



ESTÉFANY RIBEIRO LEÃO

**AVALIAÇÃO DA SUPLEMENTAÇÃO MATERNA COM
ÓLEO DE CHIA SOBRE PARÂMETROS METABÓLICOS EM
MODELO DE RESTRIÇÃO ALIMENTAR NEONATAL**

LAVRAS-MG

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Prof.^a Dra. Isabela Coelho de Castro
Orientadora

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**EVALUATION OF MATERNAL SUPPLEMENTATION WITH CHIA OIL
OVER METABOLIC PARAMETERS IN MODEL OF POSTNATAL
UNDERNUTRITION**

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RESUMO

A nutrição materna e o consumo de ácidos graxos durante os períodos gestacionais e/ou de lactação são determinantes críticos fortemente relacionados ao desenvolvimento fetal e pós-natal normal. Por outro lado, alterações na nutrição da prole nos períodos iniciais da vida podem predispor ao desenvolvimento de distúrbios metabólicos. Nesse sentido, tem se buscado compostos que atuem na prevenção e/ou revertendo esses distúrbios. O óleo de chia (OC) se destaca como alimento promissor por possuir elevado conteúdo do ácido α -linolênico (55-66%) e compostos fenólicos. O objetivo deste estudo foi avaliar a influência da suplementação materna com diferentes doses do OC sobre parâmetros metabólicos em camundongos BALB/c submetidos à restrição alimentar neonatal. Todos os procedimentos foram aprovados pelo Comitê de Ética no Uso de Animais. Foram utilizados 30 camundongos como progenitores. As fêmeas progenitoras receberam a suplementação com o óleo por gavagem, nas doses 2,5 ou 5g/kg de peso corporal, desde o acasalamento até o desmame dos filhotes. Para induzir a subnutrição durante a lactação, três dias após o nascimento o tamanho da ninhada foi ajustado para 15-16 animais por ninhada, formando os grupos Subnutrido (S), S + progenitora suplementada com óleo de chia (SOC 2,5 e SOC 5g/kg de peso corporal). Ninhadas contendo de 8-10 filhotes por mãe foram usados como Controle (C), progenitora sem suplementação. O peso corporal analisado ocorreu aos 21, 70 e 120 dias, e a ingestão alimentar foi mensurada semanalmente após o desmame. Aos 70 e 120 dias os animais foram submetidos ao teste de tolerância oral à glicose (TTOG). Após eutanásia, os tecidos e sangue foram coletados para as análises. Foram analisados os perfil lipídico e glicemia de jejum a partir do plasma, lipídeos totais hepáticos e marcadores de estresse oxidativo e enzimas antioxidantes no tecido hepático e adiposo epididimal (TAE). Os dados foram submetidos à teste de normalidade (Shapiro-Wilk), seguido de análise de variância (Oneway Anova) com nível de significância de $p < 0,05$. Aos 21 dias, os grupos S, SOC 2,5 e SOC 5 mostraram menor peso corporal em relação ao grupo controle. Os três grupos subnutridos apresentaram maior ganho de peso corporal e menores pesos nos TAE e retroperitoneal comparados ao grupo controle. No TTOG de 120 dias os animais SOC 2,5 e SOC 5 apresentaram menor área sob a curva quando comparados ao grupo S. Os animais SOC 5 apresentaram maior concentração da glicemia de jejum comparado aos animais C, e mostraram menor conteúdo de lipídios hepáticos totais em relação ao C, S e SOC 2,5. Em relação aos parâmetros de estresse oxidativo hepáticos, os animais SOC 5 apresentaram menores valores das substâncias reativas ao ácido tiobarbitúrico (TBARS) em relação aos animais S, e a atividade da catalase (CAT) foi maior no grupo SOC 2,5 em relação ao grupo controle. Em relação ao estresse oxidativo no TAE, os dois grupos suplementados apresentaram menores valores de TBARS em relação ao C, o conteúdo de hidroperóxidos foi maior no grupo SOC 2,5 em relação ao C e ao S, o grupo SOC 5 apresentou menores concentrações de hidroperóxidos em relação ao SOC 2,5 e a atividade da CAT foi maior nos grupos S e SOC 2,5 em comparação ao controle. Dessa forma, dentro das doses usadas neste estudo, percebeu-se que a suplementação materna do óleo de chia na dose de 5g/kg de peso corporal apresentou leve vantagem, pois atenuou a glicemia e reduziu TBARS no fígado e TAE, demonstrando que o óleo de chia nessa dose pode melhorar alterações metabólicas geradas pela subnutrição neonatal.

Palavras chave: Desnutrição. Programação fetal. Metabolismo. Ácidos graxos ômega-3.

ABSTRACT

Maternal nutrition and fatty acids consumption during gestational and/or lactational periods are critical determinants strongly related to normal fetal and postnatal development. Changes in offspring nutrition in the early stages of life may predispose to the development of metabolic disorders. In this sense, compounds that act in preventing and/or reversing these disorders have been sought. Chia oil (ChO) stands out as a promising food for having a high content of alpha-linolenic acid (55-66%), and phenolic compounds. The aim of this study is evaluate the influence of maternal supplementation with ChO by two doses (2.5 or 5g/kg bodyweight) over metabolic parameters in BALB/c mice subject to postnatal undernutrition. All procedures were approved by the local ethical committee for animal research. Thirty BABL/c mice were used. The females receive the supplementation with oil by gavage (2.5 or 5g/kg b.w.), since mating until weaning of the offspring. The neonatal undernutrition was induced by increasing litter size to 15-16 animals, forming the Undernutrition (UN), UN + chia oil (UN 2.5 or UN 5g/kg b.w.) groups. Litters with 8-10 animals were used as Control (C), without supplementation. The body weight was measured at 21, 70 and 120 days, and food intake was measured weekly after weaning. The oral glucose tolerance test (OGTT) was performed at 70 and 120 days of age of the offspring. After euthanasia (120 days), the tissues and blood were collected to analyses. Hepatic lipids were extracted by Folch's method and evaluated by commercial kits. Plasma lipids and fasting glucose were also analyzed by commercial kits. Oxidative stress markers (TBARS and hidroperoxydes contents) and antioxidant enzymes (SOD and CAT) were determined by colorimetric method. All statistical analyses were conducted using GraphPad Prism® software, differences between groups were evaluated by one-way analysis of variance (ANOVA). The significance level adopted was $p < 0.05$. At 21 days, the UN, UN 2.5 and UN 5 groups showed lower body weight, a higher weight gain and a lower weight of epididymal and retroperitoneal adipose tissue compared to Control. At OGTT 120 days the animals UN 2.5 and UN 5 showed a smaller area under curve compared to UN group. The UN 5 animals showed higher concentration of fasting glucose in comparison to C animals, and presented lower content of hepatic lipids compared to C, UN and UN 2,5 groups. The oxidative stress markers (TBARS) in liver were reduced in both groups supplemented, and the UN 2.5 showed higher CAT activity compared to control. Regarding the oxidative stress in the epididymal adipose tissue (EAT), the two supplemented groups had lower TBARS values compared to C, the hydroperoxide content was higher in the SOC 2.5 group compared to C and S, the UN 5 group showed lower content of hydroperoxide compared to UN 2.5 group, and the CAT activity was higher in the S and SOC 2.5 groups compared to the control. Thus, within the doses used in this study, it was noticed that maternal supplementation of chia oil at a dose of 5g/kg of body weight had a slight advantage, as it attenuated some metabolic parameters in undernourished animals, such as glyceimic curve and reduced TBARS in the liver and TAE, demonstrating that chia oil at this dose can improve metabolic changes generated by neonatal malnutrition.

Keywords: Malnutrition. Fetal programming. Metabolism. Fatty acids, omega 3.

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LISTA DE ABREVIATURAS

AGPI	Ácido graxo poliinsaturado
ALA	Ácido alfa linolênico
ARC	Núcleo arqueado
ATP	Adenosina trifosfato
AUC	Área sob a curva
B.W.	Peso corporal
C	Grupo Controle/Control group
CAT	Catalase
CEUA	Comitê de ética em experimentação animal
ChO	Óleo de chia
cm	Centímetros
DHA	Ácido docosahexaenoico
EPA	Ácido eicosapentaenoico
FABPs	Proteínas de ligação intracelular de ácidos graxos
FATPs	Proteína de transporte de ácidos graxos
IL-1 β	Interleucina 1 Beta
MDA	Malondialdeído
mg/dL	Miligrama por decilitro
mg/kg	Miligrama por kilo
mL	Mililitro
mmol/L	Milimol por litro
NF- κ B	Fator nuclear kappa-B
NPY	Neuropeptídeo Y
OGTT	Teste de tolerância oral à glicose
PPAR	Receptor ativado por proliferadores de peroxissoma
ROS	Espécies reativas de oxigênio
Rpm	Rotações por minuto
S	Grupo Subnutrido
SEM	Erro padrão da média
SOD	Superóxido dismutase

LISTA DE ABREVIATURAS (continuação)

SOC 2,5	Subnutrido óleo de chia 2,5g/kg de peso corporal
SOC 5	Subnutrido óleo de chia 5g/kg de peso corporal
TAE	Tecido adiposo epididimal
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TNF- α	Fator de necrose tumoral alfa
UCP 1	Proteína desacopladora 1
UFLA	Universidade Federal de Lavras
UN	Undernutrition
ω -3	Ômega 3

LISTA DE SÍMBOLOS

α	Alfa
Δ	Delta
κ	Kappa
ω	Ômega
γ	Gamma
\pm	Mais ou menos
$<$	Menor
μ	Micro
®	Registrado

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PRIMEIRA PARTE

1 INTRODUÇÃO

A nutrição inadequada no começo da vida, além da predisposição genética ou os fatores de estilo de vida, pode modular os processos de desenvolvimento para produzir mudanças permanentes que aumentam o risco do desenvolvimento de doenças crônicas, esse fenômeno é denominado programação metabólica (MATHIAS et al., 2014; FERGUSON et al., 2019).

Na programação metabólica um único genótipo é capaz de produzir diferentes fenótipos em resposta a diferentes ambientes, o que garante a capacidade de responder a um ambiente nutricional alterado para permitir a sobrevivência a curto prazo. A longo prazo, essas respostas são prejudiciais, pois predisõem o organismo a distúrbios metabólicos que surgem na vida adulta (HANLEY et al., 2010; HOCHBERG et al., 2011; PATEL, SRINIVASAN, 2011; SOUZA et al., 2019).

Ajustes de tamanho de ninhada de roedores têm sido usados para alterar a nutrição no período de amamentação, resultando em alterações na quantidade e qualidade de leite disponível para os filhotes recém-nascidos. Como resultado, os filhotes de grandes ninhadas apresentam baixo peso corporal, bem como hipoinsulinemia e hipoleptinemia no pós-natal (PATEL, SRINIVASAN, 2011; COLLDEN et al., 2015; XAVIER et al., 2019).

Mathias e colaboradores (2014), em sua revisão, trazem que um número considerável de estudos, majoritariamente em ratos, destacam os efeitos de algumas moléculas bioativas na modificação epigenética e indicam que pode ser possível prevenir a programação metabólica e o risco de doenças mais tarde na vida adulta (HAGGARTY, 2012).

Estudos têm demonstrado que o óleo de chia apresenta um elevado conteúdo de compostos polifenólicos e possui significativamente maior conteúdo dos ácidos α -linolênico (55-66%) e linoleico (16-22%) do que o óleo de linhaça (50-62% de ALA), canola e soja (ÁLVAREZ-CHÁVEZ et al., 2008; IXTAINA et al., 2011; MARTÍNEZ-CRUZ, PAREDES-LÓPEZ, 2014; REYES-CAUDILLO et al., 2008; MOHAMMADI-SARTANG et al., 2017). Dessa forma, a chia tem sido um alimento promissor para a sociedade e comunidade científica por seu conteúdo em ácidos graxos,

antioxidantes e bioativos, para prevenir ou modificar os distúrbios metabólicos e inflamatórios advindos de doenças crônicas.

A nutrição materna e o consumo de ácidos graxos durante os períodos gestacionais e/ou de lactação são determinantes críticos fortemente relacionados ao desenvolvimento fetal e pós-natal normal, que induzem modificações na programação fetal que levam a suscetibilidade da prole a doenças metabólicas ao longo da vida (MENNITTI et al., 2015).

Desse modo, o objetivo do presente projeto foi avaliar a influência da suplementação materna com o óleo de chia em diferentes doses sobre parâmetros metabólicos na prole submetida à subnutrição alimentar neonatal e verificar qual a dose mais eficiente.

2 REFERENCIAL TEÓRICO

2.1 Programação Metabólica

Barker (1995) em seu estudo epidemiológico trouxe pela primeira vez a possibilidade de que os acontecimentos no começo da vida em humanos poderiam exercer um papel importante na progressão de doenças crônicas na idade adulta. Em seu trabalho, Barker expôs que os recém-nascidos com baixo peso ao nascer, por desnutrição materna durante a gravidez, tinham maior predisposição a desenvolver um fenótipo adulto de excesso de peso e distúrbios cardiometabólicos.

Diversos estudos epidemiológicos revelaram que a exposição a um ambiente desfavorável no início da vida é associada a um risco consideravelmente aumentado de doença posteriormente. Esse fenômeno é denominado programação para o início da vida. O termo “programação” define uma perturbação em períodos críticos de desenvolvimento que causam danos permanentes e alterações ao longo da vida com consequências “irreversíveis”, como o risco de desenvolver obesidade, diabetes e doenças cardiovasculares (MATHIAS et al., 2014; PERSSON, BONDKE, 2018).

O ambiente nutricional e hormonal durante os períodos embrionários e fetais desempenha um papel modulador essencial, pois conduz a expressão do genoma. Dentre os mecanismos envolvidos na programação metabólica, destacam-se os mecanismos epigenéticos, considerada como a maneira pela qual o genoma interage e responde ao ambiente (COMINETTI, ROGERO, HORST, 2017).

A epigenética modula e regula a expressão gênica através de várias "marcas" epigenômicas, como metilação do DNA, alterações pós-transcricionais (acetilação, metilação, fosforilação, entre outras) que ocorrem nas proteínas designadas histonas e expressão de microRNAs, que acometem os genes e as vias essenciais durante o desenvolvimento nos períodos iniciais da vida e as funções fisiológicas da vida adulta (COMINETTI, ROGERO, HORST, 2017; TIFFON, 2018).

Estímulos ambientais de natureza física, mental ou nutricional podem interferir na expressão genômica da prole. Sinais epigenéticos possuem grande plasticidade, podendo ser modulados, e até mesmo revertidos, por atuação de diversos fatores, como alimentação, medicamentos, produtos químicos, fatores físicos e psicossociais (HAGGARTY, 2012; HABBOUT et al., 2013; COMINETTI, ROGERO, HORST, 2017).

Durante o período de lactação as condições nutricionais e metabólicas maternas podem programar o sistema de homeostase energética da prole e alterar sua função mais tarde na vida, sendo assim considerada uma janela crítica do desenvolvimento (XAVIER et al., 2019).

Em roedores, a neurogênese hipotalâmica ocorre durante o meio da gestação, enquanto as projeções neurais entre diferentes núcleos se desenvolvem durante a vida pós-natal imediata. Portanto, mudanças durante esses períodos críticos do desenvolvimento, como por exemplo, o consumo materno de dieta rica em gordura durante a gestação e lactação, pode afetar o desenvolvimento da neurogênese hipotalâmica ou das projeções neurais, e tem consequências a longo prazo no metabolismo da prole (SUN et al., 2014).

Modelos experimentais de subnutrição alimentar durante a gestação e/ou lactação são descritos na literatura e, como resultados, os animais apresentam durante a vida adulta aumento do peso corporal, alterações no controle da ingestão alimentar e resistência à insulina (BULFIN et. al., 2011; HABBOUT et al, 2013; PÔRTO et. al., 2008).

Além disso, a nutrição pode desencadear mudanças epigenéticas no desenvolvimento perinatal até a idade adulta por meio de diferentes vias, como progressão do fator de risco metabólico e geração de estresse oxidativo. Moléculas oxidantes podem interagir diretamente com bases pareadas de DNA causando tanto alterações genéticas como epigenéticas. O *status* de redox celular influencia a expressão do gene e a diferenciação celular (SAHA et al., 2017).

2.2 Restrição alimentar neonatal como modelo experimental de programação metabólica

A programação do desenvolvimento da saúde e doença em adultos é estudada ao longo de duas décadas mostrando um progresso considerável, mas ainda não há um consenso sobre os nutrientes exatos e os mecanismos envolvidos. Há ainda, a tentativa de conciliar os resultados de estudos epidemiológicos em humanos, que relacionam o peso ao nascer aos desfechos de saúde e doença na vida adulta, à estudos de intervenção nutricional em modelos animais (HANLEY et al., 2010).

A vantagem de se utilizar modelos animais é a possibilidade de manipular dietas, medicamentos e cirurgia, controlados durante o período gestacional e pós-natal, o que

seria inviável e anti-ético em estudos com humanos (TARRY-ADKINS, OZANNE, 2011; ZHU, CAO, LI, 2019).

Estudos são realizados em diferentes espécies, como ovelhas, porcos e primatas não humanos, sendo os principais em ovelhas e roedores. Entretanto, a maior parte dos estudos com animais utiliza modelos de roedores por sua menor gestação e, por possuírem embriologia, anatomia e fisiologia semelhantes ao humano. Dessa forma, é possível monitorar e modificar o ambiente intrauterino de modelos animais, a fim de obter informações sobre as bases moleculares da patogênese da doença metabólica humana (HANLEY et al., 2010; WARNER, OZANNE, 2010; SEKI et al., 2012).

Camundongos da linhagem BALB/c são largamente usados em pesquisas de imunologia e fisiologia. Além disso, são normalmente utilizados em estudos de obesidade induzida através da alimentação e seus principais sintomas, a saber, hiperlipidemia, hiperglicemia, diabetes e inflamação de baixo grau (LI et al., 2020).

Em roedores as ilhotas pancreáticas e os neurônios continuam o processo de desenvolvimento durante o período pós-natal imediato. Sendo uma janela crítica para o desenvolvimento e, portanto, alterações nutricionais, hormonais e metabólicas durante esse período funciona como pistas independentes para os efeitos de programação metabólica (PATEL, 2011; RIBEIRO et al., 2017).

O ajuste do tamanho de ninhada é comumente utilizado para investigar os efeitos a longo prazo do excesso ou restrição alimentar durante o período de amamentação. Diferentes métodos têm sido utilizados para induzir a restrição alimentar, sendo estes: separação materna temporal, manipulação do tamanho da ninhada, restrição da ingestão materna em proteína-energia, ou até mesmo, ligação cirúrgica das mamas para prejudicar a lactação (PATEL, 2011; HUBER et al., 2013).

A manipulação do tamanho da ninhada por meio do aumento de filhotes no período da lactação aparece previamente no estudo de McCance (1962), onde a ninhada conta com 15 a 18 filhotes. Neste estudo e em outros trabalhos, foi observado que os animais criados em grandes ninhadas apresentavam um ganho de peso corporal e crescimento mais lento em relação aos animais de ninhadas menores. Após o período de desmame e com acesso livre à comida os animais subnutridos obtiveram um rápido crescimento apesar de não alcançarem o peso e estatura dos animais criados em ninhadas normais (CARON et al., DAVIS, CHAMSEDDINE, HARPER, 2016).

Animais provenientes de grandes ninhadas consomem menos leite devido à competição, resultando em menor taxa de crescimento e de massa gorda. No decorrer da

amamentação, a redução da ingestão de leite é adicionada a uma diminuição na concentração de triglicerídeos no leite, que é o componente lipídico mais abundante, e, portanto, a principal fonte de energia para o recém-nascido. Esta condição de diminuição da ingestão de leite e menor teor de triglicerídeos causam distúrbios metabólicos e endócrinos nos recém-nascidos, com diminuição das concentrações plasmáticas de glicose, insulina e leptina (LÓPEZ-SOLDADO, MUNILLA, HERRERA, 2006; SUN et al., 2014).

Os animais subnutridos se tornam hiperfágicos e apresentam crescimento de recuperação na juventude. Como resultado apresentam excesso de peso na idade adulta, com aumento dos depósitos de tecido adiposo branco e aumento do índice de Lee. Além disso, apresentam diminuição de proteína desacopladora 1 (UCP1) no tecido adiposo marrom, o que pode ajudar a manter o fenótipo de sobrepeso na idade adulta (MARANGON et al 2020; CARON et al., 2012).

Devido a programação nutricional no período neonatal, esses animais parecem desenvolver resistência às ações centrais da adiponectina no peso corporal e ingestão alimentar. Esse hormônio é principalmente produzido pelo tecido adiposo branco e regula o metabolismo de lipídios e glicose (HALAH et al., 2018). Soma-se a isso, alterações nas secreções de leptina e grelina durante o período neonatal, e esses hormônios desempenham importante função na formação dos circuitos hipotalâmicos (HABBOUT et al., 2013; MARANGON et al., 2020).

A leptina é o principal modulador do neuropeptídeo Y (NPY), produzido no núcleo arqueado (ARC) e liberado em várias regiões hipotalâmicas, onde desempenha potentes efeitos estimuladores do apetite. Estudos anteriores demonstraram que a injeção central de NPY em roedores pode causar hiperfagia e obesidade acentuadas, e também pode reduzir o gasto de energia ao inibir a termogênese do tecido adiposo marrom. Foi visto que aos 21 dias, animais criados em grandes ninhadas apresentam aumento do NPY (VELKOSKA et al., 2008; CLARK et al., 1984; PLAGEMANN et al., 1999; HANSEN, JOVANOVSKA, MORRIS, 2004).

Ainda, foi observado que os animais criados em grandes ninhadas se tornam insensíveis à ação da grelina. Estudos com ratos obesos mostraram que a obesidade foi associada a resistência à grelina. Este hormônio estimula a liberação do hormônio do crescimento, aumenta a ingestão de alimentos por meio de estímulos diretos sobre o NPY/AgRP e inibição dos neurônios POMC/CART no núcleo arqueado do hipotálamo, e aumenta o ganho de peso corporal (BRIGGS et al., 2010). Além disso, após ativação

do receptor GHSR1a, a grelina ativa uma cascata intracelular, que resulta na fosforilação da AMPK e aumento na β -oxidação dos ácidos graxos, gerando espécies reativas de oxigênio (ROS) (LEBRETHON et al., 2007; CUI, LI, ZHU 2014; MARANGON et al., 2020).

Os neurônios no ARC recebem informações sobre o armazenamento corporal de energia por meio de hormônios periféricos, como insulina, leptina e adiponectina, que estão relacionados à massa de tecido adiposo branco e à homeostase da glicose. Sendo assim, esses animais podem apresentar alterações na homeostase energética, o que contribui para alterações na sensibilidade a esses hormônios, programando mecanismos metabólicos e centrais, que podem contribuir para o sobrepeso e a obesidade na idade adulta (REMMERS, DELEMARRE-VAN, 2011; HALAH et al., 2018; MARANGON et al., 2020).

López-Soldado et al (2006), estudando a subnutrição no período de lactação observaram que estes animais apresentavam diminuição da secreção de insulina estimulada por glicose. Contudo, para manter a secreção de insulina estimulada pela glicose, as células β requerem altos níveis de ATP, que é a principal fonte de energia das espécies reativas de oxigênio, no entanto as células β demonstram níveis baixos de enzimas de defesa antioxidantes, portanto, quaisquer defeitos na função mitocondrial ou aumento no estresse oxidativo impactam gravemente a função destas células (WARNER, OZANNE, 2010; FUJIMOTO et al., 2007; LENZEN, DRINKGERN, TIEDGE, 1996; TIEDGE et al., 1997).

Deve-se levar em conta também o fato de que diversos sistemas essenciais envolvidos na regulação da alimentação são imaturos ao nascimento e se desenvolvem durante o período pós-natal nos roedores (WARNER, OZANNE, 2010). Poucos trabalhos estudam a fundo os efeitos metabólicos da subnutrição durante a lactação, fazendo-se necessário mais pesquisas nesse campo.

2.3 Óleo de chia

A chia (*Salvia Hispanica L.*) é uma planta nativa do sul do México e norte da Guatemala, e sua semente é descrita como fonte de ácidos graxos poliinsaturados (AGPI), fibra alimentar, minerais, compostos fenólicos e um teor de proteínas superior a outros grãos (cerca de 19%) sendo, portanto, uma promissora fonte de peptídeos

bioativos (MARINELI et. al., 2015; da SILVA et al., 2017; GRANCIERI, MARTINO, de MEJIA, 2019).

O óleo de chia destaca-se como a principal fonte vegetal de ácidos graxos ômega-3 (ω -3), apresentando 68% da sua composição de ácido α -linolênico (C18:3, ALA), além de exibir conteúdo de tocoferóis, fitoesteróis, carotenoides e compostos fenólicos, tais como, ácido clorogênico, ácido cafeico, miricetina, quercetina e kaempferol, e compostos lipolíticos, agregando grande potencial antioxidante (REYES-CAUDILLO et al., 2008; IXTAINA et al., 2011; MARTÍNEZ-CRUZ, PAREDES-LÓPEZ, 2014; MARINELI et. al., 2015).

Os AGPI ω -3 têm sido associados com a melhora do perfil lipídico, atenuação do risco cardiometabólico e diminuição da inflamação (LESNA, et al., 2013). A alta concentração de ω -3 está associada à redução do risco de doença arterial coronariana, hipertensão, diabetes tipo 2, artrite reumatoide, desordens autoimunes e câncer (CONNOR, 2000).

O ALA é precursor do ácido eicosapentaenóico (EPA) e do ácido docosahexaenóico (DHA) no organismo, evidências tem mostrado que a suplementação de óleo rico em ALA aumenta as concentrações de DHA em vários tecidos, apesar disso, o ALA produz respostas fisiológicas diferentes do EPA e DHA, particularmente na adiposidade e no manejo da glicose. A maior parte dos efeitos do ALA é por meio da modulação das lipoproteínas, enquanto o EPA e DHA podem reduzir a síntese de triglicerídeos e reduzir a adiposidade (POUDYAL et al., 2013).

Os ω -3 parecem regular positivamente duas enzimas envolvidas na hidrólise de triglicerídeos, lipase lipoproteica e lipase de triacilglicerol, que produzem efeitos hipotriacilglicerolêmicos e lipolíticos nos adipócitos, através de ligação aos receptores ativados por proliferadores de peroxissoma α e γ (PPAR). Embora existam muitos dados sobre os mecanismos de atuação dos ácidos graxos n-3 na redução da adiposidade e dislipidemia, os mecanismos de atuação do ALA são pouco compreendidos (POUDYAL et a., 2011, 2013).

Muitas investigações têm mostrado que o consumo de fontes dietéticas naturais (frutas, nozes, vegetais) com compostos bioativos antioxidantes (polifenóis, tocoferóis, carotenoides, vitaminas) podem auxiliar na prevenção do estresse oxidativo e pode ser uma alternativa natural para prevenção e controle de doenças crônicas (AVIGNON et al., 2012; BULLO, LAMUELA-RAVENTOS; SALAS-SALVADO, 2011; LANDETE, 2012).

Na gestação, a única fonte de ácidos graxos poli-insaturados para o feto em desenvolvimento é a mãe por meio da placenta. A ingestão alimentar de ácidos graxos durante a gestação e sua subsequente transferência da mãe para o feto são cruciais para o crescimento e desenvolvimento fetal. Acredita-se que o transporte dos ácidos graxos maternos para o feto ocorra por meio de algumas proteínas transmembranas: proteína de transporte de ácidos graxos (FATPs), translocase de ácidos graxos (FAT/CD36) e proteínas de ligação intracelular de ácidos graxos (FABPs). Após os ácidos graxos estarem dentro da célula que envolve o feto, se translocam para o núcleo e alteram a expressão gênica, podem ser armazenados ou se deslocam para a mitocôndria para modular a função mitocondrial (JONES, MARK, WADDELL, 2014; LEWIS, DESOYE, 2017; SHRESTHA et al., 2020).

Questiona-se, assim, se a suplementação materna com óleo de chia, fonte do ácido graxo poli-insaturado ômega-3, durante a gestação e lactação, seria capaz de modular as alterações geradas na prole submetida a subnutrição, com consequências até a vida adulta.

2.4 Efeitos da suplementação com ômega-3 sobre marcadores de estresse oxidativo

A desnutrição desencadeia aumento do dano oxidativo ao DNA e aos lipídios, o que é atribuído a mecanismos antioxidantes danificados, incluindo alterações da atividade transcricional e enzimática (GAVIA-GARCIA et al., 2015). Nesse sentido, os animais criados em grandes ninhadas apresentam aumento nos níveis de lipoperoxidação, óxido nítrico, TNF- α e IL-1b, redução de SOD e aumento das atividades da catalase (SANTOS-JÚNIOR et al., 2016).

O estresse oxidativo ocorre devido a um desequilíbrio entre os sistemas oxidante e antioxidante, o que pode ser causado pela elevada geração de radicais livres e diminuída atividade dos antioxidantes. As espécies reativas de oxigênio (EROs) são produtos normais do metabolismo celular ou ocorrem devido à exposição a estímulos, como radiação ionizante e produtos químicos, que reagem com o DNA podendo causar diversas lesões em bases oxidadas, quebras de fitas simples e duplas, podem danificar lipídios celulares e proteínas (SAKAI et al., 2017). Sendo assim, o estresse oxidativo pode desencadear inflamação, dano celular, câncer, e desempenhar um papel importante na etiologia de vários fatores de risco relacionados à aterosclerose, hipertensão, diabetes

tipo 2 e resistência à insulina (VALKO et al., 2007; CHEN et al., 2011; POUDYAL et al., 2013; SAMARGHANDIAN, 2017).

Além disso, o consumo excessivo de alguns macronutrientes, tais como, glicose, ácidos graxos saturados ou AGPI ω -6 podem induzir um processo inflamatório por meio de vias mediadas por NF- κ B que leva ao desencadeamento de estresse oxidativo, e por outro lado, ALA, EPA e DHA podem inibir as vias mediadas pela NF- κ B, influenciando a produção de citocinas, como TNF- α , IL-6, IL-1 β (MENNITTI et al., 2015; YANG et al., 2019).

Yang e colaboradores (2019) em sua revisão sistemática descrevem que os AGPI n-3, vitamina A, E, C, selênio e outros polifenóis podem atenuar o estresse oxidativo. Corroborando este dado, Sakai et al. (2017) observaram que o EPA e o DHA diminuem o dano ao DNA causado por EROs em células endoteliais da aorta humana. Diferentes tipos de ácidos graxos têm efeitos modificáveis na produção de radicais livres e cada ácido graxo atua diretamente na eliminação de EROs e indiretamente no estímulo de enzimas antioxidantes (BULLO, LAMUELA-RAVENTOS, SALAS-SALVADO, 2011).

Diversos biomarcadores do estresse oxidativo tem sido estudados, entre eles, peróxido de hidrogênio, radical superóxido, glutatona oxidada (GSSG), malondialdeído (MDA), isoprostanos, carbonilas e nitrotirosina, que podem ser facilmente mensurados no plasma e sangue (BIRBEN et al., 2012).

Alguns estudos observaram que o ALA proveniente do óleo de chia pode modificar o perfil antioxidante hepático, visto através do aumento do conteúdo de tiol reduzido (GSH), maior razão GSH/GSSG e redução do conteúdo de MDA. Além disto, foi demonstrado que o ALA resulta em maior atividade de enzimas antioxidantes, como superóxido dismutase (SOD), glutatona peroxidase (GPx) e que o óleo de semente de chia é melhor do que a semente para restaurar o sistema antioxidante, vistos em ratos com obesidade induzida pela dieta (MARINELI et al., 2015; RINCÓN-CERVERA et al., 2016; PARKER et al., 2018; HAN et al., 2020).

A qualidade da nutrição materna durante a gravidez pode afetar permanentemente o fenótipo da prole por meio de uma interação fisiológica direta e resulta em consequências perinatais pós-natais na prole. As concentrações maternas de ácidos graxos essenciais podem modular o crescimento e desenvolvimento fetal, pois o ALA e seus metabólitos DHA e EPA, além do ácido araquidônico (AA) são transferidos através da placenta para a prole em desenvolvimento através de transportadores e

podem ter notáveis efeitos no desenvolvimento do sistema nervoso central e pós-natal (HERRERA, 2002; MATHIAS et al., 2014; BASAK, VILASAGARAM, DUTTAROY, 2020; SHRESTHA et al., 2020).

A suplementação materna com ALA durante a lactação promove hipermetilação no fígado de FADS2. O aumento dessa proteína foi relacionado às concentrações hepáticas do ácido docosapentaenóico. Assim, a regulação transcricional de FADS2 que codifica a $\Delta 5$ dessaturase, representa um mecanismo potencial pela qual a ingestão materna de ácidos graxos pode induzir mudanças permanentes no conteúdo de AGPI das membranas celulares da prole. Os AGPI ω -3 incorporados à membrana celular, afetam sua fluidez, função do receptor, atividade enzimática e produção de citocinas e eicosanoides (CHILDS et al., 2012; HOILE et al., 2013; NICULESCU, LUPU, CRACIUNESCU, 2013; SILVA, OLIVEIRA, GOMES-MARCONDES, 2018).

Foi visto que a suplementação materna de ω -3 melhorou as defesas antioxidantes da prole com tumor induzido, vistos por meio de menores níveis hepáticos de MDA e aumento da atividade da SOD (MIYAGUTI, DE OLIVEIRA, GOMES-MARCONDES, 2018).

Alguns trabalhos propuseram que o ω -3, tem a capacidade de ativar direta e indiretamente a via do NF- κ B, entre elas por meio da ativação do PPAR- α , e essa via é responsável pelo controle da expressão gênica de várias enzimas antioxidantes. Dessa forma, a modulação da via do NF- κ B pelo EPA pode modular o conteúdo de CAT e, conseqüentemente, sua atividade. Além disso, foi descrito que os produtos da peroxidação não enzimática do EPA, J3-isoprostanos, reagem com grupos sulfidríla no complexo Keap1 responsável pela ubiquitinação e posterior degradação do fator de transcrição Nrf2, levando à translocação nuclear de Nrf2 e expressão de várias enzimas antioxidantes no fígado, formação de glutathiona e diminuição da taxa de peroxidação lipídica (ZUÑIGA et al. 2011; SILVA et al., 2016).

Existem poucos estudos que avaliam a suplementação materna de ω 3 durante a gestação e lactação sobre os efeitos do estresse oxidativo, que é um fator crucial para o desenvolvimento da prole, sendo um aspecto que carece de elucidação (KAJARABILLE et al., 2017).

3 CONSIDERAÇÕES FINAIS

O ambiente pós-natal é considerado um dos períodos cruciais do desenvolvimento, onde alterações na nutrição podem influenciar o desenvolvimento normal e gerar alterações que acompanharão o indivíduo a longo prazo. A alimentação materna é fator determinante para o desenvolvimento fetal e pós-natal normal, podendo gerar modificações no feto por meio de mecanismos epigenéticos.

Poucos são os trabalhos encontrados que associam a atuação da suplementação materna com uma fonte vegetal de ômega 3 sobre parâmetros metabólicos em proles subnutridos durante o pós-natal, o que torna a proposta deste estudo altamente relevante.

4 REFERÊNCIAS

ÁLVAREZ-CHÁVEZ, L. M. *et al.* Chemical characterization of the lipid fraction of Mexican chia seed (*Salvia hispanica* L.). **International Journal of Food Properties**, v. 11, n. 3, p. 687–697, 2008.

AUGUSTO, R. L. *et al.* Fighting Oxidative Stress : Increased Resistance of Male Rat Cerebellum at Weaning Induced by Low Omega 6 / Omega 3 Ratio in a Protein-Deficient Diet. **The Cerebellum**, p. 103–117, 2017. Disponível em: <http://dx.doi.org/10.1007/s12311-016-0773-1>.

AVIGNON, A. *et al.* Dietary antioxidants: Do they have a role to play in the ongoing fight against abnormal glucose metabolism? **Nutrition**, v. 28, n. 7, p. 715–721, 2012. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0899900712000354>.

BARKER, D. J. The fetal origins of coronary heart disease. **Acta Paediatrica**, v. 86, p. 72–82, 1997.

BASAK, S.; VILASAGARAM, S.; DUTTARROY, A. K. Maternal dietary deficiency of n-3 fatty acids affects metabolic and epigenetic phenotypes of the developing fetus. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v. 158, p. 102109, 2020. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0952327820300673>.

BIRBEN, E., SAHINER, U. M., SACKESEN, C., *et al.* Oxidative Stress and Antioxidant Defense, **World Allergy Organization Journal**, n. January, p. 9–19, 2012.

BRIGGS, D. I. *et al.* Diet-Induced Obesity Causes Ghrelin Resistance in Arcuate NPY/AgRP Neurons. **Endocrinology**, v. 151, n. 10, p. 4745–4755, 1 out. 2010. Disponível em: <https://doi.org/10.1210/en.2010-0556>.

BULFIN, L. J. et al. Anxiety and hypothalamic-pituitary-adrenal axis responses to psychological stress are attenuated in male rats made lean by large litter rearing. **Psychoneuroendocrinology**, v. 36, n. 7, p. 1080–1091, ago. 2011.

BULLÓ, M.; LAMUELA-RAVENTÓS, R.; SALAS-SALVADÓ, J. Mediterranean Diet and Oxidation : Nuts and Olive Oil as Important Sources of Fat and Antioxidants. **Current Topics in Medicinal Chemistry**, v. 11, n. 14, p. 1797–1810, 2011. Disponível em:

<https://www.ingentaconnect.com/content/ben/ctmc/2011/00000011/00000014/art00005>.

CARON, E. et al. Alteration in neonatal nutrition causes perturbations in hypothalamic neural circuits controlling reproductive function. **Journal of Neuroscience**, v. 32, n. 33, p. 11486–11494, 2012. Disponível em: <https://doi.org/10.1523/JNEUROSCI.6074-11.2012>.

COMINETTI, C., ROGERO, M., HORST, M. **Genômica nutricional: dos fundamentos à nutrição molecular**. [S.l.: s.n.], 2017.

CHEN, X., TIAN, X., YOON, J. Fluorescent and luminescent probes for detection of reactive oxygen and nitrogen species, **The Royal Society of Chemistry**, v. 40, p. 4783–4804, 2011. Disponível em: <https://doi.org/10.1039/c1cs15037e>.

CHILDS, C. E. et al. Changes in rat n-3 and n-6 fatty acid composition during pregnancy are associated with progesterone concentrations and hepatic FADS2 expression. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v. 86, n. 4, p. 141–147, 2012. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0952327812000397>.

CLARK, J. T. et al. NEUROPEPTIDE Y AND HUMAN PANCREATIC POLYPEPTIDE STIMULATE FEEDING BEHAVIOR IN RATS. **Endocrinology**, v. 115, n. 1, p. 427–429, 1 jul. 1984. Disponível em: <https://doi.org/10.1210/endo-115-1-427>.

COLLDEN, G. et al. Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin. **Molecular Metabolism**, v. 4, n. October 2014, p. 15–24, 2015.

CUI, L. et al. Impact of changes in postnatal nutrition on puberty onset and the expression of hypothalamic GnRH and ghrelin. **Eur Rev Med Pharmaco Sci**, v. 18, p. 703–709, 2014.

DA SILVA, B. P. et al. Chemical composition of Brazilian chia seeds grown in different places. **Food Chemistry**, v. 221, p. 1709–1716, 2017. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0308814616317939>.

DA SILVA, E. P. et al. Omega-3 fatty acids differentially modulate enzymatic anti-oxidant systems in skeletal muscle cells. **Cell Stress and Chaperones**, v. 21, n. 1, p. 87–95, 2016. Disponível em: <https://doi.org/10.1007/s12192-015-0642-8>.

- DAVIS, K.; CHAMSEDDINE, D.; HARPER, J. M. Nutritional limitation in early postnatal life and its effect on aging and longevity in rodents. **Experimental Gerontology**, v. 86, p. 84–89, 2016. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0531556516301280>.
- DOS SANTOS-JÚNIOR, E. F. et al. Malnutrition increases NO production and induces changes in inflammatory and oxidative status in the distal colon of lactating rats. **Neurogastroenterology & Motility**, v. 28, n. 8, p. 1204–1216, 1 ago. 2016. Disponível em: <https://doi.org/10.1111/nmo.12820>.
- FERGUSON, D. P. et al. Postnatal undernutrition alters adult female mouse cardiac structure and function leading to limited exercise capacity. **Journal of Physiology**, v. 597, n. 7, p. 1855–1872, 2019.
- FUJIMOTO, S. et al. Impaired metabolism–secretion coupling in pancreatic β -cells: Role of determinants of mitochondrial ATP production. **Diabetes Research and Clinical Practice**, v. 77, n. 3, Supplement, p. S2–S10, 2007. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0168822707001568>.
- GAVIA-GARCÍA, G. et al. Oxidative damage and antioxidant defense in thymus of malnourished lactating rats. **Nutrition**, v. 31, n. 11, p. 1408–1415, 2015. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0899900715002294>.
- GRANCIERI, M. et al. Digested total protein and protein fractions from chia seed (*Salvia hispanica* L.) had high scavenging capacity and inhibited 5-LOX, COX-1-2, and iNOS enzymes. **Food Chemistry**, v. 289, n. November 2018, p. 204–214, 2019. <https://doi.org/10.1016/j.foodchem.2019.03.036>.
- HABBOU, A. et al. Postnatal overfeeding in rats leads to moderate overweight and to cardiometabolic and oxidative alterations in adulthood. **Biochimie**, v. 94, n. 1, p. 117–124, 2012. Disponível em: <http://dx.doi.org/10.1016/j.biochi.2011.09.023>.
- HABBOU, A. et al. Postnatal Overfeeding in Rodents by Litter Size Reduction Induces Major Short- and Long-Term. **The Journal of Nutrition**, p. 553–562, 2013. <https://doi.org/10.3945/jn.112.172825.embryonic>.
- HAGGARTY, P. Nutrition and the Epigenome. In: BOUCHARD, C.; ORDOVAS, J. M. B. T.-P. IN M. B. AND T. S. (Org.). **Recent Advances in Nutrigenetics and Nutrigenomics**. [S.l.]: Academic Press, 2012. v. 108. p. 427–446. Disponível em: <https://www.sciencedirect.com/science/article/pii/B9780123983978000162>.
- HALAH, M. P. et al. Neonatal nutritional programming impairs adiponectin effects on energy homeostasis in adult life of male rats. **Am J Physiol Endocrinol Metab**, v. 315, p. E29–E37, 2018. <https://doi.org/10.1152/ajpendo.00358.2017>.
- HANLEY, B. et al. A review of metabolic programming, imprinting and epigenetics S1. **Br J Nutr**, v. 104, p. S1-25, 2010.
- HANLEY, B. et al. Metabolic imprinting, programming and epigenetics – a review of present priorities and future opportunities. **British Journal of Nutrition**, v. 104, n. S1,

p. S1–S25, 2010. Disponível em: <https://www.cambridge.org/core/article/metabolic-imprinting-programming-and-epigenetics-a-review-of-present-priorities-and-future-opportunities/94311D5315F8A4FD29F863F0D72A98E4>.

HANSEN, M. J.; JOVANOVSKA, V.; MORRIS, M. J. Adaptive responses in hypothalamic neuropeptide Y in the face of prolonged high-fat feeding in the rat. **Journal of Neurochemistry**, v. 88, n. 4, p. 909–916, 1 fev. 2004. Disponível em: <https://doi.org/10.1046/j.1471-4159.2003.02217.x>.

HERRERA, E. Implications of Dietary Fatty Acids During Pregnancy on Placental, Fetal and Postnatal Development—A Review. **Placenta**, v. 23, p. S9–S19, 2002. Disponível em: <https://www.sciencedirect.com/science/article/pii/S014340040290771X>.

HOCHBERG, Z. et al. Child Health, Developmental Plasticity, and Epigenetic Programming. **Endocrine Reviews**, v. 32, n. April, p. 159–224, 2011. Disponível em: <https://doi.org/10.1210/er.2009-0039>.

HOILE, S. P. et al. Maternal fat intake in rats alters 20 : 4n-6 and 22 : 6n-3 status and the epigenetic regulation of Fads2 in offspring liver ☆ , ☆☆ , ★. **The Journal of Nutritional Biochemistry**, v. 24, n. 7, p. 1213–1220, 2013. Disponível em: <http://dx.doi.org/10.1016/j.jnutbio.2012.09.005>.

HUBER, R. C. et al. Behaviour of postnatally growth-impaired mice during malnutrition and after partial weight recovery. **Nutritional Neuroscience**, v. 16, n. 3, p. 125–134, 2013. Disponível em: <https://doi.org/10.1179/1476830512Y.0000000038>

IXTAINA, V. Y. et al. Characterization of chia seed oils obtained by pressing and solvent extraction. **Journal of food composition and analysis: an official publication of the United Nations University, International Network of Food Data Systems**, v. 24, n. 2, p. 166-174, 2011. Disponível em: <https://doi.org/10.1016/j.jfca.2010.08.006>.

JONES, M. L.; MARK, P. J.; WADDELL, B. J. Maternal dietary omega-3 fatty acids and placental function. **Reproduction**, v. 147, n. 5, p. 143–152, 2014. Disponível em: <https://doi.org/10.1530/REP-13-0376>.

KAJARABILLE, N. et al. Omega-3 LCPUFA supplement: a nutritional strategy to prevent maternal and neonatal oxidative stress. **Maternal and Child Nutrition**, v. 13, n. 2, p. 1–12, 2017.

LANDETE, J. M. Updated Knowledge about Polyphenols: Functions, Bioavailability, Metabolism, and Health. **Critical Reviews in Food Science and Nutrition**, v. 52, n. 10, p. 936–948, 2012. Disponível em: <https://doi.org/10.1080/10408398.2010.513779>

LEBRETHON, M. C. et al. Effects of In Vivo and In Vitro Administration of Ghrelin, Leptin and Neuropeptide Mediators on Pulsatile Gonadotrophin-Releasing Hormone Secretion from Male Rat Hypothalamus Before and After Puberty. **Journal of Neuroendocrinology**, v. 19, n. 3, p. 181–188, 2007. Disponível em: <https://doi.org/10.1111/j.1365-2826.2006.01518.x>.

LENZEN, S.; DRINKGERN, J.; TIEDGE, M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. **Free Radical Biology and Medicine**, v. 20, n. 3, p. 463-466, 1996. Disponível em: <https://www.sciencedirect.com/science/article/pii/0891584996020515>.

LEVIN, B. E. Metabolic imprinting: Critical impact of the perinatal environment on the regulation of energy homeostasis. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 361, n. 1471, p. 1107-1121, 2006.

LEWIS, R. M.; DESOYE, G. Placental Lipid and Fatty Acid Transfer in Maternal Overnutrition. **Annals of Nutrition and Metabolism**, v. 70, n. 3, p. 228-231, 2017. Disponível em: <https://www.karger.com/DOI/10.1159/000463397>.

LI, J. et al. High fat diet induced obesity model using four strains of mice: Kunming, C57BL/6, BALB/c and ICR. **Experimental Animals**, v. 69, p. 326-335, 2020. Disponível em: <https://doi.org/10.1538/expanim.19-0148>.

LÓPEZ-SOLDADO, I.; MUNILLA, M. A.; HERRERA, E. Long-term consequences of under-nutrition during suckling on glucose tolerance and lipoprotein profile in female and male rats. **British Journal of Nutrition**, v. 96, n. 6, p. 1030-1037, 2006.

MARANGON, P. B. et al. Perinatal Over- and Underfeeding Affect Hypothalamic Leptin and Ghrelin Neuroendocrine Responses in Adult Rats. **Physiology and Behavior**, v. 215, n. September 2019, p. 112793, 2020. Disponível em: <https://doi.org/10.1016/j.physbeh.2019.112793>.

MARINELI, S. et al. Antioxidant potential of dietary chia seed and oil (*Salvia hispanica* L.) in diet-induced obese rats. **Food Research Internacional**, v. 76, p. 666-674, 2015. Disponível em: <https://doi.org/10.1016/j.foodres.2015.07.039>.

MARTÍNEZ-CRUZ, O.; PAREDES-LÓPEZ, O. 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. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0021967314005597>.

MATHIAS, P. C. F. et al. Maternal diet, bioactive molecules, and exercising as reprogramming tools of metabolic programming. **European Journal of Nutrition**, v. 53, n. 3, p. 711-722, 2014. Disponível em: <https://doi.org/10.1007/s00394-014-0654-7>.

MCCANCE, R. A. FOOD, GROWTH, AND TIME. **The Lancet**, v. 280, n. 7258, p. 671-676, 1962. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0140673662904993>.

MENNITTI, L. V. et al. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. **The Journal of Nutritional Biochemistry**, v. 26, n. 2, p. 99-111, 1 fev. 2015. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0955286314002034>.

MIYAGUTI, N. A. DA S.; DE OLIVEIRA, S. C. P.; GOMES-MARCONDES, M. C. C. Maternal nutritional supplementation with fish oil and/or leucine improves hepatic

function and antioxidant defenses, and minimizes cachexia indexes in Walker-256 tumor-bearing rats offspring. **Nutrition Research**, v. 51, p. 29–39, 2018. Disponível em: <https://www.sciencedirect.com/science/article/pii/S027153171730619X>.

MOHAMMADI-SARTANG, M. et al. The effect of flaxseed supplementation on body weight and body composition: a systematic review and meta-analysis of 45 randomized placebo-controlled trials. **Obesity Reviews**, v. 18, n. 9, p. 1096–1107, 1 set. 2017. Disponível em: <https://doi.org/10.1111/obr.12550>

NICULESCU, M. D.; LUPU, D. S.; CRACIUNESCU, C. N. Perinatal manipulation of α -linolenic acid intake induces epigenetic changes in maternal and offspring livers. **FASEB Journal**, v. 27, n. 1, p. 350–358, 2013. Disponível em: <https://doi.org/10.1096/fj.12-210724>.

PARKER, J. et al. Therapeutic Perspectives on Chia Seed and Its Oil: A Review. **Planta Medica**, v. 84, n. 9–10, p. 606–612, 2018.

PATEL, M. S.; SRINIVASAN, M. Metabolic Programming Due to Alterations in Nutrition in the Immediate Postnatal Period 1 – 3. **The Journal of Nutrition**, p. 1–4, 2010.

PATEL, M. S.; SRINIVASAN, M. Metabolic Programming in the Immediate Postnatal Life. **Annals of Nutrition and Metabolism**, v. 58(suppl 2, n. Suppl. 2, p. 18–28, 2011. Disponível em: <https://www.karger.com/DOI/10.1159/000328040>.

PERSSON, P. B.; BONDKE PERSSON, A. Metabolism, obesity and the metabolic syndrome. **Acta Physiologica**, v. 223, n. 3, p. 1–3, 2018.

PLAGEMANN, A. et al. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. **Journal of neuroendocrinology**, v. 11, n. 7, p. 541–546, 1999. Disponível em: <http://europepmc.org/abstract/MED/10444311>.

PÔRTO, L. C. J. et al. Impairment of the serotonergic control of feeding in adult female rats exposed to intra-uterine malnutrition. **British Journal of Nutrition**, v. 101, n. 8, p. 1255–1261, 2008. Disponível em: <https://www.cambridge.org/core/article/impairment-of-the-serotonergic-control-of-feeding-in-adult-female-rats-exposed-to-intrauterine-malnutrition/A4528A4454131D9E1DFB0F30D7780397>.

REMMERS, F.; DELEMARRE-VAN DE WAAL, H. A. Developmental Programming of Energy Balance and Its Hypothalamic Regulation. **Endocrine Reviews**, v. 32, n. 2, p. 272–311, 1 abr. 2011. Disponível em: <https://doi.org/10.1210/er.2009-0028>.

REYES-CAUDILLO, E.; TECANTE, A.; VALDIVIA-LÓPEZ, M. A. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. **Food Chemistry**, v. 107, n. 2, p. 656–663, 2008. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0308814607008709>.

RIBEIRO, T. A. et al. Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. **Scientific Reports**, v. 7, n. 1, p. 7634, 2017. Disponível em: <https://doi.org/10.1038/s41598-017-07395-2>.

- SAHA, S. K. et al. Correlation between Oxidative Stress, Nutrition, and Cancer Initiation. **International Journal of Molecular Sciences**, v.18, n.7, p. 1544, 2017. Disponível em: <https://doi.org/10.3390/ijms18071544>.
- SAKAI, C. et al. Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. **PLoS ONE**, v. 12, n. 11, p. 1–13, 2017. Disponível em: <https://doi.org/10.1371/journal.pone.0187934>.
- SEKI, Y., WILLIAMS, L., VUGUIN, P. M., et al. Minireview : Epigenetic Programming of Diabetes and Obesity : Animal Models, **Endocrinology**, v. 153, n. March, p. 1031–1038, 2012. Disponível em: <https://doi.org/10.1210/en.2011-1805>.
- SHRESTHA, N. et al. Role of omega-6 and omega-3 fatty acids in fetal programming. **Clinical and Experimental Pharmacology and Physiology**, n. July 2019, p. 907–915, 2020. Disponível em: <https://doi.org/10.1111/1440-1681.13244>.
- SOUZA, A. F. P. et al. Fish oil supplementation during adolescence attenuates metabolic programming of perinatal maternal high-fat diet in adult offspring. **British Journal of Nutrition**, v. 121, n. 12, p. 1345–1356, 2019. Disponível em: <https://doi.org/10.1017/S0007114519000771>.
- SUN, B. et al. Large Litter Rearing Improves Leptin Sensitivity and Hypothalamic Appetite Markers in Offspring of Rat Dams Fed High-Fat Diet During Pregnancy and Lactation. **Endocrinology**, v. 155, n. 9, p. 3421–3433, 1 set. 2014. Disponível em: <https://doi.org/10.1210/en.2014-1051>.
- TARRY-ADKINS, J. L.; OZANNE, S. E. Mechanisms of early life programming: current knowledge and future directions. **The American Journal of Clinical Nutrition**, v. 94, n. suppl_6, p. 1765S-1771S, 1 dez. 2011. Disponível em: <https://doi.org/10.3945/ajcn.110.000620>.
- TIEDGE, M. et al. Relation Between Antioxidant Enzyme Gene Expression and Antioxidative Defense Status of Insulin-Producing Cells. **Diabetes**, v. 46, n. 11, p. 1733 LP – 1742, 1 nov. 1997. Disponível em: <http://diabetes.diabetesjournals.org/content/46/11/1733.abstract>.
- TIFFON, C. The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease. **International Journal of Molecular Sciences**, v. 19, n. 3425, 2018. Disponível em: <https://doi.org/10.3390/ijms19113425>.
- VALKO, M. et al. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology**, v. 39, n. 1, p. 44–84, 2007. Disponível em: <https://www.sciencedirect.com/science/article/pii/S1357272506002196>.
- VELKOSKA, E. et al. Early Undernutrition Leads to Long-Lasting Reductions in Body Weight and Adiposity Whereas Increased Intake Increases Cardiac Fibrosis in Male Rats. **The Journal of Nutrition**, v. 138, n. 9, p. 1622–1627, 1 set. 2008. Disponível em: <https://doi.org/10.1093/jn/138.9.1622>.

WARNER, M. J., OZANNE, S. E. Mechanisms involved in the developmental programming of adulthood disease, **Biochemical Journal**, v. 427, n. 3, p. 333–347, 14 abr. 2010. Disponível em: <https://doi.org/10.1042/BJ20091861>.

XAVIER, J. L. P. et al. Litter size reduction induces metabolic and histological adjustments in dams throughout lactation with early effects on offspring. **Anais da Academia Brasileira de Ciências**, v. 91, n. 1, p. 1–17, 2019.

YANG, J. et al. Oxidative Stress and Non-Alcoholic Fatty Liver Disease : Effects of Omega-3 Fatty Acid Supplementation. **Nutrients**, v. 11, p. 1–37, 2019. Disponível em: <https://doi.org/10.3390/nu11040872>.

ZHU, Z.; CAO, F.; LI, X. Epigenetic Programming and Fetal Metabolic Programming. **Frontiers in Endocrinology**, v. 10, n. December, p. 1–15, 2019. Disponível em: <https://www.frontiersin.org/article/10.3389/fendo.2019.00764>.

ZÚÑIGA, J. et al. N-3 PUFA supplementation triggers PPAR- α activation and PPAR- α /NF- κ B interaction: Anti-inflammatory implications in liver ischemia-reperfusion injury. **PLoS ONE**, v. 6, n. 12, 2011. Disponível em: <https://doi.org/10.1371/journal.pone.0028502>.

SEGUNDA PARTE - ARTIGO**MATERNAL SUPPLEMENTATION WITH CHIA OIL ATTENUATES
METABOLIC DISTURBANCES IN MICE SUBJECT TO POSTNATAL
UNDERNUTRITION**

O estudo é uma versão preliminar e será submetido à Revista Nutrition Research ISSN: 0271-5317, sendo apresentado de acordo com as normas de publicação desta revista.

1 **Maternal supplementation with chia oil attenuates metabolic disturbances in mice**
2 **subjected to postnatal undernutrition**

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19

20

21 **ABSTRACT**

22

23 Maternal intake of omega-3 polyunsaturated fatty acids, such as chia oil, could play a
24 key role in the metabolic programming of offspring. This work aimed to evaluate the
25 effects of maternal supplementation with two doses of chia oil (2.5 or 5g/kg body
26 weight) on the metabolic parameters of BALB/c mice subjected to postnatal
27 undernutrition and to verify which dose is most effective. At 21 days, the undernutrition
28 groups had lower body weights than the Control group, and the UN 5 group exhibited
29 lower body weights than the UN 2.5 group. The weight gain of the UN 5 group was also
30 lower than that of the UN 2.5 group. The UN, UN 2.5 and UN 5 groups showed lower
31 weights of adipose tissues than the Control group. At 120 days, glucose tolerance was

32 evaluated, and both supplemented groups showed a lower area under the curve than the
33 UN animals. Maternal supplementation with 2.5g/kg b.w. chia oil improved catalase
34 activity in the liver, but the dose was insufficient to decrease hydroperoxides in the liver
35 and epididymal adipose tissue. On the other hand, 5g/kg b.w. of chia oil decreased body
36 weight and improved markers of oxidative stress, but the dose was insufficient to
37 increase the activity of antioxidant enzymes; therefore, this dose had a slight advantage
38 in attenuating metabolic disturbances. These findings suggest that maternal
39 supplementation with chia oil could attenuate metabolic alterations in mice subjected to
40 postnatal undernutrition.

41

42

43 **Keywords:** Metabolism. Fetal development. Fatty Acids, Omega-3. Malnutrition.

44

45 1. INTRODUCTION

46

47 Epidemiological studies in animals have shown that alterations in nutrition and
48 growth during early postnatal life could have permanent effects on metabolism in later
49 life [1,2]. Alterations during suckling, such as the composition and amount of milk
50 consumed, which vary according to maternal diet, can program long-lasting metabolic
51 alterations that lead to the development of obesity [3,4].

52

53 Therefore, both a lack and an excess of nutrients during prenatal and perinatal
54 life increase the risk of obesity and metabolic alterations in adulthood. Litter size
55 manipulations have been used to model experimental under- or overnutrition in rodents
56 [4–7].

57

58 Animals raised in large litters experience alterations in leptin and ghrelin
59 secretion and changes in energetic metabolism that include reduced expression of

57 glucose transporters in cardiac and skeletal muscle and increased insulin resistance in
58 key organs controlling control glucose homeostasis in the liver, muscle and adipose
59 tissue [8–11].

60 Underfeeding during suckling can modify offspring's oxidative status, resulting
61 in increased levels of lipid peroxidation, decreased activity of superoxide dismutase
62 (SOD), and increased activity of catalase (CAT), which indicates a vulnerability of
63 these enzymes to the stressful conditions imposed by large litters. The mechanism of
64 these modifications is not yet known [12].

65 Maternal nutrition during gestation and lactation plays a key role in the
66 metabolic programming of offspring. Moreover, the quantity and quality of maternal
67 dietary fat intake have profound health implications during and after pregnancy.
68 Maternal consumption of omega-3 polyunsaturated fatty acids (PUFA ω -3) has been
69 shown to benefit offspring development and could prevent obesity, insulin resistance,
70 and cardiovascular disease through epigenetic regulation [11,13].

71 Plants are an important nutritional source of alpha-linolenic acid (ALA), which
72 is elongated to form the ω -3 eicosapentaenoic acid (EPA) and docosahexaenoic acid
73 (DHA) [14]. In the recent years, chia seeds have attracted increasing attention in human
74 health and nutrition due to their higher content of ALA (55-66%). Furthermore, they are
75 an important source of protein, dietary fiber, minerals (including iron and calcium), and
76 bioactive compounds (such as tocopherols and phenolic compounds), increasing their
77 potential benefits to human health [15,16].

78 This work aimed to evaluate the potential of maternal supplementation with chia
79 oil (2.5 g/kg or 5 g/kg), a rich vegetal source of ALA, in modulating the metabolic
80 disturbances of undernourished BALB/c mice and to verify the most effective dose.

81 **2. MATERIALS AND METHODS**

82

83 **2.1 Maternal supplementation**

84 The chia oil was obtained from a local market (Pazze, 05071-6). Alpha linolenic acid
85 constitutes ~42,9% of its total fatty acids. The oil was tested in two different doses.
86 Mice were divided into three experimental groups (two groups given varying doses of
87 chia oil and no one group without supplementation). The chia oil (ChO) was
88 administered to female mice by oral gavage, at a dose of 2.5g/kg or 5g/kg of body
89 weight, from mating to weaning of the offspring (21 days after birth). The total
90 supplementation time was between 38 and 42 days.

91

92 **2.2 Animals and experimental design**

93 All animal procedures were approved by the local ethical committee for animal
94 research (CEUA/UFLA/050/2019). Thirty BALB/c mice (twenty-two females and eight
95 males, 45-55 days old) were obtained from the Animal Care Center of the Federal
96 University of Lavras (UFLA). The animals were maintained according to the ethical
97 guidelines of the institution and had free access to water and standard chow (Nuvilab®),
98 energetic value 339 kcal/100g, carbohydrates 54g/100g, proteins 22g/100g, and lipids
99 4g/100g. Female mice were housed in groups of three animals per cage. One male was
100 placed into each female group for mating, for a period of fourteen days. After this time,
101 the male was removed, and the females remained together until the birth of offspring.
102 Three days after birth, the litters were relocated into the experimental groups. To induce
103 early postnatal undernutrition, describe by Caron et al., (2012), the original litter was
104 increased to 15-16 pups (females and males). Litters of this size composed the
105 Undernutrition (UN) and Undernutrition + Chia Oil supplementation (2.5 g/kg or 5 g/kg
106 body mass; UN 2.5 and UN 5) groups. The litters of Control group (C) groups contained

107 8-10 pups (females and males). Only male mouse pups were used in the study; thus the
108 procedure described was repeated until the minimum statistical number of mice was
109 obtained for the experimental groups. After weaning, the animals males were separated
110 from females and housed in 5 per cage. The experimental design is shown in **Figure 1**.

111 The weights of mice were measured at 21, 70 and 120 days after birth, and food
112 intake was measured weekly after the first 21 days. To calculate the percentage of body
113 weight gain, a formula was used: $(FW - IW) \times 100$, where FW represents their final
114 weight (g) at 120 days and IW represents their initial weight at 21 days. The Lee index
115 was calculated according to the formula $[\sqrt[3]{\text{weight (g)}/\text{nose-to-anus length (cm)}}]$ [49].

116 At 120 days, the animals fasted for 12 hours, anesthetized with ketamine (at 200
117 mg/kg body mass), xylazine (at 15 mg/kg body mass), and inhaled isoflurane, and
118 euthanized by cardiac exsanguination. The epididymal and retroperitoneal adipose
119 tissue and liver were collected, weighed, and stored at -80°C. Blood samples were
120 collected for analysis, and the plasma was also separated, frozen and stored in a freezer
121 at -80°C.

122

123 **2.3 Oral glucose tolerance test**

124 The oral glucose tolerance test (OGTT) was performed in mice after 70 and 120 days.
125 Mice were fasted overnight for 12 h with free access to water beforehand. Blood was
126 drawn from tail veins at 0, 30, 60, 90 and 120 min after glucose was administered by
127 oral gavage (2 g/kg body weight). Glucose levels were determined by an Accu-Chek
128 glucometer (Roche Diagnostics, Indianapolis, IN, USA) and the data were recorded as
129 mmol/L. The area under the curve (AUC) was calculated by trapezoidal approximation
130 of glucose levels over time.

131

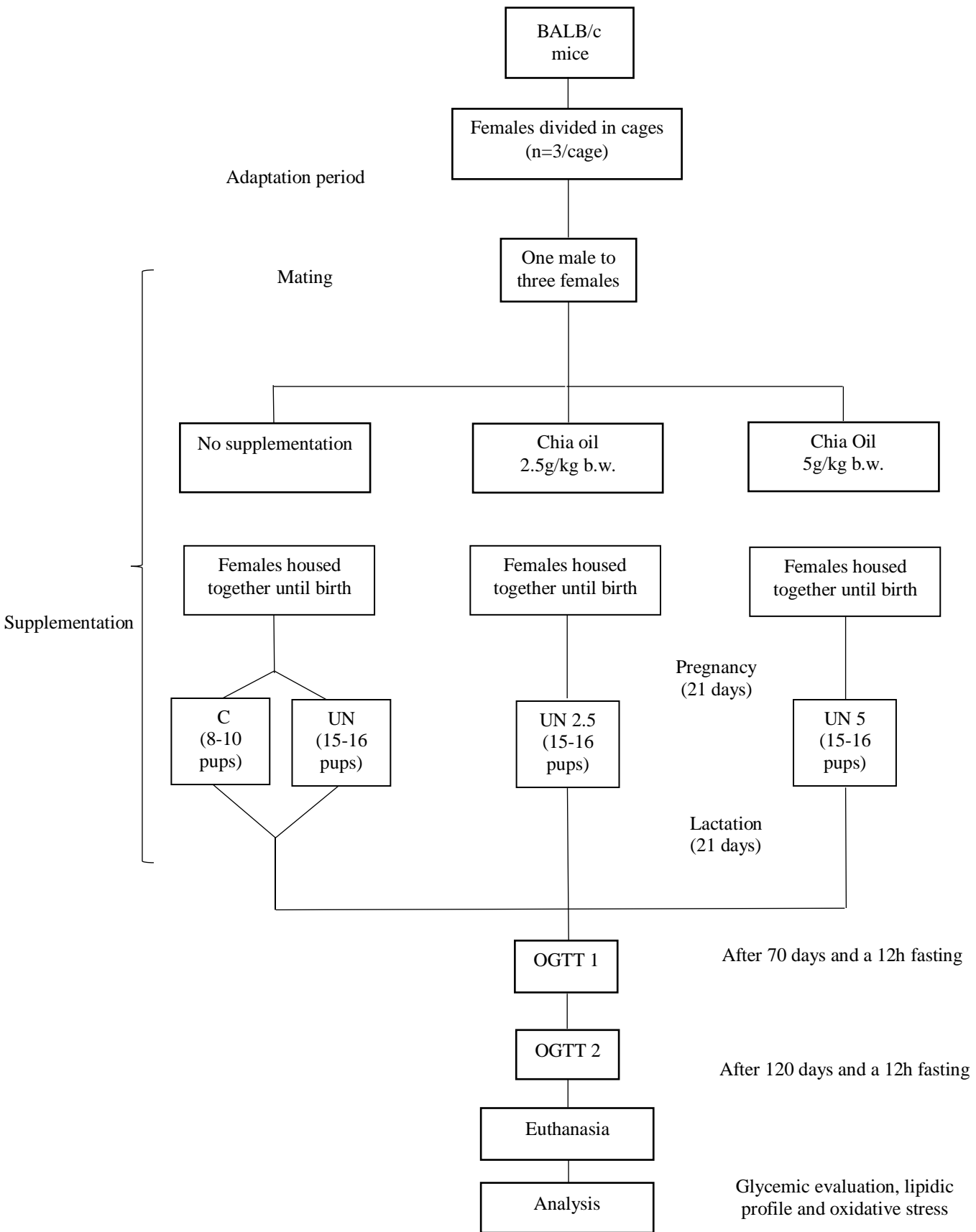


Figure 1: Experimental design – C: Control, UN: Undernutrition Group, UN 2.5: Undernutrition Group with chia oil (2.5g/kg) supplementation and UN 5: Undernutrition Group with chia oil (5g/kg) supplementation. OGTT: Oral glucose tolerance test. B.W.: body weight.

133 **2.4 Glycemic Evaluation and Lipid profile**

134 Total cholesterol, triglyceride and glucose levels were quantified after fasting by
135 colorimetric kits (Labtest, Brazil).

136

137 **2.5 Determination of hepatic lipid content**

138 Hepatic lipids were extracted by organic solvents according to Folch et al. [17].
139 Lipid extracts were dried overnight at 37 °C, and the total hepatic lipids were
140 quantified. Subsequently, lipid extracts were diluted in 500 mL of isopropanol. Total
141 cholesterol and triglyceride levels were quantified by colorimetric kits (Labtest, Brazil).

142

143 **2.6 Oxidative stress in hepatic and epididymal adipose tissue**

144 Samples of hepatic and epididymal adipose tissue (100 mg/animal) were
145 homogenized in phosphate buffered saline. The homogenate was centrifuged at 12,000
146 rpm for 10 minutes at 4 °C and the infranatant content was stored at –20 °C. Total
147 protein content was determined by the Bradford method [18] of difference analysis. A
148 standard curve was constructed from bovine serum albumin. In an ELISA plate, 10 µl of
149 the sample were pipetted in triplicate, and 250 µl of Bradford's solution were added to
150 all wells. Ten microliters of H₂O served as the blank. The reading was taken in a
151 spectrophotometer at 630 nm.

152

153 **2.7 Peroxidation Assay**

154 Lipid peroxidation was determined by the Thiobarbituric Acid Reactive
155 Substances (TBARS) technique according to Wallin et al. [19], by adding 0.5 mL
156 TBARS solution (15 g trichloroacetic acid and 0.375 g thiobarbituric acid) to 6.25 mL
157 of 4.0 M HCl. Tubes were heated at 100 °C for 15 min, cooled, and centrifuged after the
158 addition of 0.75 mL butanol. The absorbance was measured at 535 nm. The
159 concentration of TBARS was expressed as nmol of malondialdehyde (MDA)/mg of
160 protein.

161

162 **2.8 Hydroperoxide Assay**

163 Hydroperoxide concentrations were measured following the method of Banerjee
164 et al. [20]. In brief, ferrous ions (Fe^{+2}) were oxidized to ferric ions (Fe^{+3}) under acidic
165 conditions by hydroperoxides, which then bound with the xylenol orange present in the
166 reagent to form a colored complex. The absorption maximum was 550 nm. Results were
167 expressed as $\mu\text{mol}/\text{mg}$ of protein.

168

169 **2.9 Catalase activity**

170 Catalase (CAT) activity was measured according to the method described by Aebi
171 [21]. The enzyme activity was determined by the consumption of H_2O_2 at 240 nm. Each
172 assay received 100 μL sample, along with 2000 μL phosphate buffer and 50 μL of 0.3
173 M H_2O_2 . CAT activity was then calculated using the following equation: $(\text{abs}_{0\text{seg}} -$
174 $\text{abs}_{60\text{seg}}/0.1) \times \text{dilution}/\text{mg}$ protein. Results were expressed as $\Delta\text{E}/\text{min}/\text{mg}$ of protein.

175

176 **2.10 Superoxide dismutase assay**

177 Superoxide dismutase (SOD) was quantified by inhibition of auto-oxidation at
178 an absorbance of 550 nm. This method involves the generation of superoxide by

179 pyrogallol autoxidation and the inhibition of the superoxide-dependent reduction of the
180 tetrazolium dye MTT [3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide].
181 The reaction was stopped by the addition of 150 μ L dimethyl sulfoxide (DMSO).
182 Results are expressed as U SOD/mg of protein/mL.

183

184 **2.11 Statistical analysis**

185 Shapiro-Wilk tests of normality were conducted before the data were analyzed.
186 Differences between groups were evaluated by one-way analysis of variance with post
187 hoc comparisons (Bonferroni's test). All statistical analyses were conducted using
188 GraphPad Prism version 8.0 software. Data are reported as the mean \pm S.E.M. The
189 significance level was set at $p < 0.05$.

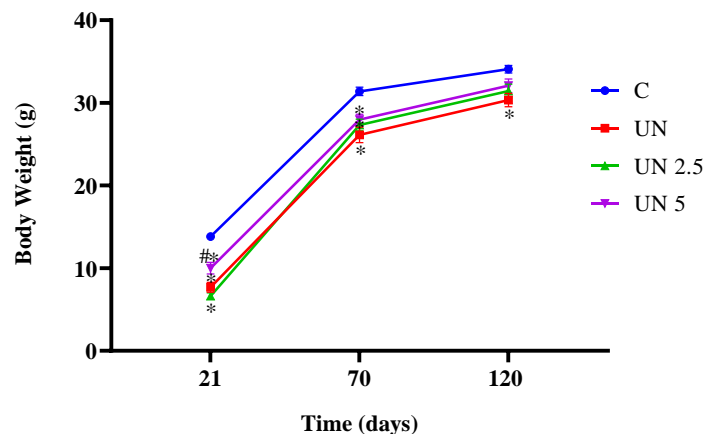
190

191 **3. RESULTS**

192 **3.1 Anthropometric parameters**

193 The animals were weighed at 21 and 70 days. The three undernutrition groups
194 (UN, UN 2.5, and UN 5) had lower body weights ($p < 0.05$) than the Control group. The
195 UN 5 group had higher weights than the UN 2.5 group ($p = 0.003$) at 21 days. At 120
196 days, only the UN group had lower body weights than the Control group ($p < 0.05$). The
197 body weight curve is presented in Figure 2.

198



199

200 **Figure 2: Body weight at 21, 70, and 120 days.** C: Control (n=9), UN: Undernutrition
 201 Group (n=5), UN 2.5: Undernutrition Group with chia oil supplementation (2.5g/kg)
 202 (n=8) and UN 5: Undernutrition Group with chia oil supplementation (5g/kg) (n=10).
 203 The data represent the mean \pm standard error of the mean (SEM). * indicates $p < 0.05$
 204 versus C. # indicates $p < 0.05$ versus UN 2.5.

205

206 Gains in body weight were calculated, and the UN, UN 2.5 and UN 5 groups
 207 showed significant increases in body weight compared to the Control group. Among the
 208 UN groups, the UN 2.5 group gained more weight than the UN 5 group ($p < 0.05$). There
 209 was no difference in food intake between the experimental groups. None of the groups
 210 showed differences in the Lee index. The UN groups had lower epididymal adipose
 211 tissue weights than the Control group ($p < 0.05$). The UN ($p < 0.05$), UN 2.5 ($p < 0.05$) and
 212 UN 5 ($p < 0.0001$) groups had lower retroperitoneal adipose tissue weights than the
 213 Control group (Table 1). Liver weights were significantly higher in the UN groups than
 214 the Control group ($p < 0.05$).

215

216 **Table 1: Body weight (B.W.) gain (%), food intake (g/day/animal), Lee index,**
 217 **epididymal adipose tissue (EAT) (mg/g b.w.), liver (mg/g b.w.) and retroperitoneal**
 218 **adipose tissue (mg/g b.w.) weight of experimental groups.**

	C	UN	UN 2,5	UN 5
B.W. gain (%)	147.9 \pm 7.38	306.0 \pm 37.63*	377.9 \pm 29.19*	242.2 \pm 34.17* &

Food intake (g/day/animal)	5.10±0.2	5.79±0.5	6.18±0.4	5.69±0.4
Lee index at 120 days	1.13±0.01	1.06±0.02	1.08±0.03	1.09±0.03
EAT weight (mg/g B.W.)	16.32±0.95	8.52±1.19*	8.87±1.07*	8.03±1.35***
Liver weight (mg/g B.W.)	38.38±0.6	41.15±0.8*	40.82±0.6*	47.76±2.9*
Retroperitoneal adipose tissue weight (mg/g B.W.)	3.35±0.2	1.81±0.3*	2.10±0.3*	1.24±0.2***

219 *p<0.05 and ***p<0.0001 versus Control; &p<0.05 versus UN 2.5. Values presented as
 220 the mean ± SEM. Groups included the Control (C, n=9), Undernutrition (UN, n=5),
 221 Undernutrition with 2.5 g/kg chia oil supplementation (UN 2.5, n=8) and Undernutrition
 222 with 5g/kg chia oil supplementation (UN 5, n=10).

223

224 3.2 Metabolic parameters

225 The fasting glucose and lipid profile of the experimental groups are presented in
 226 Table 2. The UN 5 group showed higher values of fasting glucose when compared to
 227 the Control group (p<0.05). No differences were observed among the groups in plasma
 228 triglycerides and total cholesterol. Only the UN 5 group had lower total hepatic lipids
 229 than Control group (p=0.0008), UN group (p=0.04) and UN 2.5 group (p=0.005). The
 230 hepatic cholesterol and triglycerides levels were not different between the groups.

231 **Table 2: Fasting glucose and lipidic profile of experimental groups.**

	C	UN	UN 2.5	UN 5
<i>Blood Plasma</i>				
Fasting Glucose (mmol/L)	3.7±0.3	5.5±1.2	5.0±0.7	6.8±0.6*
Triglycerides (mmol/L)	0.7±0.1	0.9±0.2	0.7±0.1	1.2±0.2
Total cholesterol (mmol/L)	1.6±0.2	2.0±0.3	1.7±0.3	2.0±0.4
<i>Hepatic Lipids</i>				
Total lipids (mg of lipids/g of liver)	64.1±3.0	55.7±6.4	59.1±7.5	27.8±6.9*#&
Cholesterol hepatic (mmol/L)	3.8±0.4	3.7±0.1	3.8±0.6	3.8±1.0
Triglycerides hepatics (mmol/L)	1.9±0.2	1.8±0.3	2.6±0.1	1.4±0.2

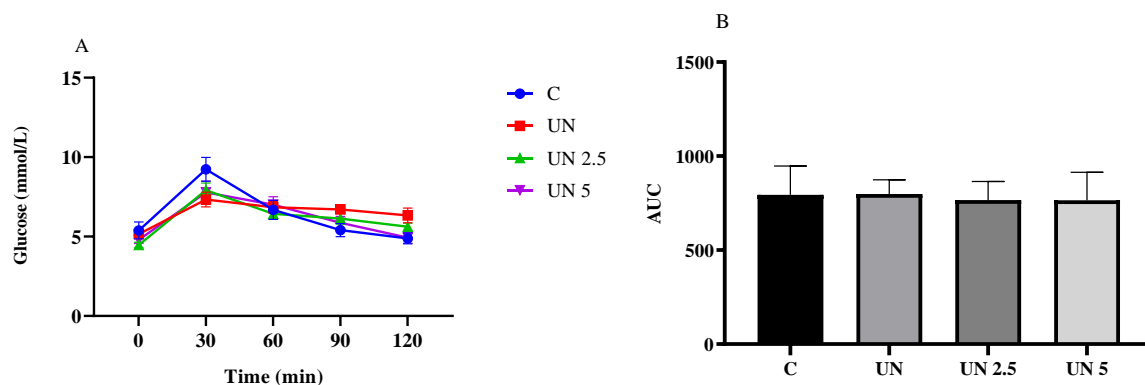
232 * $p < 0.05$ versus control; # $p < 0.05$ versus UN; & $p < 0.05$ versus UN 2.5. Values presented
233 as mean \pm SEM.

234

235 At 70 days and 120 days, the oral glucose tolerance test (OGTT) was conducted
236 to assess glucose tolerance (Figure 3). There were no differences in the glycemic curve
237 of the groups at 70 days. At 120 days, a point-by-point analysis of the glycemic curve
238 revealed that the UN group had higher glycemic values at 30 and 60 minutes than the C
239 group ($p < 0.05$). The UN 2.5 and UN 5 groups presented lower glycemic curves at 30,
240 60, 90, and 120 minutes compared to those of the UN group ($p < 0.05$). When compared
241 to the Control group, the UN 2.5 and UN 5 groups had lower glycemic values at 90 and
242 120 minutes ($p < 0.05$). The UN 2.5 and UN 5 groups had a smaller area under the curve
243 (AUC) at 120 days than the UN group ($p < 0.05$).

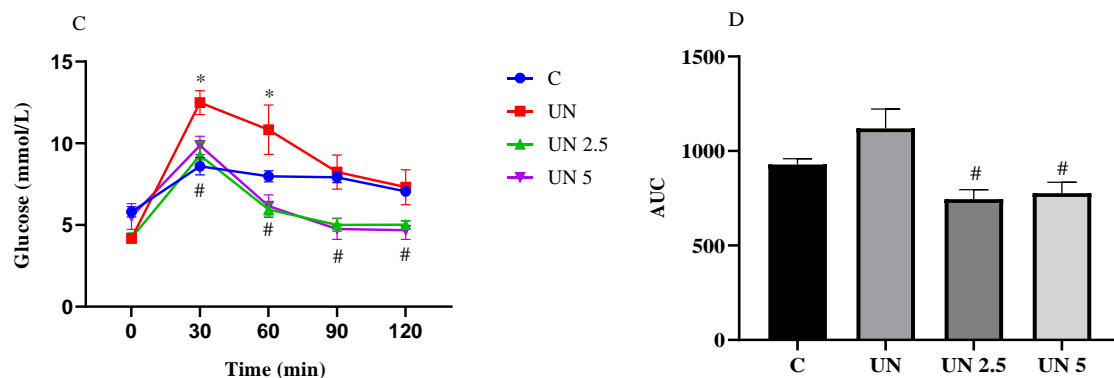
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249 **Figure 3: Glycemic curve (mmol/L) vs. time (min) from the oral glucose tolerance**
 250 **test (OGTT) and the AUC at 70 (A-B) and 120 (C-D) days.** Groups included the
 251 Control (C, n=9), Undernutrition (UN, n=5), Undernutrition with 2.5 g/kg chia oil
 252 supplementation (UN 2.5, n=8) and Undernutrition with 5g/kg chia oil supplementation
 253 (UN 5, n=10). Values presented as mean \pm SEM. * p <0.05 versus C. # p <0.05 versus UN.

254

255

256 The UN 5 group showed lower TBARS in hepatic tissue than the C ($p=0.04$),

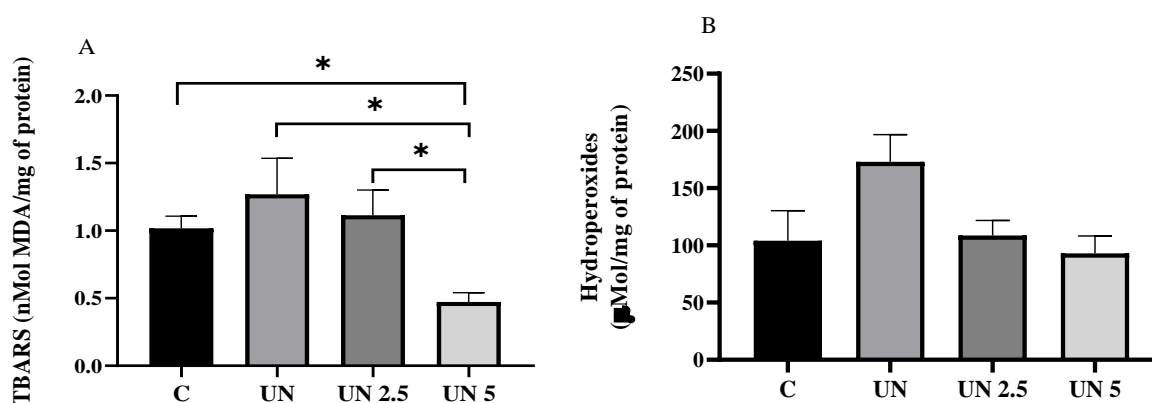
257 UN ($p=0.007$), and UN 2.5 ($p=0.02$) groups. No differences among the groups were

258 found in hydroperoxide and SOD content in the liver. The UN 2.5 group had higher

259 catalase activity in the liver than the C ($p=0.02$) and UN 5 ($p=0.0001$) groups (Figure

260 4).

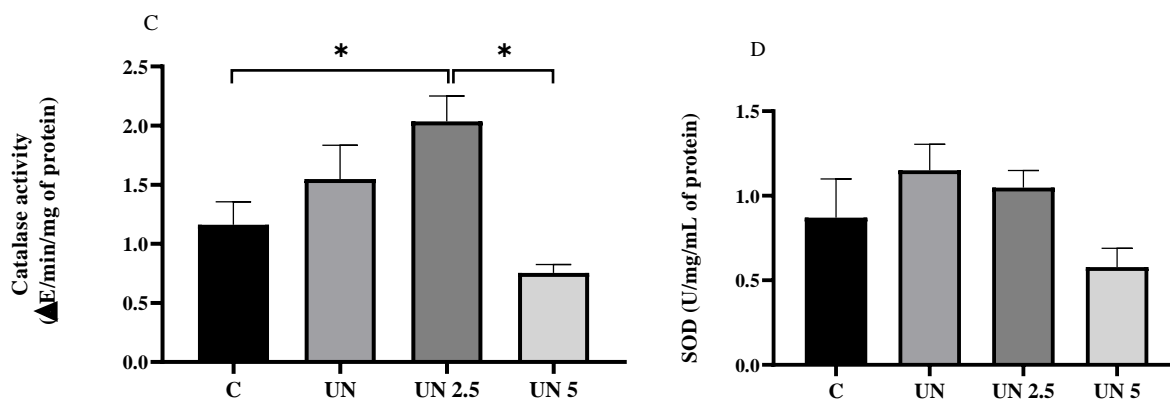
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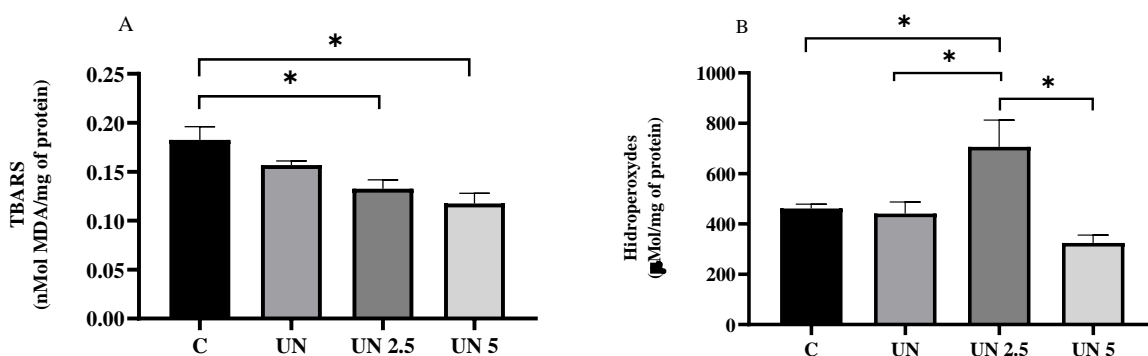
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266 **Figure 4: Oxidative stress in the liver. Determination of TBARS (A),**
 267 **hydroperoxides (B), the activity of antioxidant enzyme CAT (C), and SOD (D) in**
 268 **the liver, normalized by protein concentration. *p<0.05.** Groups included the Control
 269 (C, n=9), Undernutrition (UN, n=5), Undernutrition with 2.5 g/kg chia oil
 270 supplementation (UN 2.5, n=8) and Undernutrition with 5g/kg chia oil supplementation
 271 (UN 5, n=10).

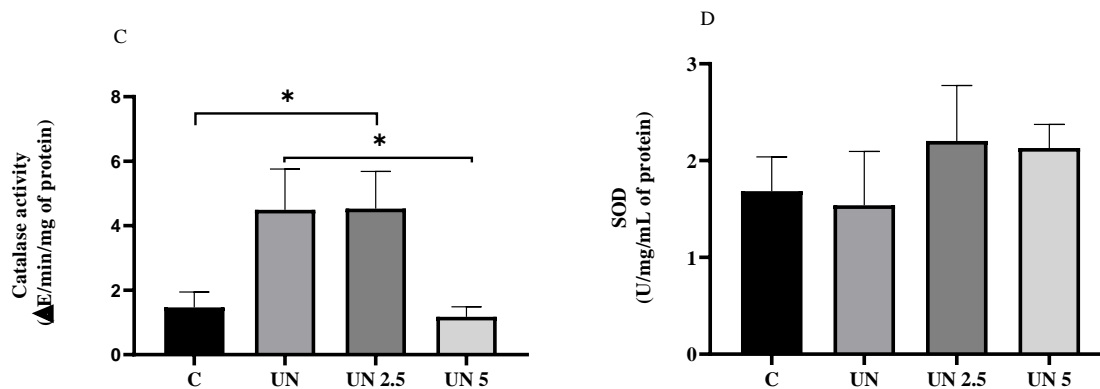
272

273 This study also analyzed the oxidative stress in epididymal adipose tissue
 274 (Figure 5). The TBARS concentrations were lower in the UN 2.5 (p=0.03) and UN 5
 275 (p=0.003) groups than the Control group. The levels of hydroperoxides were higher in
 276 the UN 2.5 group than the C, UN and UN 5 groups (p<0.05). The UN 2.5 group had
 277 higher CAT activity than the C group (p<0.05), and the UN 5 group had lower CAT
 278 activity than the UN (p=0.005) group. No differences in the SOD content in the liver
 279 were observed.

280



281



282

283 **Figure 5: Oxidative stress in the epididymal adipose tissue (EAT). Determination**
 284 **of TBARS (A), hydroperoxides (B), the activity of antioxidant enzyme CAT (C),**
 285 **and SOD (D) in the EAT, normalized by protein concentration. *p<0.05. Groups**
 286 **included the Control (C, n=9), Undernutrition (UN, n=5), Undernutrition with 2.5 g/kg**
 287 **chia oil supplementation (UN 2.5, n=8) and Undernutrition with 5g/kg chia oil**
 288 **supplementation (UN 5, n=10).**

289

290 4. DISCUSSION

291 The present study evaluated the effect of maternal supplementation with chia oil
 292 (2.5 or 5g/kg body weight) on the metabolic parameters of mice reared in large litters
 293 and verified the most effective dose.

294 Our results demonstrate that postnatal undernutrition results in decreased body
 295 weight early in life. Despite their higher weight gain, undernourished offspring did not
 296 reach the body weight of the Control group, which aligns with previous findings
 297 [7,8,22,23].

298 The most abundant lipids in milk are triglycerides. During the suckling period,
 299 there is a reduction in milk triglyceride content, in addition to a competition-driven
 300 reduction in milk consumption by mice reared in large litters. This undernutrition causes
 301 metabolic and endocrine disturbances in offspring, such as reductions in plasma
 302 glucose, triglyceride, insulin, and leptin concentrations [22,24].

303 Although the Lee index found no differences between groups, the weight of
304 epididymal and retroperitoneal adipose tissue was relatively low in the UN groups, and
305 especially lower in the UN 5 group than the Control group. Kozak et al. [25] have
306 demonstrated that undernourished animals have smaller adipocytes, indicating reduced
307 lipid accumulation. In rodents, the development of white adipose tissue occurs
308 exclusively during the postnatal period, which is the period of lactation. Additionally,
309 maternal dietetic supplementation with ALA (7g/100g of diet of linseed oil) has been
310 linked with lower fat accumulation, which is seen both in lower fat mass and in reduced
311 adipocyte size in offspring. ALA has been suggested to promote alterations in gene
312 expression involved in the regulation of fat oxidation and adipogenesis in adipose tissue
313 and the suppression of adipocyte differentiation [26–28].

314 Previous studies have found alterations in body composition, such as
315 redistribution of fat, decreases of fat mass, and increases of lean mass, in animals
316 supplemented with ALA derived from chia oil [29,30]. Moreover, ALA decreased body
317 weight gain and liver fat accumulation in the offspring of mothers supplemented with
318 6g/100g dietary flaxseed oil [31]. Similar results were found in this study, as offspring
319 from dams supplemented with chia oil had lower weight gain, especially offspring from
320 dams supplemented with 5g/kg b.w. ChO.

321 The UN 2.5 and UN 5 groups had a lower AUC at 120 days than the UN group,
322 although the UN 5 group exhibited higher fasting glucose than the Control group.
323 López-Soldado et al. [22] previously demonstrated that undernourished animals exhibit
324 a normal glucose tolerance, despite a reduction in glucose-stimulated insulin secretion.

325 Enes et al. [32] found that chia oil (ChO) positively regulated the mRNA of
326 insulin receptors. Furthermore, the phenolic compounds of ChO seem to contribute to
327 the control of glucose by decreasing gluconeogenesis. Souza et al. [33] observed

328 improved glucose responses in Swiss rats fed with a high-fat diet and supplemented
329 with 1.5g/kg b.w. ChO for six weeks. Poudyal et al. [29] supplemented Wistar rats with
330 ChO at 30mL/kg of diet for eight weeks and found improvements in glucose tolerance
331 and insulin sensitivity without changes in lipid plasma. Moreover, another study
332 supplemented rat diet with 10% flaxseed oil and observed greater reductions in hepatic
333 mRNA expression of PPAR- γ , suggesting that ALA may activate the PPAR- γ
334 dependent pathway to alter liver lipid metabolism and to increase insulin sensitivity
335 [20].

336 Maternal supplementation with PUFA ω -3 during gestation and lactation has
337 been reported to enhance the activities of citrate synthase, isocitrate dehydrogenase, and
338 α -ketoglutarate dehydrogenase in the liver mitochondria of adult offspring. These
339 enzymes are key enzymes of the tricarboxylic acid cycle (TCA), and this increase of
340 enzymatic activity supplies evidence of improved TCA cycle efficiency . The
341 improvement of mitochondrial function by PUFA ω -3 supplementation prevents insulin
342 resistance through decreasing glycemia. Another proposed mechanism is that omega-3
343 from maternal supplementation improves glucose metabolism in offspring by increasing
344 the expression of genes related to fatty acid oxidation (CPT1a and Acox1) and
345 glycolysis/gluconeogenesis (GPD1) and by reducing the expression of genes related to
346 the synthesis of fatty acids (Acy and Scd1) in offspring, resulting in higher catabolism
347 of triglycerides and lower hepatic synthesis of fatty acids. In addition, omega-3 was
348 reported to reduce pyruvate kinase activity, which suggests an increase in glucose
349 oxidation [34,35].

350 In this study, we observed that TBARS decreased in the liver and the epididymal
351 adipose tissue of undernourished animals whose mothers were supplemented with 5g/kg
352 b.w. ChO. Previous works have demonstrated that omega-3 supplementation decreases

353 levels of MDA, which is an end product of lipid peroxidation and a marker of oxidative
354 stress. It was proposed that omega-3 alters the composition of prostaglandins, as omega-
355 3 is a potent inhibitor of the arachidonic acid prostaglandin production pathway.
356 Arachidonic acid has inflammatory properties. Moreover, some works have shown that
357 omega-3 inhibits the activity of the cyclooxygenase-2 (COX-2) enzyme, which could
358 explain the effect of PUFA on reduction of MDA because COX-2 produces
359 inflammatory and oxidative prostaglandins that could cause lipid peroxidation [36–41].

360 The UN 2.5 group showed increased CAT activity in both tissues analyzed,
361 despite presenting higher hydroperoxide content in the epididymal tissue. Earlier work
362 has established that hydroperoxides are absent in normal plasma due to the presence of
363 degradative systems such as catalase [42]. Despite improving the activity of this
364 antioxidant enzyme, ChO supplementation seems insufficient to decrease the presence
365 of this stress marker.

366 Rincón-Cervera et al. [43] provided 21 days of chia oil (ALA 63%)
367 supplementation and observed high antioxidant enzyme (SOD, CAT, glutathione
368 peroxidase, and glutathione reductase) activity in the liver. They also found that the
369 higher the content of dietetic ALA, the higher the hepatic conversion of EPA and DHA.
370 These results suggest that ALA supplementation in large dosages could regulate the
371 activity of antioxidant enzymes.

372 Previous works have proposed that omega-3, through activation of PPAR- α ,
373 could activate the NF- κ B pathway, which is responsible for controlling gene expression
374 of various antioxidant enzymes. The non-enzymatic peroxidation of omega-3 generates
375 a product called J3-isoprostanes, which leads to the expression of several antioxidant
376 enzymes in the liver, the formation of glutathione, and a decrease in lipid peroxidation
377 rates [44,45].

378 Previous evidence has demonstrated that 15-30 days of supplementation with
379 10-24% ALA in dietary lipids is sufficient to increase levels of ALA and
380 eicosapentaenoic acid (EPA) in the bloodstream; in this way, the enrichment of plasma
381 lipids by the consumption of ALA influence the quality of lipoproteins synthesized by
382 the liver (VLDL, HDL, LDL) and the peripheral distribution of ALA by increasing the
383 bioaccessibility of PUFA n-3 in the body [48].

384 Enrichment of the maternal diet with PUFA ω -3 was associated with higher ω -3
385 PUFA content in the liver of offspring. Moreover, in diabetic mothers (Wistar rats),
386 supplementation with PUFA ω -3 (2.5%) during pregnancy and lactation can prevent
387 disturbances in the lipid profile and antioxidants of the liver in macrosomic offspring
388 [46,47].

389 More works are required to evaluate the antioxidant profile of these
390 undernourished mice, such as glutathione reductase and SOD, and to assess the area
391 under the curve of insulin and leptin levels, which show alterations in this experimental
392 model.

393 According to the findings of the present work, maternal supplementation with
394 ChO seems to attenuate some metabolic alterations in undernourished mice, such as
395 weight gain, glucose tolerance, and hepatic and epididymal levels of TBARS,
396 hydroperoxides, and CAT activity. Maternal supplementation of ChO at a dose of
397 2.5g/kg b.w. promoted improvement in glucose tolerance and CAT activity. On the
398 other hand, maternal supplementation with 5g/kg b.w. decreased weight gain, improved
399 glucose tolerance and markers of oxidative stress but was insufficient to increase the
400 activity of antioxidant enzymes and reduced the content of hepatic lipids; therefore, this
401 dose had a slight advantage in attenuating metabolic disturbances. However, further

402 research is necessary, as there is a lack of concrete evidence regarding maternal
403 supplementation with ALA on undernourished offspring and their metabolic outcomes.

404

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409

410 **CONFLICT OF INTEREST**

411 None.

412

413 **5. REFERENCES**

414 [1] Gluckman PD, Hanson MA, Beedle AS. Early life events and their
415 consequences for later disease: A life history and evolutionary perspective. *Am J Hum*
416 *Biol* 2007;19:1–19. <https://doi.org/10.1002/ajhb.20590>.

417 [2] Kayser BD, Goran MI, Bouret SG. Perinatal Overnutrition Exacerbates Adipose
418 Tissue Inflammation Caused by High-Fat Feeding in C57BL / 6J Mice. *PLoS One*
419 2015;1–15. <https://doi.org/10.1371/journal.pone.0121954>.

420 [3] Levin BE. Metabolic imprinting: Critical impact of the perinatal environment on
421 the regulation of energy homeostasis. *Philos Trans R Soc B Biol Sci* 2006;361:1107–21.
422 <https://doi.org/10.1098/rstb.2006.1851>.

423 [4] Debarba LK, Marangon PB, Borges BC, Veida-silva H, Venâncio JC, Almeida-
424 pereira G, et al. Neonatal nutritional programming induces gliosis and alters the
425 expression of T-cell protein tyrosine phosphatase and connexins in male rats. *Horm*
426 *Behav* 2020;120:1–12. <https://doi.org/10.1016/j.yhbeh.2020.104690>.

427 [5] Bulfin LJ, Clarke MA, Buller KM, Spencer SJ. Anxiety and hypothalamic-
428 pituitary-adrenal axis responses to psychological stress are attenuated in male rats made
429 lean by large litter rearing. *Psychoneuroendocrinology* 2011;36:1080–91.
430 <https://doi.org/10.1016/j.psyneuen.2011.01.006>.

- 431 [6] Habbout A, Li N, Rochette L, Vergely C. Postnatal Overfeeding in Rodents by
432 Litter Size Reduction Induces Major Short- and Long-Term. *J Nutr* 2013;553–62.
433 <https://doi.org/10.3945/jn.112.172825.embryonic>
- 434 [7] Ferguson DP, Monroe TO, Heredia CP, Fleischmann R, Rodney GG, Taffet GE,
435 et al. Postnatal undernutrition alters adult female mouse cardiac structure and function
436 leading to limited exercise capacity. *J Physiol* 2019;597:1855–72.
437 <https://doi.org/10.1113/JP277637>.
- 438 [8] Marangon PB, Mecawi AS, Antunes-Rodrigues J, Elias LLK. Perinatal over-
439 and underfeeding affect hypothalamic leptin and ghrelin neuroendocrine responses in
440 adult rats. *Physiol Behav* 2020;215:112793.
441 <https://doi.org/10.1016/j.physbeh.2019.112793>.
- 442 [9] Sarmento V, Vasconcelos D, Salgueiro S, Carlos R, Guedes A, Carneiro B, et al.
443 *Neuroscience Letters* Large litters rearing changes brain expression of GLUT3 and
444 acetylcholinesterase activity in adult rats. *Neurosci Lett* 2012;525:34–8.
445 <https://doi.org/10.1016/j.neulet.2012.07.054>.
- 446 [10] Solis AJ De, Baquero AF, Bennett CM, Grove KL, Zeltser LM. Postnatal
447 undernutrition delays a key step in the maturation of hypothalamic feeding circuits. *Mol*
448 *Metab* 2016;5:198–209. <https://doi.org/10.1016/j.molmet.2016.01.003>.
- 449 [11] Ong TP, Ozanne SE. Developmental programming of type 2 diabetes: early
450 nutrition and epigenetic mechanisms. *Curr Opin Clin Nutr Metab Care* 2015;18:354–60.
451 <https://doi.org/10.1097/MCO.000000000000177>.
- 452 [12] dos Santos-Júnior E, Gonçalves-Pimentel C, De Araujo L, Da Silva T, de
453 Melo-Júnior M, Moura-Neto V, et al. Malnutrition increases NO production and
454 induces changes in inflammatory and oxidative status in the distal colon of lactating
455 rats. *Neurogastroenterol Motil* 2016;1204–16. <https://doi.org/10.1111/nmo.12820>.
- 456 [13] Leikin-frenkel AI. Is there A Role for Alpha-Linolenic Acid in the Fetal
457 Programming of Health ? *J Clin Med* 2016;5:1–10. <https://doi.org/10.3390/jcm5040040>.
- 458 [14] Niculescu MD, Lupu DS, Craciunescu CN. Perinatal manipulation of α -linolenic
459 acid intake induces epigenetic changes in maternal and offspring livers. *FASEB J* 2013.
460 <https://doi.org/10.1096/fj.12-210724>.
- 461 [15] Ayerza R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.)
462 from five northwestern locations in Argentina. *J Am Oil Chem Soc* 1995;72:1079--
463 1081. <https://doi.org/10.1007/BF02660727>

- 464 [16] Fernández-lópez J, Lucas-gonzález R, Viuda-martos M, Sayas-barberá E, Pérez-
465 alvarez JA. Chia Oil Extraction Coproduct as a Potential New Ingredient for the Food
466 Industry : Chemical , Physicochemical , Techno-Functional and Antioxidant Properties.
467 Plant Foods Hum Nutr 2018;73:130–6. [https://doi.org/https://doi.org/10.1007/s11130-](https://doi.org/https://doi.org/10.1007/s11130-018-0670-5)
468 [018-0670-5](https://doi.org/https://doi.org/10.1007/s11130-018-0670-5).
- 469 [17] Folch J, Less M, Stanley G. A simple method for the isolation and purification
470 of total lipides from animal tissue. J Biol Chem 1957;226:497–509.
471 [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5).
- 472 [18] Bradford MM. A rapid and sensitive method for the quantitation of microgram
473 quantities of protein utilizing the principle of protein-dye binding. Anal Biochem
474 1976;72:248–54. <https://doi.org/10.1006/abio.1976.9999>.
- 475 [19] Wallin B, Rosengren B, Shertzer HG, Camejo G. Lipoprotein Oxidation and
476 Measurement of Thiobarbituric Acid Reacting Substances Formation in a Single
477 Microtiter Plate: Its Use for Evaluation of Antioxidants. Anal Biochem 1993;208:10–5.
- 478 [20] Mohammadi-Sartang M, Mazloom Z, Raeisi-Dehkordi H, Barati-Boldaji R,
479 Bellissimo N, Totosy de Zepetnek JO. The effect of flaxseed supplementation on body
480 weight and body composition: a systematic review and meta-analysis of 45 randomized
481 placebo-controlled trials. Obes Rev 2017;18:1096–107.
482 <https://doi.org/https://doi.org/10.1111/obr.12550>.
- 483 [21] Banerjee D, Kumar PA, Kumar B, Madhusoodanan UK, Nayak, Jacob J.
484 Determination of absolute hydrogen peroxide concentration by spectrophotometric
485 method. Curr Sci 2002;83:1193–4.
- 486 [22] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
487 [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3).
- 488 [23] López-Soldado I, Munilla MA, Herrera E. Long-term consequences of under-
489 nutrition during suckling on glucose tolerance and lipoprotein profile in female and
490 male rats. Br J Nutr 2006;96:1030–7. <https://doi.org/10.1017/BJN20061949>.
- 491 [24] Caron E, Ciofi P, Prevot V, Bouret SG. Alteration in neonatal nutrition causes
492 perturbations in hypothalamic neural circuits controlling reproductive function. J
493 Neurosci 2012;32:11486–94. <https://doi.org/10.1523/JNEUROSCI.6074-11.2012>.
- 494 [25] Neville MC, Picciano MF. REGULATION OF MILK LIPID 1997.
- 495 [26] Kozak LP, Newman S, Chao P, Mendoza T, Koza RA. The Early Nutritional
496 Environment of Mice Determines the Capacity for Adipose Tissue Expansion by

- 497 Modulating Genes of Caveolae Structure. *PLoS One* 2010;5:e11015.
498 <https://doi.org/10.1371/journal.pone.0011015>.
- 499 [27] Buckley JD, Howe PRC. Long-Chain Omega-3 Polyunsaturated Fatty Acids
500 May Be Beneficial for Reducing Obesity—A Review. *Nutr* 2010;2.
501 <https://doi.org/10.3390/nu2121212>.
- 502 [28] Okuno M, Kajiwara K, Imai S, Kobayashi T, Honma N, Maki T, et al. Perilla
503 Oil Prevents the Excessive Growth of Visceral Adipose Tissue in Rats by Down-
504 Regulating Adipocyte Differentiation 1, 2. *Am Soc Nutr Sci* 1997;1752–7.
- 505 [29] Korotkova M, Gabrielsson B, Lönn M, Hanson L-åke, Strandvik B. Leptin
506 levels in rat offspring are modified by the ratio of linoleic to α -linolenic acid in the
507 maternal diet. *J Lipid Res* 2002;43:1743–9. [https://doi.org/10.1194/jlr.M200105-](https://doi.org/10.1194/jlr.M200105-JLR200)
508 [JLR200](https://doi.org/10.1194/jlr.M200105-JLR200).
- 509 [30] Poudyal H, Panchal SK, Ward LC, Brown L. Effects of ALA, EPA and DHA in
510 high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. *J Nutr Biochem*
511 2013;24:1041–52. <https://doi.org/10.1016/j.jnutbio.2012.07.014>.
- 512 [31] Fonte-Faria T, Citelli M, Atella G.C, Raposo HF, Zago L, de Souza T, da Silva
513 SV, Barja-Fidalgo C. Chia oil supplementation changes body composition and activates
514 insulin signaling cascade in skeletal muscle tissue of obese animals. *Nutrition*
515 2019;58:167–74. <https://doi.org/10.1016/j.nut.2018.08.011>.
- 516 [32] Hollander KS, Brami CT, Konikoff FM, Fainaru M. Dietary enrichment with
517 alpha-linolenic acid during pregnancy attenuates insulin resistance in adult offspring in
518 mice. *Arch Physiol Biochem* 2014;3455:99–111.
519 <https://doi.org/10.3109/13813455.2014.940352>.
- 520 [33] Enes BN, Moreira L de PD, Toledo RCL, Moraes ÉA, Moreira ME de C,
521 Hermsdorff HHM, et al. Effect of different fractions of chia (*Salvia hispanica* L.) on
522 glucose metabolism, in vivo and in vitro. *J Funct Foods* 2020;71.
523 <https://doi.org/10.1016/j.jff.2020.104026>.
- 524 [34] de Souza T, Vargas da Silva S, Fonte-Faria T, Nascimento-Silva V, Barja-
525 Fidalgo C, Citelli M. Chia oil induces browning of white adipose tissue in high-fat diet-
526 induced obese mice. *Mol Cell Endocrinol* 2020;507:110772.
527 <https://doi.org/10.1016/j.mce.2020.110772>.
- 528 [35] Kasbi-Chadli F, Ferchaud-Roucher V, Krempf M, Ouguerram K. Direct and
529 maternal n-3 long-chain polyunsaturated fatty acid supplementation improved
530 triglyceridemia and glycemia through the regulation of hepatic and muscle sphingolipid

- 531 synthesis in offspring hamsters fed a high-fat diet. *Eur J Nutr* 2016;55:589–99.
532 <https://doi.org/10.1007/s00394-015-0879-0>.
- 533 [36] Novak EM, Keller BO, Innis SM. Metabolic development in the liver and the
534 implications of the n-3 fatty acid supply. *Am J Physiol - Gastrointest Liver Physiol*
535 2012;302:250–9. <https://doi.org/10.1152/ajpgi.00189.2011>.
- 536 [37] Egan K.M, A. LJ, Susanne F, Beverley K, J. RD, M. SE, et al. COX-2-Derived
537 Prostacyclin Confers Atheroprotection on Female Mice. *Science* (80) 2004;306:1954–7.
538 <https://doi.org/10.1126/science.1103333>.
- 539 [38] Harizi H, Corcuff J-B, Gualde N. Arachidonic-acid-derived eicosanoids: roles in
540 biology and immunopathology. *Trends Mol Med* 2008;14:461–9.
541 <https://doi.org/https://doi.org/10.1016/j.molmed.2008.08.005>.
- 542 [39] Corey EJ, Shih C, Cashman JR. Docosahexaenoic acid is a strong inhibitor of
543 prostaglandin but not leukotriene biosynthesis. *Proc Natl Acad Sci* 1983;80:3581 LP –
544 3584. <https://doi.org/10.1073/pnas.80.12.3581>.
- 545 [40] Kobayashi N, Barnard RJ, Henning SM, Elashoff D, Reddy ST, Cohen P, et al.
546 Effect of Altering Dietary ω -6/ ω -3 Fatty Acid Ratios on Prostate Cancer Membrane
547 Composition, Cyclooxygenase-2, and Prostaglandin E₂; *Clin Cancer Res* 2006;12:4662
548 LP – 4670. <https://doi.org/10.1158/1078-0432.CCR-06-0459>.
- 549 [41] Heshmati J, Morvaridzadeh M, Marou S, Akbari A. Omega-3 fatty acids
550 supplementation and oxidative stress parameters: A systematic review and meta-
551 analysis of clinical trials. *Pharmacol Res* 2019;149:104462.
552 <https://doi.org/10.1016/j.phrs.2019.104462>.
- 553 [42] Capobianco E, Fornes D, Lorena S, Powell TL, Jansson T, Jawerbaum A.
554 Supplementation with polyunsaturated fatty acids in pregnant rats with mild diabetes
555 normalizes placental PPAR γ and mTOR signaling in female offspring developing
556 gestational diabetes. *J Nutr Biochem* 2018;53:39–47.
557 <https://doi.org/10.1016/j.jnutbio.2017.10.006>.
- 558 [43] Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body.
559 *FEBS Lett* 2000;486:10–3. [https://doi.org/https://doi.org/10.1016/S0014-](https://doi.org/https://doi.org/10.1016/S0014-5793(00)02197-9)
560 [5793\(00\)02197-9](https://doi.org/https://doi.org/10.1016/S0014-5793(00)02197-9).
- 561 [44] Rincón-Cervera MÁ, Valenzuela R, Hernandez-Rodas MC, Barrera C, Espinosa
562 A, Marambio M, et al. Vegetable oils rich in alpha linolenic acid increment hepatic n-3
563 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats.

- 564 Prostaglandins Leukot Essent Fat Acids 2016;111:25–35.
565 <https://doi.org/10.1016/j.plefa.2016.02.002>.
- 566 [45] Zuñiga J, Cancino M, Medina F, Varela P, Vargas R, Tapia G, et al. N-3 PUFA
567 Supplementation Triggers PPAR- α Activation and PPAR- α / NF- κ B Interaction :
568 Anti-Inflammatory Implications in Liver Ischemia-Reperfusion Injury. PLoS One
569 2011;6. <https://doi.org/10.1371/journal.pone.0028502>.
- 570 [46] Jr EPS, Nachbar RT, Hirabara SM. Omega-3 fatty acids differentially modulate
571 enzymatic anti-oxidant systems in skeletal muscle cells. Cell Stress Chaperones
572 2016;21:87–95. <https://doi.org/10.1007/s12192-015-0642-8>.
- 573 [47] Chechi K, Herzberg GR, Cheema SK. Maternal dietary fat intake during
574 gestation and lactation alters tissue fatty acid composition in the adult offspring of
575 C57Bl / 6 mice. Prostaglandins Leukot Essent Fat Acids 2010;83:97–104.
576 <https://doi.org/10.1016/j.plefa.2010.06.001>.
- 577 [48] Soulimane-Mokhtari NA, Guermouche B, Saker M, Merzouk S, Merzouk H,
578 Hichami A, et al. Serum lipoprotein composition, lecithin cholesterol acyltransferase
579 and tissue lipase activities in pregnant diabetic rats and their offspring receiving
580 enriched n-3 PUFA diet. Gen Physiol Biophys 2008;27:3–11.
- 581 [49] Couëdelo L, Buaud B, Abrous H, Chamekh-coelho I, Majou D, Boué-vaysse C.
582 Effect of increased levels of dietary α -linolenic acid on the n -3 PUFA bioavailability
583 and oxidative stress in rat. Br J Nutr 2021:1–14.
584 <https://doi.org/10.1017/S0007114521002294>.
- 585 [50] Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F, et
586 al. Anthropometrical parameters and markers of obesity in rats. Lab Anim
587 2007;41:111–9. <https://doi.org/10.1258/00236770779399518>.