced area compared with the CChO group ($p < 0.05$). No statistically significant differences were observed among the remaining groups (Figure 2D).

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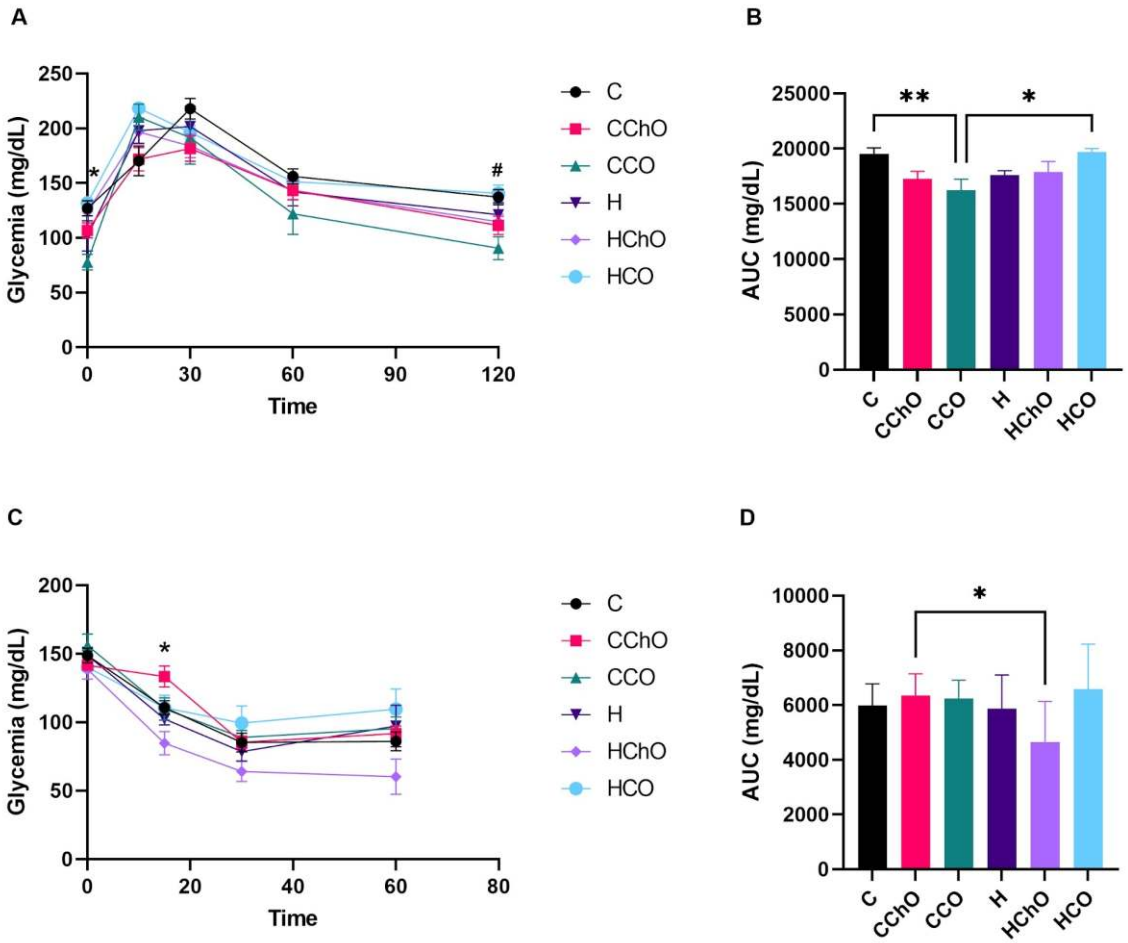

Figure 2. Oral glucose tolerance test (OGTT) and insulin sensitivity test (IST) with area under the curve (AUC) in adult offspring exposed to postnatal hyperalimentation. A) Glycemic curve (mg/dL) vs time (min) of the Oral Glucose Tolerance Test (OGTT) * C vs. CCO / CCO vs. HCO, # C vs. CCO / CCO vs. HCO $p < 0.001$; B) Area Under Curve (AUC) at 120 days of life in the OGTT experimental groups, * $p<0,05$ and ** $p<0,001$; C) Glycemic curve (mg/dL) vs time (min) of the Insulin Sensitivity Test (IST), * CChO vs. HChO; D) Area Under Curve (AUC) at 120 days of life of the IST experimental groups, * $p<0.05$ and ** $p<0.001$. C: control group, CChO: control chia oil group, CCO: control corn oil group, H: hyperfed group, HChO: hyperfed chia oil group, HCO: hyperfed corn oil group.

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Parameters related to oxidative stress (TBARS, SOD, ROOH, or CAT) did not differ significantly among the groups (Figure 3A-D).

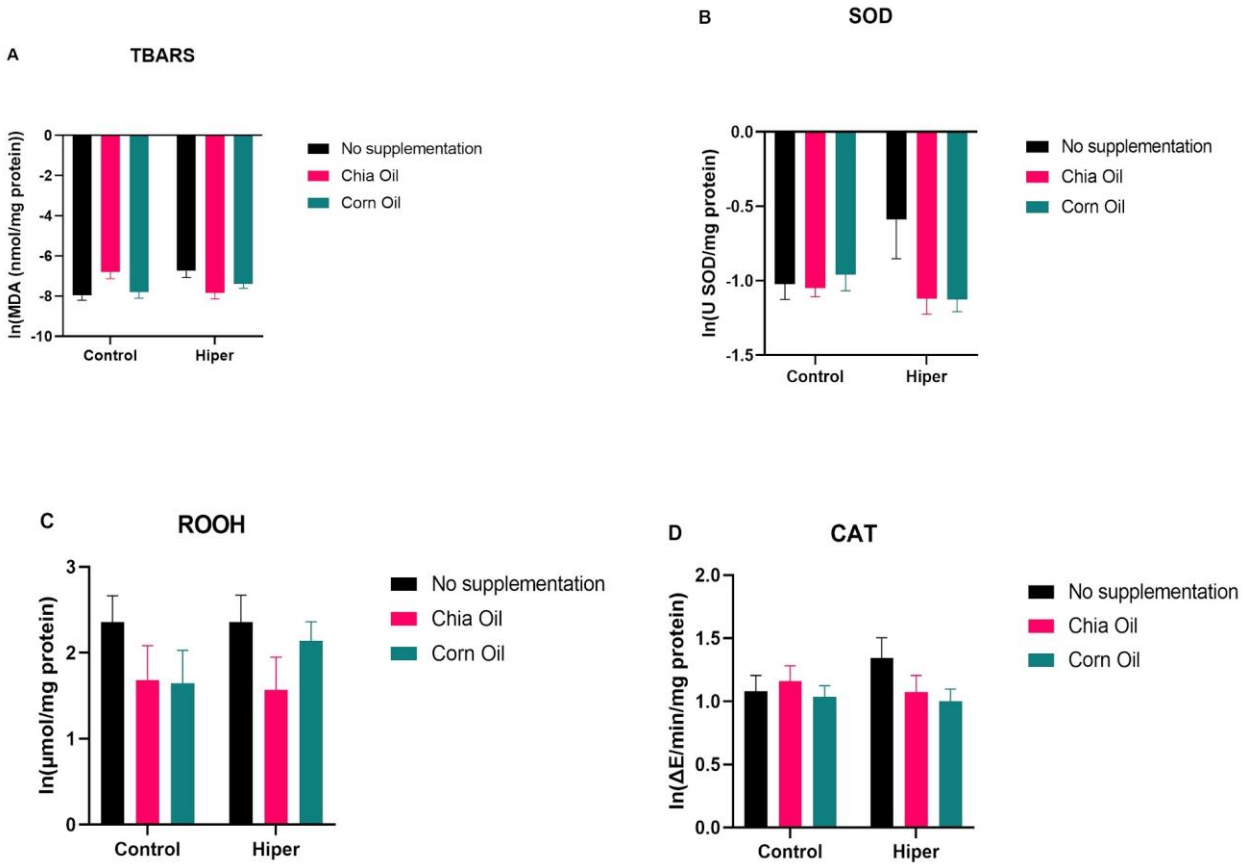

Figure 3. Hepatic oxidative stress. A) Substances reactive to thiobarbituric acid (TBARS) B) Superoxide dismutase (SOD) concentration. C) Concentration of lipid hydroperoxides (ROOH). D) Catalase (CAT) Activity.

4. Discussion

Changes in nutrition and growth during early postnatal life can have permanent effects on metabolism in adulthood (Alves; Alves, 2024). The present work used litter size reduction in order to investigate the effects of maternal supplementation with ChO, focusing on the analysis of the previously defined dosage (5 g/kg) of the oil,

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using corn oil as an omega-6-rich oil and water (placebo). Overfeeding in early postnatal life is a critical window for development, resulting in long-term consequences for weight gain and energy balance, contributing to the increased incidence of metabolic diseases in adulthood, which are not yet completely explained by genetic and lifestyle factors (Enes-Marques *et al.*, 2020).

In the current study, hyperfed animals, regardless of ChO supplementation, exhibited greater body weight at 21 days compared with their respective controls. However, by 120 days of age, this difference persisted only in the ChO-supplemented group, with the HChO group showing higher body weight than its respective control group (CChO, $p < 0.001$). In contrast, previous studies employing hyperalimentation models in Wistar rats induced by litter size reduction have reported early and sustained increases in body weight, detectable from weaning and persisting into adulthood (Conceição *et al.*, 2013; Huang *et al.*, 2021; Junior *et al.*, 2019; Lopes *et al.*, 2025; Yzydorczyk *et al.*, 2017). Such a persistent pattern was not observed in the present study.

No significant differences were observed in the Lee index among the experimental groups. This index can be used as a simple way to indirectly measure obesity in experiments subject to a weight gain method. However, the association of the Lee index with other parameters, such as metabolic, is necessary for better interpretation of the results (Bernardis; Patterson, 1968; Novelli *et al.*, 2007). Increased adiposity is closely related to increased Reactive Oxygen Species (ROS) generation and oxidative stress, and may be an early condition in the development of chronic diseases that are related to obesity. Dietary fat intake influences the fatty acid profile of adipose tissue, potentially altering its function and contributing to meta-inflammation associated with obesity and metabolic diseases. This inflammatory environment in adipose tissue may promote the development of obesity-related conditions (Del Cornò *et al.*, 2021). Studies using postnatal hyperalimentation models have demonstrated reductions in the activity of antioxidant enzymes, including SOD, CAT, and glutathione peroxidase, accompanied by increased oxidative damage in liver tissue compared with animals from normal litters (Conceição *et al.*, 2015, 2013; Lopes *et al.*, 2025; Yzydorczyk *et al.*, 2017). Although lipid peroxidation is commonly

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associated with metabolic dysfunction and obesity-related oxidative stress, no significant differences in hepatic TBARS levels were detected among the experimental groups in the present study. Previous experimental evidence has shown increased hepatic lipid peroxidation following postnatal overfeeding, as indicated by higher MDA and hydroperoxide concentrations (Lopes *et al.*, 2025). These findings suggest that the effects of neonatal overnutrition on oxidative stress may vary according to the experimental model and assessment conditions. Future studies evaluating hepatic gene expression and transcriptomic responses may provide further insight into the molecular mechanisms underlying the response to maternal chia oil supplementation

Despite being a rich source of polyunsaturated fatty acids, particularly ALA, ChO supplementation did not produce marked changes in most of the metabolic parameters in the present study. ALA is a precursor of the long-chain n-3 PUFAs EPA and DHA; however, its conversion to these fatty acids is limited (Takic *et al.*, 2022). During pregnancy and lactation, maternal dietary fatty acids are selectively transferred to the offspring through the placenta and subsequently via breast milk, thereby influencing the lipid environment during critical periods of development (Duttaroy; Basak, 2022; Khor, 2022). Therefore, maternal ChO supplementation may contribute to metabolic programming in the offspring despite the absence of marked changes in conventional metabolic markers. Previous evidence suggests that the biological impact of n-3 PUFA supplementation may depend on the physiological context. For instance, supplementation with high-dose n-3 PUFA in healthy individuals has shown limited effects on adipose tissue gene expression under resting conditions, but may modulate the adipose transcriptomic response during inflammatory stress. These findings indicate that n-3 PUFA supplementation prior to an inflammatory challenge may modify specific inflammatory and metabolic pathways, revealing adipose tissue genes and molecular pathways as potential targets for nutritional modulation (Ferguson *et al.*, 2016). In addition to its high ALA content, ChO contains several bioactive compounds, including tocopherols, phytosterols, carotenoids, phospholipids, and residual phenolic compounds, which may contribute to its biological activity through complementary antioxidant and

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metabolic effects (Martínez-Cruz; Paredes-López, 2014). Supporting this hypothesis, Poudyal *et al.* (2012) demonstrated that supplementation with ALA-rich ChO improved glucose tolerance and insulin sensitivity without reducing total body fat, suggesting that its metabolic benefits may occur independently of changes in overall adiposity.

In contrast, a study conducted in Swiss mice fed a high-fat diet and supplemented with chia oil (1.5 g/kg of diet) reported improvements in glucose metabolism in obese animals. The authors suggested that these effects were associated with increased browning of subcutaneous adipose tissue, which may contribute to improved glucose metabolism and reduced obesity-related insulin resistance (Fonte-Faria *et al.*, 2019).

In models of postnatal hyperalimentation, changes in glucose and lipid homeostasis can be found, with increased glycemia and high glucose tolerance (Costa *et al.*, 2019; Rigal *et al.*, 2024; Tejera-Muñoz *et al.*, 2023; Yzydorczyk *et al.*, 2017), but what was not found in the present study. Fonte-Faria *et al.* (2019) supplemented C57BL/6 mice fed a high-fat diet with ChO (1 g/kg diet) and demonstrated that supplementation improved glycemic levels and insulin tolerance. Additionally, ChO reduced fat mass accumulation and increased lean mass, as assessed by nuclear magnetic resonance, in obese mice. In the present study, the ChO supplementation did not significantly alter glucose metabolism and body weight gain. In the study by Marineli *et al.* (2015), the daily consumption of chia seeds or ChO improved plasma and hepatic antioxidant status and reduced lipid peroxidation in obese rats. Both forms of chia exhibited similar in vivo antioxidant properties, likely due to the presence of polyphenols, ALA, and other bioactive compounds, suggesting a protective effect against oxidative stress associated with obesity (Marineli *et al.*, 2015). In contrast, Moreira *et al.* (2022) reported that the inclusion of chia flour or oil in a high saturated fat and fructose diet improved metabolic parameters, restoring antioxidant defenses, increasing fatty acid oxidation, improving glucose and triglyceride levels, reducing body weight, and attenuating hepatic steatosis in Wistar rats (Rattus norvegicus domestica), although no direct effect on liver inflammation was observed. In addition, ChO did not show significant effects in the present study.

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It is admitted that the model explored in this investigation had limitations, as some expected metabolic changes were not significantly detected in animals exposed to postnatal hypernutrition, a fact that limited, in part, a conclusion about the modulatory potential of ChO under study. It is believed that this limitation is justified, in part, by the strain used.

In the present study, BALB/c mice were used as the experimental model. This inbred strain is widely employed in biomedical research due to its well-characterized genetic and physiological background, particularly in oncology and infectious disease studies, where its defined immune profile and susceptibility to specific pathogens make it highly valuable (Boi *et al.*, 2016). However, most studies investigating metabolic programming have traditionally utilized Wistar rats or C57BL/6 mice, which are considered more susceptible to diet-induced metabolic alterations. In contrast, evidence suggests that BALB/c mice may exhibit relative resistance to metabolic disturbances (Saha *et al.*, 2020; Silva-Santana *et al.*, 2020). This characteristic may partially explain why postnatal overnutrition did not result in overt metabolic dysfunction in adulthood in the present study, as fasting glucose, insulin, lipid profile, hepatic lipid accumulation, and oxidative stress markers remained largely unchanged in the hyperfed offspring. Although neonatal overfeeding promoted increased body weight at weaning, this difference was no longer evident in adulthood in the H group. Therefore, the present findings should not be interpreted as evidence that maternal ChO prevented established metabolic disease. Rather, maternal ChO supplementation appeared to modulate insulin sensitivity in offspring exposed to early postnatal overnutrition despite the absence of marked metabolic impairment. It is also possible that the relatively young age at evaluation (120 days) allowed the detection of early metabolic adaptations before the development of more overt metabolic abnormalities, which have been reported to emerge later in life in developmental programming models. In addition, although the sample size was defined based on previous study using this experimental model (Leão *et al.*, 2025), future studies with larger sample sizes are warranted to increase statistical power and reduce the risk of Type II error, particularly for detecting subtle metabolic alterations. Therefore, although the use of BALB/c mice represents an innovative approach in this field, it

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also constitutes a limitation of the present study, as possible strain-specific metabolic resilience may have attenuated the magnitude of the observed effects and potentially limits direct comparison with previous literature.

A relevant limitation is the absence of maternal metabolic characterization during lactation. Maternal body weight, glucose homeostasis, and lipid profile were not evaluated, precluding assessment of whether maternal metabolic adaptations contributed to the offspring metabolic outcomes. Likewise, milk production and breast milk composition were not directly assessed. Although litter size reduction increases milk availability per pup, it was not possible to determine whether maternal supplementation with ChO or CO altered milk quantity or fatty acid composition, which may have influenced the metabolic phenotype of the offspring. Consequently, the present experimental design does not allow us to disentangle the effects of maternal ChO supplementation occurring during gestation from those occurring during lactation, and therefore the contribution of each developmental period to the observed offspring outcomes cannot be determined. Also, the exclusive use of female offspring represents a limitation of the study, as sex-specific differences in the metabolic response to maternal nutritional exposure cannot be assessed, limiting the generalizability of the findings to male offspring.

Another limitation concerns the lack of an isocaloric control for the oil-supplemented groups. Although all groups underwent gavage, controlling in part for procedural stress, the control animals received water, which does not provide the energy supplied by chia oil (ChO) or corn oil (CO). This is particularly relevant in studies of oil supplementation, as dietary oils are biologically active and their effects may depend on their fatty acid composition. Thus, comparisons of CChO or CCO with C reflect both the additional energy intake and the biological effects of the respective oils. Therefore, the present design does not allow these effects to be completely disentangled, highlighting the methodological challenge of establishing an appropriate inert oil control in such studies.

Finally, complementary mechanistic approaches, including cross-fostering experiments, artificial milk supplementation, and analyses of insulin signaling pathways, inflammatory markers, or tissue-specific molecular responses, were

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beyond the scope of the present study. The absence of these analyses limits mechanistic interpretation of the improved insulin sensitivity observed in the offspring. Future studies incorporating maternal metabolic characterization together with these experimental approaches will be important to clarify the biological pathways through which maternal ChO supplementation influences metabolic programming during early life.

5. Conclusion

Maternal chia oil supplementation produced limited metabolic effects in male BALB/c offspring exposed to postnatal overnutrition. Litter-size reduction increased body weight at weaning, whereas the male adult metabolic phenotype was comparatively mild and was not accompanied by consistent alterations in circulating lipids, hepatic lipid content, or hepatic oxidative-stress markers. Yet, male offspring of females supplemented with chia oil showed no changes in glucose metabolism, tissue or body weight, or oxidative stress, which does not indicate a generalised protective effect against postnatal overnutrition.

References

AEBI, Hugo. Catalase in vitro. *In*: Methods in enzymology. Elsevier, 1984. v. 105, p. 121–126. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0076687984050163. Acesso em: 11 mar. 2026.

AHMED, Akheruz Zaman *et al.* Chia Seed Oil Ameliorates Doxorubicin-Induced Cardiotoxicity in Female Wistar Rats: An Electrocardiographic, Biochemical and Histopathological Approach. **Cardiovascular Toxicology**, v. 21, n. 7, p. 533–542, 2021.

ALVES, João Guilherme Bezerra; ALVES, Lucas Victor. Early-life nutrition and adult-life outcomes. **Jornal De Pediatria**, v. 100 Suppl 1, n. Suppl 1, p. S4–S9, 2024.

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REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

BAE, Jae Hyun; LIM, Hyunjung; LIM, Soo. The Potential Cardiometabolic Effects of Long-Chain ω-3 Polyunsaturated Fatty Acids: Recent Updates and Controversies. **Advances in Nutrition (Bethesda, Md.)**, v. 14, n. 4, p. 612–628, 2023.

BALAKRISHNAN, Gayathri *et al.* A Comprehensive Review of Phenolic Compounds in Chia Seeds and Their Applications in the Food Industry. **Plant Foods for Human Nutrition (Dordrecht, Netherlands)**, v. 80, n. 1, p. 46, 2025.

BERNARDIS, L. L.; PATTERSON, B. D. Correlation between “lee index” and carcass fat content in weanling and adult female rats with hypothalamic lesions. **Journal of Endocrinology**, v. 40, n. 4, p. 527–528, 1968.

BOI, Shannon K. *et al.* Obesity alters immune and metabolic profiles: New insight from obese-resistant mice on high-fat diet. **Obesity**, v. 24, n. 10, p. 2140–2149, 2016.

BRADFORD, Marion M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1, p. 248–254, 1976.

BUEGE, John A.; AUST, Steven D. Microsomal lipid peroxidation. *In*: METHODS IN ENZYMOLOGY. Elsevier, 1978. v. 52, p. 302–310. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0076687978520326. Acesso em: 11 mar. 2026.

CAVALLI, Juliana *et al.* Chia oil prevents chemical and immune-mediated inflammatory responses in mice: Evidence for the underlying mechanisms. **Food Research International**, v. 149, p. 110703, 2021.

CONCEIÇÃO, E. P. S. *et al.* Early redox imbalance is associated with liver dysfunction at weaning in overfed rats. **The Journal of Physiology**, v. 593, n. 21, p. 4799–4811, 2015.

CC BY

REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

CONCEIÇÃO, Ellen P.S. *et al.* Oxidative stress programming in a rat model of postnatal early overnutrition — role of insulin resistance. **The Journal of Nutritional Biochemistry**, v. 24, n. 1, p. 81–87, 2013.

COSTA, V. M. G. *et al.* Effect of postnatal overfeeding on the male and female Wistar rat reproductive parameters. **Journal of Developmental Origins of Health and Disease**, v. 10, n. 6, p. 667–675, 2019.

DEL CORNÒ, Manuela *et al.* Dietary Fatty Acids at the Crossroad between Obesity and Colorectal Cancer: Fine Regulators of Adipose Tissue Homeostasis and Immune Response. **Cells**, v. 10, n. 7, p. 1738, 2021.

DIETERICH, Daniela C *et al.* Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino-acid tagging. **Nature Protocols**, v. 2, n. 3, p. 532–540, 2007.

DONATO, Jose. Programming of metabolism by adipokines during development. **Nature Reviews Endocrinology**, v. 19, n. 7, p. 385–397, 2023.

DUTTAROY, Asim K.; BASAK, Sanjay. Maternal Fatty Acid Metabolism in Pregnancy and Its Consequences in the Feto-Placental Development. **Frontiers in Physiology**, v. 12, p. 787848, 2022.

ENES-MARQUES, Silvia *et al.* Neonatal overnutrition programming impairs cholecystokinin effects in adultmale rats. **The Journal of Nutritional Biochemistry**, v. 86, p. 108494, 2020.

FERGUSON, Jane F. *et al.* Adipose tissue RNASeq reveals novel gene–nutrient interactions following n-3 PUFA supplementation and evoked inflammation in humans. **The Journal of Nutritional Biochemistry**, v. 30, p. 126–132, 2016.

FOLCH, J.; LEES, M.; SLOANE STANLEY, G. H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of Biological Chemistry**, v. 226, n. 1, p. 497–509, 1957.

CC BY

REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

FONTE-FARIA, Thaís *et al.* Chia oil supplementation changes body composition and activates insulin signaling cascade in skeletal muscle tissue of obese animals. **Nutrition**, v. 58, p. 167–174, 2019.

GHAFOOR, Kashif *et al.* An evaluation of bioactive compounds, fatty acid composition and oil quality of chia (Salvia hispanica L.) seed roasted at different temperatures. **Food Chemistry**, v. 333, p. 127531, 2020.

HABBOUT, Ahmed *et al.* Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences. **The Journal of Nutrition**, v. 143, n. 5, p. 553–562, 2013.

HUANG, Fang *et al.* Postnatal overfeeding induces hepatic microRNA-221 expression and impairs the PI3K/AKT pathway in adult male rats. **Pediatric Research**, v. 89, n. 1, p. 143–149, 2021.

JUNIOR, Marcos Divino Ferreira *et al.* Postnatal early overfeeding induces cardiovascular dysfunction by oxidative stress in adult male Wistar rats. **Life Sciences**, v. 226, p. 173–184, 2019.

KHOR, Geok Lin. Implications of maternal diet on breast milk docosahexaenoic acid 22:6n-3 (DHA) and arachidonic acid 20:4n-6 (AA) contents: A narrative review. **Asia Pacific Journal of Clinical Nutrition**, v. 31, n. 4, 2022.

LEÃO, Estéfany Ribeiro *et al.* Maternal supplementation with chia oil attenuates hepatic metabolic disturbances in mice subjected to postnatal undernutrition. **Frontiers in Nutrition**, v. 12, p. 1636396, 2025.

LEÃO, E.R. *et al.* Metabolic effects of chia oil in experimental models: a narrative review. **Grasas y Aceites**, v. 75, n. 2, p. 1995, 2024.

LOPES, Isabela Queiroz Perígolo *et al.* Maternal fish oil supplementation improves metabolic and inflammatory markers in mice overfed during the postnatal period. **Frontiers in Nutrition**, v. 12, p. 1685437, 2025.

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REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

MARINELI, Rafaela Da Silva *et al.* Antioxidant potential of dietary chia seed and oil (Salvia hispanica L.) in diet-induced obese rats. **Food Research International**, v. 76, p. 666–674, 2015.

MARTÍNEZ-CRUZ, Oliviert; PAREDES-LÓPEZ, Octavio. Phytochemical profile and nutraceutical potential of chia seeds (Salvia hispanica L.) by ultra high performance liquid chromatography. **Journal of Chromatography A**, v. 1346, p. 43–48, 2014.

MOREIRA, Luiza De Paula Dias *et al.* Chia (Salvia hispanica L.) Flour and Oil Ameliorate Metabolic Disorders in the Liver of Rats Fed a High-Fat and High Fructose Diet. **Foods**, v. 11, n. 3, p. 285, 2022.

NELSON, Dennis P.; KIESOW, Lutz A. Enthalpy of decomposition of hydrogen peroxide by catalase at 25° C (with molar extinction coefficients of H2O2 solutions in the UV). **Analytical Biochemistry**, v. 49, n. 2, p. 474–478, 1972.

NOUROOZZADEH, J.; TAJADDINISARMADI, J.; WOLFF, S.P. Measurement of Plasma Hydroperoxide Concentrations by the Ferrous Oxidation-Xylenol Orange Assay in Conjunction with Triphenylphosphine. **Analytical Biochemistry**, v. 220, n. 2, p. 403–409, 1994.

NOVELLI, E. L. B. *et al.* Anthropometrical parameters and markers of obesity in rats. **Laboratory Animals**, v. 41, n. 1, p. 111–119, 2007.

PLAGEMANN, A. A matter of insulin: developmental programming of body weight regulation. **The Journal of Maternal-Fetal & Neonatal Medicine: The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians**, v. 21, n. 3, p. 143–148, 2008.

POUDYAL, Hemant *et al.* Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. **The Journal of Nutritional Biochemistry**, v. 23, n. 2, p. 153–162, 2012.

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ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

RIGAL, Eve *et al.* Short-Term Postnatal Overfeeding Induces Long-Lasting Cardiometabolic Syndrome in Mature and Old Mice Associated with Increased Sensitivity to Myocardial Infarction. **Molecular Nutrition & Food Research**, v. 68, n. 15, p. 2400136, 2024.

ROGERS, P.; WEBB, G.P. Estimation of body fat in normal and obese mice. **British Journal of Nutrition**, v.43, n.1, p.83-86, 1980.

RUN, Qi *et al.* The Effects of Chia Supplementation on Lipid Profile in Patients Suffering from Metabolic Disorders: A Systematic Review and Meta-Analysis. **Journal of Food Biochemistry**, v. 2024, n. 1, p. 5587140, 2024.

SAHA, Piu *et al.* Distinct iron homeostasis in C57BL/6 and Balb/c mouse strains. **Physiological Reports**, v. 8, n. 9, 2020. Disponível em: https://onlinelibrary.wiley.com/doi/abs/10.14814/phy2.14441. Acesso em: 11 mar. 2026.

SCIENCEINSIGHTS. **What Makes BALB/c Mice Essential for Research?**. 2025. Disponível em: https://scienceinsights.org/what-makes-balb-c-mice-essential-for-research/?utm_source=chatgpt.com. Acesso em: 11 fev. 2026.

SHAHIDI, Fereidoon; AMBIGAIPALAN, Priyatharini. Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. **Annual Review of Food Science and Technology**, v. 9, p. 345–381, 2018.

SILVA-SANTANA, Giorgio *et al.* Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 *Mus musculus*. **Animal Models and Experimental Medicine**, v. 3, n. 4, p. 304–315, 2020.

SUN, Jingchi; ZHANG, Weishe. Supplementation with dietary omega-3 PUFA mitigates fetal brain inflammation and mitochondrial damage caused by high doses of sodium nitrite in maternal rats. **PLOS ONE**, v. 17, n. 3, p. e0266084, 2022.

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REMUNOM
ISSN 2178-6925

Received: 13/08/2026 - **Accepted:** 11/09/2026
Vol: 13.19
DOI: 10.66104/qq05f385
Pages: 1-31

TAKIC, Marija *et al.* Effects of Dietary α-Linolenic Acid Treatment and the Efficiency of Its Conversion to Eicosapentaenoic and Docosahexaenoic Acids in Obesity and Related Diseases. **Molecules**, v. 27, n. 14, p. 4471, 2022.

TEJERA-MUÑOZ, Antonio *et al.* Postnatal Overfeeding during Lactation Induces Endothelial Dysfunction and Cardiac Insulin Resistance in Adult Rats. **International Journal of Molecular Sciences**, v. 24, n. 19, p. 14443, 2023.

YZYDORCZYK, Catherine *et al.* Transient postnatal overfeeding causes liver stress-induced premature senescence in adult mice. **Scientific Reports**, v. 7, n. 1, p. 12911, 2017.

ZHENG, Wen *et al.* Early postnatal overnutrition as a contributor to metabolic dysregulation: Insights into hepatic epigenetic mechanisms. **The Journal of Nutritional Biochemistry**, v. 145, p. 110022, 2025.

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