UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO ACADÊMICO DE VITÓRIA

ALINE MARIA NUNES DE LIRA GOMES BLOISE

EFEITOS DA INGESTÃO DE UMA DIETA COM ALTO TEOR DE ÁCIDOS GRAXOS SATURADOS ENRIQUECIDA COM ÔMEGA-3 DURANTE A GESTAÇÃO E LACTAÇÃO SOBRE OS PARÂMETROS RESPIRATÓRIOS E METABÓLICOS DE RATOS JOVENS

Vitória de Santo Antão

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Dissertação apresentada à Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, como requisito para a obtenção do título de Mestre em Nutrição, Atividade Física e Plasticidade Fenotípica.

Área de concentração: Bases Experimentais e Clínicas da Plasticidade Fenotípica

Orientador: Prof. Dr. João Henrique da Costa Silva

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RESUMO

O consumo materno de dieta rica em ácidos graxos saturados durante a gestação e lactação está associado ao aumento do risco de desenvolvimento de doenças crônicas não transmisssiveis na vida adulta da prole. Em contrapartida, o consumo de uma dieta enriquecida com ômega-3 durante o período de gestação e lactação reduz a adiposidade, melhora a sensibilidade à insulina, tem efeito anti-inflamatório e pode desempenhar um papel hipotensor na prole. Diante dessas evidências, neste estudo testou-se a hipótese de que ratos provenientes de mães que foram submetidas à dieta rica em ácidos graxos saturados no período de gestação e lactação apresentam alterações na murinometria, dislipidemia, hiperglicemia, intolerância à glicose, diminuição na sensibilidade à insulina, desbalanço oxidativo hepático e prejuízo em parâmetros ventilatórios. Além disso, testamos a hipótese de que o enriquecimento com ômega-3, a partir do óleo de linhaça, pode interferir nestes parâmetros e impedir as alterações decorrentes do consumo de dieta com alto teor de ácidos graxos saturados. Ratos *Wistar* foram alimentados com uma dieta controle (GC; 19% de lipídeos e razão ω6:ω3=12,66) ou dieta rica em ácidos graxos saturados (HL; 33% de lipídeos e razão ω6:ω3=21,22) ou dieta com alto teor de ácidos graxos saturados enriquecida com ômega-3, a partir do óleo de linhaça (HLW3; 33% de lipídeos e razão ω6:ω3=9,45) durante a gestação e lactação. Após o desmame, os animais passaram a receber dieta padrão de biotério. Foram avaliadas: as medidas murinométricas, análises bioquímicas; teste de tolerância à glicose (TTG), teste de tolerância ao piruvato (TTP), teste de sensibilidade à insulina (TSI), enzimas e marcadores relacionados ao estresse oxidativo no tecido hepático e medidas de ventilação pulmonar. Nossos resultados demonstram que as mães que consumiram dieta rica em ácidos graxos saturados apresentaram maiores níveis de colesterol quando comparado ao grupo controle e HLW3. Quanto aos parâmetros bioquímicos avaliados na prole, o grupo HL apresentou dislipidemia e hiperglicemia aos 22 e 90 dias de vida, com o grupo HLW3 apresentando melhores valores no perfil lipídico e glicêmico. Os animais do grupo HL também apresentaram níveis elevados de ALT, enquanto a prole HLW3 apresentou menores níveis de ALT aos 22 dias de vida. E aos 90 dias de vida, o grupo HLW3 apresentou menores níveis de AST em relação ao grupo controle e HL. Foi verificado no grupo HL, um desequilíbrio oxidativo a nível hepático, com redução na atividade das enzimas antioxidantes GST e SOD, não sendo encontrada diferença em relação ao grupo controle e HLW3 nestas enzimas avaliadas. Desta forma, o presente estudo sugere que o consumo materno de dieta rica em ácidos saturados está associado a prejuízos em alguns parâmetros bioquímicos e ao desequilíbrio oxidativo a nível hepático, com redução de enzimas que participam do sistema

de defesa antioxidante. Além disso, nossos achados confirmam que o consumo de ômega-3, a partir do óleo de linhaça é capaz de atenuar estas alterações observadas nos animais que receberam dieta rica em ácidos graxos saturados.

Palavras-chave: Plasticidade fenotípica. Dieta hiperlipídica. Ácido alfa-linolênico. Doenças crônicas não transmissíveis.

ABSTRACT

Maternal dietary intake rich in saturated fatty acids during gestation and lactation is associated with an increased risk of developing non-transmissible chronic diseases in adult offspring. In contrast, studies have shown that consumption of an omega-3-enriched diet during gestation and lactation reduces adiposity, improves insulin sensitivity, has an anti-inflammatory action and may play a hypotensive role in the offspring. In light of this evidence, in this study tested the hypothesis that rats from dams who were submitted to the diet rich in saturated fatty acids in the gestation and lactation period present alterations in the murinometry, dyslipidemia, hyperglycemia, glucose intolerance, decrease in insulin sensitivity, hepatic oxidative imbalance and impairment in ventilatory parameters. In addition, we tested the hypothesis that the enrichment with omega-3, from flaxseed oil, can interfere in these parameters and prevent the alterations resulting from the consumption of diet with high content of saturated fatty acids. Wistar rats were fed a control diet (GC, 19% of lipids and ratio ω6:ω3 = 12, 66) or diet rich in saturated fatty acids (HL, 33% lipids and ratio ω6: ω3 = 21.22) or diet high-saturated fatty acid enriched with omega-3, from flaxseed oil (HLW3, 33% lipids and ratio ω6: ω3 = 9.45) during gestation and lactation. After weaning, the animals began to receive standard animal feed. Were evaluated: murinometric measurements; biochemical analyzes; glucose tolerance test (GTT), pyruvate tolerance test (PTT), insulin sensitivity test (IST), enzymes and markers related to oxidative stress in hepatic tissue and measurement of pulmonary ventilation. Our results demonstrate that dams who consumed a diet rich in saturated fatty acids had higher cholesterol levels when compared to the control and HLW3 groups. As for the biochemical parameters evaluated in the offspring, HL group presented dyslipidemia and hyperglycemia at 22 and 90 days of life, with the HLW3 group presenting better values in the lipid and glycemic profile. Animals from the HL group also had elevated ALT levels, whereas HLW3 offspring had lower ALT levels at 22 days of age. At 90 days of age, the HLW3 group had lower levels of AST than the control group and HL. An oxidative imbalance was observed in the HL group, with a reduction in the activity of the antioxidant enzymes GST and SOD, and no difference was found in relation to the control and HLW3 groups in these enzymes evaluated. Thus, the present study suggests that the maternal consumption of a diet rich in saturated acids is associated to impair in some parameters biochemical and imbalances oxidative to hepatic level, with reduction of enzymes that participate in the antioxidant defense system. In addition, our findings confirm that consumption of omega-3 from flaxseed oil is able to attenuate these changes observed in animals that received a diet rich in saturated fatty acids.

Keywords: Phenotypic plasticity. Hyperlipid diet. Alpha-linolenic acid. Chronic non transmissible diseases.

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LISTA DE ABREVIATURAS E SIGLAS

AIN *American Institute of Nutrition*

ALA Alpha-linolenic acid

ALT Alanina aminotransferase

AgRP Agouti-related peptide

ARA Arachidonic acid

ARC Núcleo arqueado

AST Aspartato aminotransferase

BDNF Brain Derived Neurotrophic Factor

BHE Barreira hematoencefálica

CAT Catalase

CCR2 Chemokine receptor type 2

COX Ciclooxigenase

CT Colesterol total

DHA Docosaenoic acid

DNA Deoxyribonucleic acid

DOHaD *Developmental Origins of Health and Disease*

EPA Eicosapentaenoic acid

EROS Espécies Reativas de Oxigênio

FAS Fatty acid synthase

FFA Free fatty acid

FR Frequência respiratória

GLUT-4 Glucose transporter, type 4

GSH Glutationa reduzida

GSH-Rd Glutationa redutase

GSH-PX Glutationa peroxidase

GSSG Glutationa oxidada

GPR40 G-protein-coupled receptor

HDL High density lipoprotein

HF High frequency bands

HL Hiperlipídica (com alto teor em ácidos graxos saturados)

HLW3 Hiperlipídica enriquecida com ômega 3

IL Interleucina

INF- γ Interferon Gama

JNK1 c-Jun N-terminal kinase 1

LA Linoleic acid

LDL Low-density lipoprotein

LH Low frequency bands

LOX Lipoxigenase

MaR1 Maresina R1

MDA Malondialdeído

MCP-1 Chemotactic monocyte protein

MUFA Monounsaturated fatty acids

NPY Neuropeptídeo Y

NTS Núcleo do trato solitário

PCR-as Proteína C-reativa de alta sensibilidade

PGE_2 Prostaglandina E_2

POMC Pró-opiomelanocortina

PPAR Peroxisome proliferator-activated receptor

PUFA Polyunsaturated fatty acids

PUFAS-LC Long chain polyunsaturated fatty acids

PVN Paraventricular nucleus

RNAm RNA mensageiro

RVLM Rostral ventrolateral medulla

SFA Saturated Fatty Acid

SFA- LC Long chain saturated fatty acid

SFA-MC Medium chain saturated fatty acid

SNC Sistema nervoso central

SOD Superóxido dismutase

SREBP Sterol regulatory element-binding proteins

TG Triglicerídeos

TNF-α Tumor necrosis factor-alpha

TSI Teste de sensibilidade à insulina

TTG Teste de tolerância à glicose

TTP Teste de tolerância ao piruvato

VLDL Very low density lipoprotein

VE Ventilação pulmonar

VT Volume corrente

SUMÁRIO

1 INTRODUÇÃO

Na rápida alteração do modelo de morbimortalidade, caracterizado pela transição epidemiológica, o fator nutricional tem um papel de grande importância. Com a transição nutricional, observa-se a prevalência principalmente entre a população madura e os idosos, das doenças crônicas não transmissíveis: *diabetes mellitus* tipo 2 (DM2), obesidade, hipertensão arterial sistêmica, comorbidades associadas à alimentação hipercalórica, gorduras trans, aos ácidos graxos saturados, ao consumo exacerbado do açúcar e sal, álcool, fumo, falta de atividade física e outras práticas não saudáveis relacionadas ao estilo de vida ocidental (BATISTA FILHO; BATISTA, 2010). Visto que a dieta ocidental tem como características, níveis elevados de gorduras saturadas, sódio, açúcares simples e baixos níveis de fibras (POPKIN; GORDON-LARSEN, 2004; POPKIN; ADAIR; NG, 2012).

A *Developmental Origins of Health and Disease* (DOHaD) destacou a relação entre as fases pré-concepcional, fetal e infantil precoce da vida e o desenvolvimento de obesidade e doenças metabólicas na vida adulta (VICKERS, 2014). A exposição precoce à dieta hiperlipídica, independente de obesidade materna, induz a prole ao aumento de peso e maior ingestão calórica, sem redução no consumo de leite materno, sugerindo um padrão de hiperfagia e propiciando o desenvolvimento de um fenótipo obeso (KOJIMA; CATAVERO; RINAMAN, 2016). Na prole de ratos que foram submetidas à dieta hiperlipídica durante gestação e lactação, foram verificadas alterações metabólicas, tais como hiperglicemia, hipertrigliceridemia, aumento dos níveis de LDL *(low-density lipoprotein)* e diminuição de HDL *(high density lipoprotein)* (VIDAL-SANTOS *et al.*, 2017).

O alto consumo de dieta materna rica em gordura aumenta os níveis de ácidos graxos livres circulantes e induz a ativação de vias inflamatórias, aumentando o risco de inflamação crônica na prole (GRUBER *et al.*, 2015). É evidenciado que marcadores circulantes de inflamação sistêmica, tais como proteína C-reativa de alta sensibilidade (PCR-as), *tumor necrosis factor-alpha* (TNF-α) e algumas interleucinas (IL-6, IL-1) são elevados em indivíduos com hiperlipidemia e que se correlacionam com o aumento do risco de doenças cardiovasculares (ESTEVE; RICART; FERNÁNDEZ-REAL, 2005).

É observado em ratos que sofreram um insulto nutricional, em períodos críticos da vida (gestação e lactação), uma hiperatividade simpático-respiratória e respostas quimiorreceptoras periféricas amplificadas, que precedem o início da hipertensão em ratos

jovens (BRITO ALVES *et al.*, 2014; COSTA-SILVA *et al.*, 2015; BRITO ALVES *et al.*, 2015). Evidências experimentais também demostram que as alterações no padrão respiratório, induzidas pela hipóxia, desempenham um papel fundamental no aumento da atividade simpática e da pressão arterial em ratos (ZOCCAL; MACHADO, 2011; MORAES *et al.*, 2014).

A ingestão de gorduras tem um impacto importante durante a gestação e lactação, interferindo no crescimento, desenvolvimento e saúde da prole. Embora a gravidez seja um período que requer um aumento da demanda energética, não há evidências que suportem a maior ingestão dietética de lipídios nesta condição. Também não há uma recomendação diferente para gestantes e lactantes em comparação ao recomendado para população em geral, em relação aos ácidos graxos e seus diferentes tipos [SFA (*saturated fatty acid*), trans, MUFA (*monounsaturated fatty acids*) e PUFA (*polyunsaturated fatty acids*)], com exceção quanto ao consumo dos PUFAs, *linoleic acid* (LA; 18:2), também conhecido como ômega-6 e o *alpha-linolenic acid* (ALA; 18:3), conhecido como ômega-3, e seus derivados, *eicosapentaenoic acid* (EPA; 20:5) e o *docosaenoic acid* (DHA; 22:6), considerados PUFAs de cadeia muito longa (KOLETZKO *et al.*, 2007). O *Institute of Medicine* recomenda uma AI (*Adequate Intake*) de 13g/dia de ácido linoléico (18:2) para gestantes e lactantes, 1,4g /dia de ácido α-linolênico (18:3) para gestantes e 1,3g /dia de ácido α-linolênico (18:3) para lactantes (MEDICINE, 2005). É sugerida uma recomendação de suplementação em gestantes de 200mg de ácido docosahexaenóico (DHA; 22:6), a partir de fontes seguras (ALMEIDA *et al.*, 2014). Alguns autores recomendavam a ingestão mínima em mulheres lactantes de 200mg de ácido docosahexaenóico (DHA; 22:6), seja através de fontes alimentares ou suplementação (KOLETZKO *et al.*, 2008). Estudos mais recentes recomendam uma ingestão maior em lactantes, na ordem de 600mg/dia (CARLSON *et al.*, 2013).

O consumo de uma dieta enriquecida com ômega-3 durante o período de gestação e lactação esta relacionado com a redução da adiposidade e melhora da sensibilidade à insulina na prole (KOROTKOVA *et al.*, 2002; SARDINHA *et al.*, 2012). Os ácidos graxos poli-insaturados de cadeia muito longa (*long chain polyunsaturated fatty acids* ou LC-PUFA) através de sua ligação ao PPARs (receptor ativado por proliferador de peroxissomas) podem regular o metabolismo de lipídios, atuando na melhora da expressão de genes relacionados à oxidação de ácidos graxos (KHAIRE; KALE; JOSHI, 2015).

Tem sido demonstrado também que a ingestão de ácido α-linolênico (ALA; 18:3), também conhecido como ômega-3, é capaz de desempenhar um papel hipotensor dose-

dependente em indivíduos hipertensos (HOWE, 1997) devido, principalmente, à sua ação anti-inflamatória ao diminuir a síntese de derivados do ácido araquidônico: prostaglandina E2, tromboxano A2, prostaciclina e leucotrieno B4 (KREMER, 2000; KHATIB *et al.*, 2016). Em uma meta-análise, foi encontrada uma redução significativa nos níveis de pressão arterial (entre 2.0 a 3.4 mmHg) em pacientes hipertensos que consumiram 5,6 g de ácidos graxos ômega-3 por dia (MORRIS; SACKS; ROSNER, 1993). Da mesma forma, outro estudo (APPEL *et al.*, 1993) descobriu que a pressão arterial de hipertensos não-tratados reduziu entre 3,5-5,5 mmHg com o consumo diário de 3g de ácidos graxos ômega-3. Além disso, o ácido α-linolênico (ALA; 18:3) atua na redução de mediadores inflamatórios circulantes no plasma: IL-6, TNF-α e PCR (OLLIVER *et al.*, 2016).

É verificado que o ômega-3 e seus derivados EPA e DHA, podem atuar como citoprotetores, através da regulação positiva das enzimas antioxidantes e inibição da expressão gênica pró-inflamatória (DOSSI *et al.*, 2014).

Dessa forma, este estudo justifica-se pela necessidade de investigar se o ômega-3 associado à dieta rica em ácidos graxos saturados, no período da gestação e lactação, diminui o risco para o desenvolvimento de doenças crônicas não transmissíveis na vida adulta da prole, como a DM2, obesidade e hipertensão arterial sistêmica, atenuando os efeitos deletérios sobre a murinometria, parâmetros respiratórios e balanço oxidativo, o que pode ser uma possível estratégia na prevenção dessas doenças.

2 REVISÃO DE LITERATURA

2.1 Epigenética e Plasticidade Fenotípica

A epigenética é descrita como uma alteração reversível e hereditária que atinge a expressão gênica sem alterar a sequência de DNA *(Deoxyribonucleic acid).* Com o avanço das ferramentas epigenéticas e tecnológicas, tentativas têm sido feitas para descobrir os mecanismos epigenéticos subjacentes à perturbação metabólica e inflamação crônica causada pela obesidade induzida pela dieta (YOON *et al.*, 2017). Estudos sugerem que o período fetal é o mais sensível à instalação das alterações epigenéticas, as quais podem influenciar a expressão gênica celular, o dismorfismo sexual e a predisposição de distúrbios e doenças ao longo da vida (WEST-EBERHARD, 1986; WEST-EBERHARD, 2005; BOGDARINA *et al.*, 2007).

O conceito de “thrifty phenotype” ou “fenótipo poupador” fundamenta a hipótese da origem fetal da doença no adulto, no qual o feto é capaz de se adaptar e sobreviver a um ambiente de restrição nutricional, ainda que seja preciso reduzir o seu crescimento somático (HALES; BARKER, 1992; 2001) A flexibilidade de ajustar a trajetória de crescimento em resposta às diferentes condições ambientais é denominada ‘plasticidade fenotípica’, na qual um único genótipo pode ser capaz de produzir fenótipos variados em resposta ao ambiente que se desenvolve (WEST-EBERHARD, 1989; HANSON *et al.*, 2011). Estas adaptações podem ser benéficas a curto prazo, garantindo a sobrevivência da espécie, no entanto os custos dessa adaptação, pode ser verificada a longo prazo, com o aparecimento de doenças crônicas, como diabetes tipo 2, hipertensão e doença coronariana (GLUCKMAN *et al.*, 2009).

Os estudos epidemiológicos de Barker e colaboradores foram os primeiros a mostrarem claramente a relação entre baixo peso ao nascer, usado como modelo para nutrição fetal prejudicada e riscos aumentados de doenças não transmissíveis, como diabetes e hipertensão (BARKER; OSMOND, 1986; HALES *et al.*, 1991). A "programação fetal", agora conhecida como *Developmental Origins of Health and Disease* (DOHaD), é demonstrada como a consequência de perturbações induzidas pelo meio ambiente durante o desenvolvimento, afetando não apenas os bebês pequenos para idade gestacional, mas também os grandes para idade gestacional A prevalência de distúrbios metabólicos se elevou consideravelmente nos últimos anos. Apesar dos hábitos e estilo de vida influenciarem claramente essas epidemias, há evidências crescentes que sugerem que o ambiente nutricional

materno durante os estágios críticos do desenvolvimento no útero ocasiona um maior risco de doenças metabólicas na vida adulta (ALFARADHI; OZANNE, 2011; CHAVATTE-PALMER; TARRADE; ROUSSEAU-RALLIARD, 2016).

A presença de obesidade materna e consumo de dieta hiperlipídica no período da gestação e/ou lactação predispõe a prole ao aumento de adiposidade, obesidade, diminuição no percentual de massa magra e níveis elevados de triglicerídeos, corticosterona, insulina e glicose, além de hipertensão, que são fortes fatores de risco para o desenvolvimento de síndrome metabólica na vida adulta (DESAI *et al.*, 2014).

2.2 Dieta hiperlipídica, inflamação e alterações metabólicas

A obesidade é caracterizada por uma elevação excessiva de tecido adiposo branco, em razão do aumento da ingestão de alimentos, redução do gasto energético e alterações no estilo de vida (MOKDAD *et al.*, 2003). Segundo a Organização Mundial da Saúde, no ano de 2016, mais de 1.900 milhões de adultos de 18 anos ou mais tinham sobrepeso, dos quais mais de 650 milhões eram obesos, com 39% dos adultos maiores de 18 anos classificados com excesso de peso e 13% obesos. Em relação às crianças, 41 milhões de crianças menores de cinco anos apresentavam sobrepeso ou obesidade e havia mais de 340 milhões de crianças e adolescentes (5 a 19 anos) com excesso de peso ou obesidade (WORLD HEALTH ORGANIZATION, 2016). É verificado que uma quantidade significativa de pessoas obesas são resistentes à insulina e que a obesidade está associada com inflamação sistêmica de baixo grau (BELKINA; DENIS, 2010), com o sistema imunológico relacionado no desenvolvimento da resistência à insulina (CASTOLDI *et al.*, 2017).

Em camundongos, foi observado que a obesidade induzida pela dieta materna leva a hipertrofia dos adipócitos, hipertensão e aumento dos níveis plasmáticos de insulina na prole (SAMUELSSON; MATTHEWS; ARGENTON, 2008). A obesidade materna tem uma grande influência na vida fetal intrauterina que se estende aos distúrbios metabólicos na infância, adolescência e vida adulta (CATALANO; EHRENBERG, 2006; ARMITAGE; POSTON; TAYLOR, 2008). Os adipócitos regulam os estoques de energia e secretam muitas adipocinas que têm um grande impacto na homeostase energética e na resistência à insulina (SCHERER, 2006). Estudos mostram que a obesidade materna predispõe a prole à obesidade e à síndrome

metabólica, possivelmente através da programação metabólica fetal tardia (MURABAYASHI *et al.*, 2013).

O aumento de ácidos graxos durante a obesidade podem atuar nos macrófagos, alterando a relação celular M1 (pró-inflamatória) / M2 (anti-inflamatória). Os ácidos graxos saturados estimulam o desenvolvimento de células M1, enquanto os tipos insaturados ajudam a elevar as células M2 no tecido adiposo. Durante o desenvolvimento da obesidade e hipertrofia dos adipócitos, ocorre infiltração no tecido adiposo de macrófagos pró-inflamatórios M1 e subconjuntos de células T. Enquanto a obesidade se desenvolve acontece uma elevação, tanto no tecido adiposo como sistemicamente, de muitas citocinas pró-inflamatórias, como interleucina-1β (IL-1β), TNF-α, interleucina-17 (IL-17) e interleucina-6 (IL-6) que estão relacionadas à progressão do fenótipo obeso ligado à síndrome metabólica. O estabelecimento do fenótipo pró-inflamatório é caracterizado como a relação entre o desenvolvimento da obesidade, a evolução desta para a resistência à insulina, consequentemente ao DM2 e doença cardiovascular associada (PIROLA; FERRAZ, 2017).

A obesidade leva a hipertrofia de adipócitos pela acumulo de triglicerídeos. Os adipócitos hipertrofiados liberam a *chemotactic monocyte protein* (MCP-1) (KANDA *et al.*, 2006), esta ativa os monócitos sanguíneos através do *chemokine receptor type 2* (CCR2) e induz a migração de monócitos para o tecido adiposo e diferenciação em macrófagos. O CCR2 tem um papel fundamental no recrutamento de monócitos / macrófagos e na resposta inflamatória dependente de macrófagos (BORING *et al.*, 1998). Os macrófagos no tecido adiposo são ativados em resposta aos *free fatty acid* (FFA), liberados a partir de adipócitos hipertrofiados, produzindo maiores quantidades de mediadores inflamatórios, como TNF-α, IL-6, MCP-1 (SUGANAMI; NISHIDA; OGAWA, 2005). O nível de expressão de RNAm do TNF- α no tecido adiposo é significativamente elevado na obesidade e fortemente correlacionado com o nível de hiperinsulinemia (HOTAMISLIGIL *et al.*, 1995).

É observado hiperinsulinemia e menor tolerância à glicose, alterações nos níveis de mediadores inflamatórios e adipocinas em camundongos gestantes obesas alimentadas com dieta hiperlipídica (KĘPCZYŃSKA *et al.*, 2013; MURABAYASHI *et al.*, 2013). A diminuição de adiponectina materna encontrada pode estar relacionada a um maior peso fetal devido à diminuição da sensibilidade à insulina e elevação dessa no plasma. Também foi encontrada uma redução de resistina plasmática (KĘPCZYŃSKA *et al.*, 2013) e aumento nos níveis de TNF-α e maior expressão de RNAm de CD-68 (*cluster of differentiation 68* -

glicoproteína transmembranar, altamente expressa por monócitos e macrófagos de tecidos humanos, considerado assim um marcador de macrófagos), MCP-1, CCR2 no tecido adiposo materno (MURABAYASHI *et al.*, 2013). O tecido adiposo subcutâneo fetal (aos 17 dias de gestação) apresentou hipertrofia e infiltração de macrófagos, com aumento na expressão de marcadores inflamatórios como o TNF-α, aumento da expressão de RNAm de CCR2 e diminuição na expressão de *glucose transporter, type 4* (GLUT-4), associando-se ao desenvolvimento de resistência a insulina na prole. Essas mudanças podem estar relacionadas ao aumento do risco de obesidade na adolescência e distúrbios metabólicos (MURABAYASHI *et al.*, 2013).

Uma dieta materna rica em *long chain saturated fatty acid* (SFA- LC) (com 15,7% de ácido palmítico e 13,8% de ácido esteárico do total de ácidos graxos), contendo também 12% de ácidos graxos trans, com uma razão de PUFA: SFA de 0,86, no período da gestação e lactação leva a inflamação hipotalâmica na prole de roedores (PIMENTEL *et al.*, 2012). A ingestão materna de dieta rica em gordura (35% de calorias provindas de gordura) também demonstrou induzir inflamação na prole em primatas não humanos (GRAYSON *et al.*, 2010). Dentro de alguns dias, ao ingerir uma dieta rica em calorias e gorduras (60% de calorias provindas das gorduras), uma quantidade aumentada de SFA da periferia cruza a BHE (barreira hematoencefálica) e induz uma resposta inflamatória nos neurônios hipotalâmicos, sendo um evento precoce no desenvolvimento da obesidade (THALER *et al.*, 2012), envolvendo a ativação da microglia, os macrófagos teciduais residentes no SNC (TIMPER; BRUNING, 2017).

Foi verificado em roedores que uma dieta materna com alto teor de gordura, contendo SFA-LC, mostrou prejudicar a sinalização de leptina a nível do núcleo arqueado, levando a uma atenuação da ação da leptina na supressão do apetite (KIRK *et al.*, 2009). A alimentação materna com elevado teor de gordura, 35,5% de gorduras (55,2% de calorias provindas de gorduras) durante a lactação em camundongos altera as projeções dos neurônios pró-opiomelanocortina (POMC) e *agouti-related peptide* (AgRP) para neurônios de segunda ordem, resultando em aumento da obesidade e diminuição da homeostase da glicose na prole (VOGT *et al.*, 2014). A inflamação local no hipotálamo (que engloba o núcleo arqueado, a parte anterior do *paraventricular nucleus* (PVN) e a eminência mediana) promove o estresse no retículo endoplasmático dos neurônios hipotalâmicos, levando à resistência à insulina e leptina (KLEINRIDDERS; KONNER; BRUNING, 2009; PIMENTEL; GANESHAN; CARVALHEIRA, 2014). A ativação constitutiva da via pró-inflamatória *c-Jun N-terminal*

kinase 1 (JNK1) nos neurônios AgRP aumenta o disparo espontâneo nessas células, juntamente com a resistência neuronal e sistêmica da leptina, resultando em hiperfagia , aumento de peso e ganho de adiposidade (TSAOUSIDOU *et al.*, 2014).

Uma diminuição na relação CT (colesterol total)/HDL (marcador de risco global, que fornece indicação geral de possíveis efeitos sobre o risco de doença cardíaca coronariana) e da apolipoproteína LDL (relacionado com o risco de doença cardiovascular), foi verificado com a substituição de SFA por PUFA (SACKS; KATAN, 2002; MICHA; MOZAFFARIAN, 2010). Numa análise conjunta de estudos de coorte prospectivos, que não foi considerado apenas a ingestão de SFA, sendo considerados também os nutrientes de substituição, a substituição com PUFA foi relacionado à diminuição do risco de doença cardiovascular (JAKOBSEN *et al.*, 2009; ASTRUP *et al.*, 2011). Sendo isso associado aos efeitos nas mudanças do CT/HDL, devido ao aumento dos níveis de HDL (ASTRUP *et al.*, 2011).

2.3 Estresse oxidativo e ômega 3

O estresse oxidativo é decorrente da existência do desequilíbrio entre compostos oxidantes e antioxidantes, devido a geração excessiva ou em detrimento da velocidade de remoção de radicais livres. Esse processo conduz à oxidação de biomoléculas e consequentemente perda de suas funções biológicas e/ou desequilíbrio homeostático, causando dano oxidativo potencial em células e tecidos (HALLIWELL; WHITEMAN, 2004). As espécies reativas de oxigênio (EROS) conhecidas como radicais livres, são produzidas a partir das reações químicas e pela cadeia transportadora de elétrons nas mitocôndrias (BETTERIDGE, 2000; BIRBEN *et al.*, 2012), contêm elétrons desemparelhados que reagem, podendo levar a reações em cadeias, oxidando biomoléculas e contribuindo para o desenvolvimento de patologias como câncer, aterosclerose, hipertensão, diabetes (BIRBEN *et al.*, 2012) e complicações na doença hepática gordurosa não alcoólica (VALENZUELA *et al.*, 2012; DOSSI *et al.*, 2014). Diversos estudos têm demonstrado que o excesso de lipídeos está associado com a formação de espécies reativas e o processo de estresse oxidativo, sendo um fator importante na fisiopatologia da obesidade, esteatose hepática, aterosclerose e doenças cardiovasculares (FURUKAWA *et al.*, 2004; PELUSO *et al.*, 2012; ESTADELLA *et al.*, 2013).

Alguns exemplos de radicais livres são: OH^- (íon hidroxila), HOH^- (íon peroxil), O_2^- (ânion superóxido), NO_3^- (óxido nítrico) e O_2 (oxigênio molecular) (MAGDER, 2006;

CAROCHO; FERREIRA, 2013) e metais de transição como ferro e cobre (PRUCHNIAK; ARAZNA; DEMKOW, 2016). Os antioxidantes naturais ou sintéticos atuam na prevenção da oxidação ocasionada pelos radicais livres, pois possuem elevada estabilidade oxidativa devido sua estrutura molecular. Os sistemas antioxidantes podem ser classificados em enzimáticos e não enzimáticos. Do sistema antioxidante não enzimático fazem parte a glutationa, ácido úrico, compostos obtidos diretamente da dieta como o *a*-tocoferol (vitamina-E), β-caroteno (pro-vitamina-A), ácido ascórbico (vitamina-C) (BIRBEN *et al.*, 2012) . O sistema antioxidante enzimático integra a superóxido dismutase (SOD) que atua na remoção do ânion superóxido ($O2^{-}$), glutationa peroxidase (GSH-PX) que atua na remoção dos hidroperóxidos orgânicos, glutationa redutase (GSH-Rd) e catalase (CAT) que atuam na remoção do peróxido de hidrogênio (H_2O_2) (YU, 1994; PIETTA, 2000) e a glutationa transferase (GST) que participa da inativação de metabólitos secundários como aldeídos insaturados, epóxidos e hidroxiperóxidos (BIRBEN *et al.*, 2012)

A SOD, acoplada ao cobre e zinco (Cu-Zn-SOD), encontrada no citosol e contendo manganês (Mn-SOD) presente na matriz mitocondrial. A SOD dismuta o radical $O2^{\cdot -}$, formando H_2O_2 e O_2. O H_2O_2 é uma molécula menos instável, mas produz danos celulares quando produzidos em excesso, podendo reagir com metais de transição como ferro e cobre, produzindo o radical hidroxila, no qual o organismo não dispõe de mecanismos de defesa. A glutationa peroxidase encontrada no citosol neutraliza o peróxido de hidrogênio pela oxidação da glutationa (GSH). A GSH é um tripeptídeo com funções essenciais, posssui ação de cofator da família de enzimas glutationa peroxidases (GSH-PX), em que desempenha papel protetor contra o estresse oxidativo, com sua oxidação a dissulfeto de glutationa (GSSG). Já a catalase (CAT) presente nos peroxissomos, atua na decomposição do peróxido de hidrogênio a O_2 e H_2O (BETTERIDGE, 2000; BIRBEN *et al.*, 2012)

A aferição das EROs e das das lesões oxidativas pode ser realizada através da medição da atividade enzimática (superóxido dismutase - SOD, catalase, glutationa peroxidase - GSH-Px e glutationa redutase - GSH-Rd) e/ou a concentração de tripeptídeos (glutationa reduzida-GSH) e aldeídos (malondialdeído - MDA) e o estresse oxidativo, por dosagens de GSSG (forma oxidada da glutationa) e/ou pelo cálculo da razão GSH/GSSG. A razão entre a glutationa reduzida e oxidada (GSH / GSSG) é um importante parâmetro do estresse oxidativo. A proporção alta de GSH / GSSG protege a célula contra danos oxidativos, o rompimento dessa razão leva a ativação de fatores de transcrição sensitivos redox, como NF-κB, que atua na resposta inflamatória (BIRBEN *et al.*, 2012).

O estresse oxidativo pode ser mensurado, através dos produtos finais das reações de peroxidação lipídica, oxidação de proteínas e oxidação do DNA (BETTERIDGE, 2000). É verificado que o ômega 3 e seus derivados EPA e DHA, podem atuar na integridade deste sistema, como citoprotetores, através da regulação positiva das enzimas antioxidantes e inibição da expressão gênica pró-inflamatória (DOSSI *et al.*, 2014). Os ácidos graxos poli-insaturados de cadeia muito longa podem regular a expressão de enzimas antioxidantes (glutationa peroxidase, glutationa redutase, glutationa transferase e catalase) através da ativação do Fator 2 relacionado a NF-E2, por meio dos seus produtos de oxidação (VALENZUELA *et al.*, 2012).

Foi encontrado que a suplementação por gavagem com EPA e DHA em camundongos submetidos a dieta hiperlipídica (60% da energia provindas dos lipídios) por 12 semanas após o desmame, melhorou os níveis de citocinas inflamatórias (IL-6, IL-1β e TNF-α) e recuperou os níveis de GSH hepático e glutationa (VALENZUELA *et al.*, 2012). Outro estudo verificou que a suplementação de EPA em camundongos submetidos a uma dieta hiperlipídica (32% de lipídios) aumentou a atividade de SOD e GSH, que foi encontrada reduzida no grupo que não recebeu a suplementação, com diminuição também dos níveis de glicose, insulina, colesterol total, transaminases, depósito de colesterol e triglicerídeos hepático (HIROTANI *et al.*, 2015).

2.4 Dieta hiperlipídica e alterações cardiorrespiratórias

A ingestão elevada de gordura favorece o desenvolvimento de diversas patologias como dislipidemias, doenças cardiovasculares e hipertensão arterial (FEOLI *et al.*, 2003; COSTA-SILVA ; SIMOES-ALVES ; FERNANDES, 2016). Estudos em ratos têm demonstrado que o alto consumo de lipídios na dieta materna durante a gestação, lactação e/ou após o desmame leva ao aumento nos níveis basais de pressão arterial na prole (FEOLI *et al.*, 2003; KHAN *et al.*, 2005; XUE *et al.*, 2015), o qual persiste na vida adulta.

Alguns estudos indicam uma relação entre processos inflamatórios e disfunções no sistema nervoso autonômico, por demonstrarem que a condição inflamatória em algumas regiões do cérebro responsáveis pelo controle da atividade nervosa simpática, como o núcleo do trato solitário (NTS), a região *rostral ventrolateral medula* (RVLM) e o paraventricular nucleus (PVN) podem contribuir para o estabelecimento de patologias, como a hipertensão arterial (PATON; WAKI, 2009; ADLAN *et al.*, 2014), demonstrando que alterações no sistema imunológico são um dos fatores contribuintes para o desenvolvimento do aumento de pressão arterial (TIMPER; BRUNING, 2017). Estudos relatam que em resposta a inflamação,

as células de defesa do organismo, como os macrófagos e linfócitos, residentes na área lesada liberam citocinas pró-inflamatórias na circulação [interleucina 1-Beta (IL- 1β), IL-6 e TNF-α], as quais são capazes de aumentar a atividade simpática e promover o aumento de pressão arterial (PATON; WAKI, 2009; WAKI *et al.*, 2010). Sugerindo que na inflamação, o aumento da aderência de leucócitos na microvasculatura do tronco encefálico ou especificadamente do NTS (região fundamental no controle da pressão arterial), pode levar a liberação de espécies reativas de oxigênio e citocinas pró-inflamatórias pelos leucócitos, que alteram a atividade neuronal. A inflamação e o acúmulo dos leucócitos nesta microvasculatura do tronco encefálico podem elevar a resistência vascular. A acumulação de leucócitos nos capilares pode obstruir parcialmente o fluxo sanguíneo, diminuindo a perfusão sanguínea e resultando em uma hipóxia localizada, levando consequentemente ao aumento da atividade simpática e da pressão arterial (PATON; WAKI, 2009). Dentro da RVLM, região geradora de atividade destinada aos neurônios motores pré-ganglionares simpáticos, há neurônios intrinsecamente sensíveis à hipóxia (WANG *et al.*, 2001). Fornecendo um mecanismo de transdução pelo qual a baixa perfusão do tronco encefálico resulta em excitação simpática para tentar assegurar uma perfusão adequada (PATON; WAKI, 2009).

O desbalanço do sistema nervoso autonômico, com predominância do tônus simpático, constitui importante fator de risco para o desenvolvimento da hipertensão arterial em modelos de restrição proteica (BARROS *et al.*, 2015; BRITO ALVES *et al.*, 2015; ALVES *et al.*, 2016). Estudos demonstram que a dieta hiperlipídica também pode ter um impacto significativo na regulação da atividade simpática em neurônios de regiões-chave do controle cardiorrespiratório, como o NTS (BARDGETT ; SHARPE ; TONEY, 2014). No NTS ocorrem as primeiras sinapses das aferências periféricas relacionadas ao controle cardiorrespiratório (CIRIELLO *et al.*, 1994). Ratos provenientes de mães que consumiram dieta hiperlipídica (rica em ácidos graxos saturados) durante gestação e lactação apresentaram alterações hemodinâmicas como hipertensão arterial, com redução da sensibilidade barorreflexa, associada ao desbalanço autonômico e evidente aumento no tônus do sistema nervoso simpático para o coração. Sugerindo que as alterações decorrentes do consumo excessivo de lipídios, através de uma dieta com 32% de energia fornecida pelos lipídios (68% de ácidos graxos saturados, 16% monoinsaturados e 16% poli-insaturados) consumida pelas mães favorecem a instalação da hipertensão arterial em ratos deste modelo experimental. Foram verificadas também nesses animais, alterações metabólicas, tais como hiperglicemia,

hipertrigliceridemia, aumento dos níveis de LDL e diminuição do HDL (VIDAL-SANTOS *et al.*, 2017).

Em ratos adultos que consumiram dieta hiperlipídica durante um período de 3 ou 6 semanas, foi encontrado um aumento dos níveis pressóricos associado a uma maior ativação de citocinas pró-inflamatórias e ao aumento da expressão gênica de componentes do sistema renina-angiotensina no NTS, com a expressão diferindo de acordo com o tempo de exposição ao insulto nutricional (SPERETTA *et al.*, 2014). Tem sido proposto que modificações na geração e modulação do ritmo respiratório basal (MORAES *et al.*, 2014; COSTA-SILVA *et al.*, 2015) e alterações na quimiossensibilidade periférica e central ao O_2 e CO_2 podem desencadear a hiperativação do sistema nervoso simpático e, consequentemente, a hipertensão arterial (COSTA-SILVA; ZOCCAL; MACHADO, 2012; MORAES *et al.*, 2014; COSTA-SILVA *et al.*, 2015), sugerindo que mecanismos respiratórios também podem estar envolvidos no processo de instalação dessa patologia.

Estudos do nosso laboratório, verificaram que animais submetidos à restrição proteica durante a gestação e lactação apresentaram aumento de frequência respiratória aos 30 dias de vida, permanecendo na vida adulta, associada com elevação da pressão arterial aos 90 dias de vida. Também foi encontrado maior sensibilidade ao O_2 e CO_2 em condições de hipóxia e hipercapnia nesses animais, com elevação da resposta ventilatória aos 30 e 90 dias (BRITO ALVES *et al.*, 2014), sendo observada a participação do sistema respiratório nos mecanismos relacionados ao desenvolvimento da hipertensão arterial relacionada à desnutrição durante a gestação e lactação. Em relação ao controle autonômico vascular, foi sugerido um aumento da atividade simpática nesses animais, pois foi verificada uma elevação da razão entre as *low frequency bands* e *high frequency bands* (LF/HF) aos 90 dias de vida, que contribui para o aumento da pressão arterial relacionada a um desbalanço no controle autonômico cardiovascular (BARROS *et al.*, 2015).

Foram encontrados também em ratos que foram submetidos a um insulto nutricional durante a gestação e lactação, níveis mais elevados de malondialdeído, acompanhado com a diminuição das atividades das enzimas superóxido dismutase e catalase na medula ventral. Sugerindo que a elevação da pressão arterial encontrada nesses ratos também está associada à disfunção oxidativa medular a nível transcricional e funcional da capacidade antioxidante na medula ventral (BRITO ALVES *et al.*, 2016).

2.5 Efeitos da ingestão de ômega-3 em parâmetros metabólicos e cardiorrespiratórios

Estudos têm demonstrado que a ingestão de ácidos graxos α-linolênico (ALA; 18:3) tem ação anti-inflamatória (SALTIEL, 2010; INOUE *et al.*, 2017). Os eicosanoides que são uma grande variedade de produtos lipídicos bioativos de 20 carbonos, classificados em prostanóides, leucotrienos e lipoxinas (O'DONNELL; MASKREY; TAYLOR, 2009), são moléculas altamente potentes e de curta duração, que atuam localmente e têm sido relacionados a uma variedade de processos fisiológicos e patológicos, incluindo câncer, doenças inflamatórias, cicatrização de feridas, etc. (TUNCER; BANERJEE, 2015). Os PUFAs ácido α-linolênico (ALA; 18:3) e ácido linoleico (LA; 18:2) são clivados a partir de fosfolípidos de membrana celular pela fosfolipase A_2. Em seguida, as enzimas ciclooxigenase (COX) e lipoxigenase (LOX) metabolizam os PUFAs. Além de produzir eicosanoides pró-inflamatórios, que incluem prostaglandina E_2 (PGE_2), tromboxano A_2 e leucotrieno B4, a partir do ácido araquidônico (ARA; 20:4 PUFA derivado do ácido linoleico, LA; 18:2), a ação das COX e LOX também produz mediadores anti-inflamatórios, como as resolvinas e protectinas que são originados a partir do ácido eicosapentaenóico (EPA; 20:5) e ácido docosahexaenóico (DHA; 22:6). Um consumo adequado da razão de ômega-6: ômega-3 reduz a formação de ácido araquidônico (ARA; 20:4) a partir do ácido linoleico (LA; 18:2), aumentando assim, a disponibilidade de ácido eicosapentaenóico (EPA; 20:5) e ácido docosahexaenóico (DHA; 22:6) e consequentemente, a formação de mediadores anti-inflamatórios via COX e LOX. A absorção e incorporação de ácido linolênico (ALA; 18:3) em fosfolípidos de membrana tem também ação de inibir a utilização da via da COX e LOX no ácido araquidônico (ARA; 20:4), reduzindo assim a síntese de metabólitos pró-inflamatórios: prostaglandina E_2 (PGE_2), o tromboxano A_2 e leucotrieno B4 (KHATIB *et al.*, 2016). O ácido α-linolênico (ALA; 18:3) também atua na redução de mediadores inflamatórios circulantes no plasma: IL-6, TNF-α e PCR (OLLIVER *et al.*, 2016).

O último trimestre da gestação e os primeiros seis meses de vida pós-natal apresentam maiores necessidades de ácido docosahexaenóico (DHA; 22:6), acontecendo nesse período seu depósito na retina e córtex cerebral, que deve ser garantido pelas reservas tissulares da mãe, principalmente no tecido adiposo. Visto que o feto não tem capacidade de sintetizar ácidos graxos poli-insaturados de cadeia longa a partir dos precursores ácido α-linolênico (18:3) e ácido linoléico (18:2). O fígado também ainda não tem atividade biossintética de elongação e dessaturação para a formação de PUFA-LC (SILVA; MIRANDA JÚNIOR; SOARES, 2007). É nesse período que acontece um maior pico na velocidade de crescimento e

desenvolvimento do sistema nervoso central. O ácido docosahexaenóico (DHA; 22:6) e o ácido araquidônico (ARA; 20:4) são componentes estruturais do SNC e a deficiência desses ácidos graxos essenciais durante esse período crítico pode acarretar em danos irreparáveis. O ácido docosahexaenóico (DHA; 22:6) também mantém a fluidez da membrana das células nervosas, participa da propagação do impulso nervoso e transmissão sináptica e, sinalização para expressão de genes (DUTTAROY, 2009).

O consumo materno de ácido docosahexaenóico (DHA; 22:6) através de pescados e/ou suplementação com óleo de pescados tem uma ação importante no desenvolvimento do sistema nervoso central, na gestação de alto risco, no aumento do peso ao nascer, comprimento e circunferência da cabeça, acuidade visual, coordenação mãos-olhos, atenção, resolução de problemas e processamento de informações, na imunidade e na resposta do sistema nervoso autônomo (ALMEIDA *et al.*, 2014).

A transferência de ácidos graxos através da placenta ocorre mais por transporte mediado por proteínas, no entanto pode ocorrer por difusão passiva. O aumento no consumo de ácidos graxos maternos ocasiona uma maior transferência destes para o feto. Diversas proteínas localizadas na membrana placentária têm sido identificadas por sua função no transporte de ácidos graxos poli-insaturados de cadeia longa, derivados do ácido α-linolênico (18:3) e ácido linoléico (18:2), através da placenta, como: a proteína de ligação de ácidos graxos (*placental membrane fatty acid binding protein*, p-FABPpm) que possui alta afinidade por PUFA-LC, principalmente o ácido docosahexaenóico (DHA; 22:6); translocase de ácidos graxos altamente glicosilada (*highly glycosylated fatty acid translocase*, FAT/CD 36), com maior atividade na captação de ácidos graxos livres; proteínas de transporte de ácidos graxos (*fatty acid transport proteins*, FATP), acilCoA sintetase (*fatty acylCoA synthetase*, ACS) e proteínas intracelulares de ligação de ácidos graxos (*intracellular fatty acid binding proteins*, FABPs) (WEN-JU LIN; JAMES; DARRELL, 1984; AKERELE; CHEEMA, 2016). Ocorre no terceiro trimestre da gravidez um aumento na expressão e atividade da lipase lipoproteica placentária que hidrolisa triacilgliceróis de baixa densidade (LDL) e muito baixa densidade (VLDL) presentes no plasma materno a ácidos graxos livres que são transportados ao feto e utilizados principalmente como fonte de energia. Outra enzima atuante no citosol de células placentárias é a fosfolipase citosólica A2 que participa da liberação de ácidos graxos livres para a síntese de prostaglandinas (DUTTAROY, 2009).

O *American Institute of Nutrition* preconiza por meio da AIN-93 a oferta de 4,8g/Kg de dieta do ácido α-linolênico (ALA; 18:3) e 35,7 g/Kg de ácido linoléico (LA; 18:2) para

fase de crescimento e reprodução de roedores. Na fase de manutenção esses valores são de 2,7 g/kg e 10,4 g/kg, respectivamente (REEVES; NIELSEN; FAHEY, 1993). Em relação às recomendações para razão de consumo ômega 6: ômega 3 existe um conflito na literatura, havendo uma tendência para uma razão de 4:1 a 5:1. Alguns autores recomendam as razões 2:1 a 3:1 pela sugestão de maior conversão do ácido α-linolênico (ALA; 18:3) em ácido docosahexaenóico (DHA; 22:6), alcançando um valor máximo em torno de 2,3: 1. A razão 1:1 não é recomendada por inibir a transformação de ácido α-linolênico (ALA; 18:3) em ácido docosahexaenóico (DHA; 22:6) ou ácido eicosapentaenóico (EPA; 20:5). Em humanos é verificado um maior nível de conversão entre as mulheres, supostamente devido a influencia do estrogênio sobre a atividade das enzimas dessaturases (MARTIN *et al.*, 2006).

O consumo de dietas enriquecidas com ácido α-linolênico (ALA; 18:3), no período da gestação e lactação foi relacioanada com a diminuição da adiposidade, do tamanho do adipócito e dos níveis séricos de leptina na prole (KOROTKOVA *et al.*, 2002). Além da redução da adiposidade, a ingestão de ácido α-linolênico (ALA; 18:3) está associada com a melhora da sensibilidade à insulina, reduzida com a idade, na prole de ratos machos (SARDINHA *et al.*, 2012). O perfil de ácidos graxos em dietas hiperlípídicas durante a gestação e/ou lactação pode contribuir com o desenvolvimento de alterações metabólicas na vida adulta. Foi observado que o número de ilhotas pancreáticas foi maior na prole de ratos adultos que as mães receberam uma dieta rica em ácido α-linolênico (ALA; 18:3), sem alteração no volume do pâncreas. Uma dieta rica em gorduras saturadas (contendo uma grande quantidade de ácido láurico (12:0), *medium chain saturated fatty acid* – SFA-MC) parece diminuir o desenvolvimento de ilhas, enquanto que uma dieta rica em gorduras poli-insaturadas demonstrou promover o desenvolvimento das ilhotas pancreáticas. A plasticidade e o turnover das ilhotas e células beta pancreáticas são diminuídos após o desmame, o que mostra a importância do período perinatal no desenvolvimento pancreático (SIEMELINK *et al.*, 2002).

Os PUFAS-LC através de sua ligação ao PPARs (*peroxisome proliferator-activated receptor*) podem regular o metabolismo de lipídios, atuando na modulação da expressão de genes relacionados à oxidação de ácidos graxos. Os PPARs têm ação na regulação da expressão de genes críticos para o metabolismo de lipídios, na oxidação dos ácidos graxos, no desenvolvimento dos adipócitos e metabolismo das lipoproteínas. A expressão de PPAR através de PUFA é acompanhada de supressão de SREBP (*sterol regulatory element-binding proteins*), inibindo a lipogênese com redução na expressão de ácido graxo sintetase (*fatty acid*

synthase, FAS) e acetil CoA carboxilase (*acetyl CoA carboxylase* , ACC), e consequentemente promovendo a diminuição nos níveis séricos de ácidos graxos, triacilgliceróis e VLDL (KHAIRE, KALE e JOSHI, 2015). A suplementação materna de ácido docosahexaenóico (DHA; 22:6) durante a gravidez e lactação proporciona a normalização das alterações na deposição de tecido adiposo que são provocadas pela restrição do crescimento intrauterino (RCIU), a expressão no tecido adiposo visceral de PPAR-gama, aumento nos níveis séricos de adiponectina e a expressão dos receptores dessa adipocina no tecido adiposo de ratos que sofreram RCIU (BAGLEY *et al.*, 2013).

O aumento da proporção materna ômega-3 / ômega-6 é importante, uma vez que o PUFA, ácido α-linolênico (ALA; 18:3), percussor do DHA, são nutrientes essenciais para o desenvolvimento do feto (CRAWFORD, 2000). Em modelos de camundongos transgênicos Fat-1 (capaz de converter endogenamente o PUFA ômega-6 (LA; 18:2) em PUFA ômega-3 (ALA; 18:3)) que consumiram dieta hiperlipídica, com 45% de calorias provenientes das gorduras, aumentaram a proporção materna de PUFA ômega-3 / ômega-6 e reduziram a inflamação metabólica materna. Verificando através da exposição lipídica fetal e placentária associada à HFD, que mesmo na ausência de mudança na obesidade materna, que as placentas e os fígados fetais das mães Fat1-HFD foram protegidos do excesso de crescimento placentário e deposição lipídica fetal-placentária. A proteção materna do excesso de inflamação levou a melhores resultados metabólicos na prole desses animais (HEERWAGEN *et al.*, 2013).

Estudos têm demonstrado que o ácido α-linolênico (ALA; 18:3) está associado com a redução de triglicerídeos (SMITH *et al.*, 2011; PIEPOLI *et al.*, 2016) e um menor risco de doenças cardiovasculares (HARRIS; CONNOR; LINDSEY, 1984; KRIS-ETHERTON *et al.*, 2002). No entanto, os mecanismos subjacentes ainda não estão esclarecidos. Não se sabe ao certo se o efeito cardioprotetor é exercido pelo ácido α-linolênico (ALA; 18:3) ou pelos produtos metabólicos eicosanoides. Embora, o ácido docosahexaenóico (DHA; 22:6) pareça ser mais eficaz na diminuição da pressão arterial quando comparado ao ácido eicosapentaenóico (EPA; 20:5) (MORI *et al.*, 2000). Um estudo com ácido eicosapentaenóico (EPA; 20:5) e ácido docosahexaenóico (DHA; 22:6) em células endoteliais da aorta humana com dano de DNA induzido por peróxido de hidrogênio (H_2O_2), demonstraram que estes, o ácido eicosapentaenóico (EPA; 20:5) e o ácido docosahexaenóico (DHA; 22:6) reduziram significativamente as espécies reativas de oxigênio intracelular em condições basais e a estimulação por H_2O_2. Os níveis de RNAm de moléculas antioxidantes, como heme

oxigenase-1, tioredoxina redutase 1, cadeia leve de ferritina, cadeia pesada de ferritina e superóxido dismutase de manganês, aumentaram significativamente com o ácido eicosapentaenóico (EPA; 20:5) e o ácido docosahexaenóico (DHA; 22:6) (SAKAI *et al.*, 2017).

Tem sido demonstrado que a ingestão de ácido α-linolênico (ALA; 18:3) é capaz de desempenhar um papel hipotensor dose-dependente em indivíduos hipertensos (HOWE, 1997) devido, principalmente, à sua ação anti-inflamatória ao diminuir a síntese de derivados do ácido araquidônico: prostaglandina E2, tromboxano A2, prostaciclina e leucotrieno B4 (KREMER, 2000). Em uma meta-análise, foi encontrada uma redução significativa nos níveis de pressão arterial (entre 2.0 a 3.4 mmHg) em pacientes hipertensos que consumiram 5,6 g de ácidos graxos ômega-3 por dia (MORRIS; SACKS; ROSNER, 1993). Da mesma forma, outro estudo (APPEL *et al.*, 1993) descobriu que a pressão arterial de hipertensos não-tratados reduziu entre 3,5-5,5 mmHg com o consumo diário de 3g de ácidos graxos ômega-3.

A suplementação em camundongos com óleos dietéticos de peixe, fonte de ácido eicosapentaenóico (EPA; 20:5) e ácido docosahexaenóico (DHA; 22:6) podem diminuir a expressão gênica pró-inflamatória e aumentar a expressão anti-inflamatória e de adiponectina (IM, 2016). O ácido α-linolênico (ALA; 18:3) pode estimular a polarização M2 (o aumento de macrófagos anti-inflamatórios) relacionada à desregulação de mediadores pró-inflamatórios em tecido adiposo inflamado de camundongos obesos (TITOS *et al.*, 2011). Em adição, ácido α-linolênico (18:3) pode ser metabolizado em moléculas bioativas: resolvinas, protectinas e maresinas (PIROLA; FERRAZ, 2017). Essas moléculas não se assemelham aos eicosanóides clássicos, são novas estruturas que funcionam exclusivamente através de alvos celulares e moleculares (BANNENBERG; SERHAN, 2010). A resolvina D1 pode reduzir a produção de interferon-γ (IFNγ), elevando a expressão de arginase-1 (marcador de macrófagos M2), em macrófagos no tecido adiposo (TITOS *et al.*, 2011). A incubação de macrófagos em cultura com o mediador lipídico maresina R1 (MaR1) reduziu a produção de espécies reativas de oxigênio (EROS) e citocinas pró-inflamatórias [IL-1, TNF-α, IL-6, IFN-γ] e promoveu a regulação positiva da expressão do RNAm do receptor de manose tipo 1, marcador M2 (MARCON *et al.*, 2013). A função dos PUFAs como nutrientes benéficos ou agentes terapêuticos estão sendo investigados na prevenção e tratamento de obesidade, DM2 e numerosas doenças relacionadas à inflamação (IM, 2016).

As gorduras poli-insaturadas podem reduzir a inflamação hipotalâmica induzida por uma dieta rica em ácidos graxos saturados, a partir da presença e função da resolvina

produzida a partir de DHA, como mostra um estudo com camundongos. As enzimas envolvidas na síntese de resolvinas foram detectadas no hipotálamo e foram moduladas em resposta ao consumo de gorduras saturadas, causando uma redução da resolvina hipotalâmica. GPR18, o receptor para resolvina, que foi encontrado em neurônios POMC e NPY (Neuropeptídeo Y), também foi modulado por gorduras dietéticas. A substituição de gorduras saturadas por gorduras poli-insaturadas na dieta levou a um aumento de resolvina hipotalâmica, diminuição de massa corporal e melhora da tolerância à glicose. O tratamento intracerebroventricular com DHA obteve como resultado um aumento da expressão das enzimas sintéticas resolvinas e de citocinas anti-inflamatórias, adiposidade reduzida, com um melhor fenótipo metabólico (PASCOAL *et al.*, 2017).

Quando administrados via dieta ou injeção diretamente no hipotálamo, em ratos alimentados com uma dieta hiperlípidica, os PUFAs (ômega-3), ácido α-linolênico (18:3), foram capazes de aumentar a neurogênese hipotalâmica, grande parte da atividade neurogênica induzida por PUFAs levou um aumento do número de neurônios POMC, mas não de NPY. Sendo acompanhada por aumento da expressão do BDNF (*brain derived neurotrophic fator)* que induz a neurogênese e GPR40 (G-protein-coupled receptor receptores acoplados a proteína G 40) (NASCIMENTO *et al.*, 2016). Demonstrando que os PUFAs (ômega-3) também estão relacionados com a neurogênese, através da ligação aos recepoteres GPR40 que são associados à neurogênese no hipotálamo e em outras regiões do cérebro (BONEVA NADEZHDA; YAMASHIMA, 2011).

3 HIPÓTESES

- Ratos provenientes de mães que foram submetidas à dieta rica em ácidos graxos saturados no período de gestação e lactação apresentam alterações na murinometria, dislipidemia, hiperglicemia, intolerância à glicose, diminuição na sensibilidade à insulina, desbalanço oxidativo hepático e prejuízo em parâmetros ventilatórios.

- O enriquecimento desta dieta com ômega-3, a partir do óleo de linhaça, pode interferir nestes parâmetros e impedir as alterações decorrentes do consumo de dieta com alto teor de ácidos graxos saturados.

4 OBJETIVOS

4.1 Objetivo geral

Avaliar as alterações na murinometria, metabolismo e parâmetros ventilatórios na prole de ratas submetidas à dieta com alto teor em ácidos graxos saturados durante a gestação e lactação. Além disso, avaliar se estas alterações são atenuadas na prole de ratas submetidas a uma dieta com alto teor de ácidos graxos saturados enriquecida com ômega-3.

4.2 Objetivos específicos

Nas mães:

- Avaliar os parâmetros bioquímicos aos 19 dias de gestação.

Na prole:

- Avaliar o ganho de peso e a murinometria;
- Obter os níveis séricos em jejum de glicose, triglicerídeos, colesterol, albumina, proteínas totais e transaminases hepáticas;
- Avaliar o teste de tolerância à glicose (TTG), teste de tolerância ao piruvato (TTP) e o teste de sensibilidade à insulina (TSI);
- Analisar a frequência respiratória e a ventilação pulmonar.
- Avaliar enzimas e metabólitos relacionados ao estresse oxidativo no tecido hepático.

5 MÉTODOS

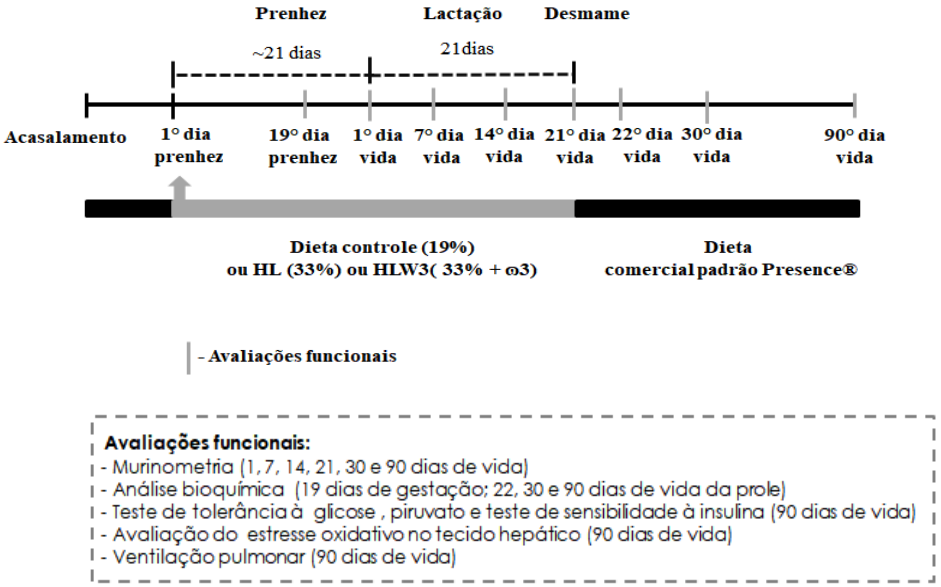
5.1 Animais

Ratas albinas primíparas (n=20) da linhagem *Wistar* (*Rattus norvegicus*) foram obtidas do biotério do Centro Acadêmico de Vitória. Aos 85 a 90 dias de vida e peso corporal compreendido entre 220 e 260 g, as ratas foram colocadas para acasalamento na proporção de 1:3 (macho:fêmea). A determinação da prenhez foi realizada a partir da observação da presença de espermatozóides no esfregaço vaginal, definindo-se o primeiro dia de gestação. A partir do primeiro dia de gestação até o 21º dia de lactação, as ratas progenitoras foram separadas e colocadas em gaiolas individuais e alocadas aleatoriamente em suas respectivas dietas com água e ração *ad libitum*, sendo compostos os seguintes grupos: grupo dieta controle (n=35), grupo dieta com alto teor ácidos graxos saturados, HL (n=27); e grupo dieta com alto teor de ácidos graxos saturados enriquecida com ômega-3, a partir do óleo de linhaça, HLW3 (n=27).

A temperatura e a umidade foram mantidas dentro dos limites de 22 a 24 °C e 55 a 65%, respectivamente, com 12h de claro e escuro (luzes acesas das 06:00 às 18:00h). A prole proveniente de cada fêmea foi reduzida a 8 ratos machos por ninhada. Nos casos da ninhada ter sido composta por menos de 8 ratos machos, ratas fêmeas foram utilizadas para padronização do tamanho da ninhada até o desmame. A figura 1 demonstra o desenho experimental, conforme os períodos das avaliações funcionais realizadas nos animais.

Os protocolos e procedimentos experimentais foram aprovados pelo Comitê de Ética de Uso Animal (CEUA) de nº 23076.049500/2016-37 (Anexo A) e seguiu as recomendações do Colégio Brasileiro de Experimentação Animal (COBEA).

Figura 1 - Desenho experimental de acordo com o período de avaliação de ratas Wistar e sua prole de machos submetidos à dieta controle (19% de lipídios), HL (33% de lipídios) e HLW3 (33% de lipídios enriquecida com ômega-3) durante a gestação e lactação e da prole de machos submetidos à dieta comercial padrão após desmame.



Um número equivalente a 20 ratas foram colocadas para acasalar e após a detecção da prenhez foram separadas em três grupos de acordo com a dieta consumida (controle, HL e HLW3), sendo submetidos a esta dieta até o desmame. Aos 21 dias de vida, as proles foram desmamadas e receberam dieta comercial padrão Presence® até os 90 dias de vida. As avaliações funcionais ocorreram aos 19 dias de prenhez das mães e no 1º, 7º, 14º, 21º, 30º e 90º dia de vida da prole. A quantidade de animais por grupo experimental variou de 27 a 35 animais.

5.2 Manipulação nutricional

Para a manipulação nutricional foram definidas as seguintes dietas: grupo controle recebeu uma dieta padrão cuja composição estava de acordo com a AIN-93 (REEVES; NIELSEN; FAHEY, 1993) com 19% da energia proveniente das gorduras, 20% das proteínas e 61% dos carboidratos; o grupo HL recebeu uma dieta com alto teor em ácidos graxos saturados adaptada a partir da composição da dieta utilizada no estudo de Ferro Cavalcante (FERRO CAVALCANTE *et al.*, 2013), com 33% da energia proveniente das gorduras, 20% das proteínas e 47% dos carboidratos; e o grupo HLW3 recebeu uma dieta com alto teor em ácidos graxos saturados, enriquecida com ômega-3 com 33% da energia proveniente das gorduras, 20% das proteínas e 47% dos carboidratos (tabela 1). A dieta padrão continha em torno de 3.5 quilocalorias por grama e as dietas hiperlipídicas em torno de 4,5 quilocalorias por grama. As dietas hiperlipídicas apresentavam maior teor de ácidos graxos saturados e a dieta enriquecida com ômega-3 continha menor quantidade de ômega 6 (tabela 2). Após o

desmame, aos 21 dias de vida, os animais receberam dieta padrão comercial para ratos (Presence®, Grupo Neovia, São Paulo, Brasil), formando três grupos: prole das mães alimentadas com dieta padrão (controle), prole das mães alimentadas com dieta com alto teor ácidos graxos saturados (HL) e prole mães alimentadas com dieta com alto teor de ácidos graxos saturados enriquecida com ômega-3 (HLW3).

Tabela 1 - Composição quanto aos ingredientes das dietas experimentais

Ingrediente/quantidade por 100g de dieta	AIN-93 G	Dieta hiperlipídica	Dieta Hiperlipídica enriquecida com ômega 3
Amido de milho	39,7	15	15
Amido dextrinizado	13,2	-	-
Farinha de trigo	-	12	12
Biscoito maisena	-	7	7
Farinha de soja	-	6	6
Banha de porco	-	2	2
Manteiga	-	8	8
Caseína (>85%)	20	20	20
Goma guar	-	0,5	0,5
Sacarose	10	18	18
Óleo de linhaça	-	-	3,5
Óleo de soja	7	7	3,5
Fibra (celulose)	5	0,3	0,3
Vitaminas	1	0,7	0,7
Mineral mix	3,5	2,5	2,5
DL-metionina	0,3	0,25	0,25
Bitartarato de colina	0,25	0,25	0,25
BTH	0,0014	0,014	0,014
Glutamato monossódico (12,3%)	-	0,2	0,2
Cloreto de sódio	-	0,3	0,3
Total (g)	100	100	100
Kcal /100g	3,69	4,52	4,51
% Gorduras totais	18,6	33,6	32,3
% Proteínas	20,2	19,6	18,2
%Carboidratos	61	46,8	49, 4

AIN-93G de acordo com Reeves et al., 1993; dieta hiperlipídica adaptada de Ferro Cavalcante et al., 2013; *Composição nutricional calculada a partir da análise centesimal das dietas realizada no laboratório de Bromatologia do Centro Acadêmico de Vitória, Universidade Federal de Pernambuco.

Tabela 2 - Composição percentual de ácidos graxos quanto à presença de dupla ligação na cadeia carbônica das dietas experimentais

Ácidos graxos	Composição percentual dos ácidos graxos Dietas		
	AIN-93G	Hiperlipídica	Hiperlipídica com ômega 3
Saturados			
Ácido octanoico (C8:0)	0	0	0
Ácido decanoico (C10:0)	0	0,85	0,74
Ácido láurico (C12:0)	0	1,16	1,15
Ácido mirístico (C14:0)	0	4,22	4,37
Ácido pentadecanoato (C15:0)	0	0,47	0
Ácido palmítico (C16:0)	11,97	22,78	21,73
Ácido heptadecanoato (C17:0)	0	0	0
Ácido esteárico (C18:0)	4,62	7,53	8,07
Ácido araquídico(C20:0)	0,36	0	0
Ácido behênico(C22:0)	0,37	0	0
Total	17,32	37,01	36,06
Monoinsaturados			
Ácido miristoleico (C14:1)	0	0,46	0
Acido palmitoleico (C16:1)	0	0,95	0,87
Ácido heptadecanoico (C17:1)	0	0	0
Ácido oleico (C18:1)	29,93	28,32	38,47
Total	29,93	29,73	39,34
Poli-insaturados			
Ácido linolênico (C18:3)	3,89	1,64	2,32
Ácido linoleico (C18:2)	48,87	31,63	22,28
Total	52,76	33,27	24,6
Ômega 3 (g/100g)	0,27	0,27	0,4
Ômega 6 (g/100g)	3,42	5,73	3,78
Razão ômega 6:3	**12,66**	**21,22**	**9,45**

Os ácidos graxos foram identificados segundo padrão externo (FAME Supelco™ mix C4-C24, Bellefonte, PA, USA) e o percentual (%) calculado conforme normalização das áreas dos picos pelo método de cromatografia gasosa no Laboratório de Fitoquímicos e procesos do Centro de Tecnologias e Estratégias do Nordeste. A partir da determinação percentual de ácido linolênico (ômega 3) e linoléico (ômega 6) foram calculadas as quantidades (g/100g de dieta) desses ácidos graxos e obteve se a razão de ômega 6 / 3. A nomenclatura dos ácidos graxos seguiu a classificação determinada pela I Diretriz sobre o consumo de gorduras e saúde cardiovascular (Santos et al., 2013).

5.3 Avaliação das medidas murinométricas

Foram realizadas as medições murinométricas (peso corporal, comprimento naso-anal e circunferência abdominal) no 1º, 7º, 14º, 21º, 30º e 90º dia de vida. Após obtenção das medidas, foi calculado o índice de Lee no 90º dia de vida a partir da relação entre a raiz

cúbica do peso corporal e o comprimento naso-anal do animal. As medições foram realizadas de acordo com o estudo de Novelli (NOVELLI *et al.*, 2007).

5.4 Análise bioquímica

As mães aos 19 dias de prenhez e a prole aos 22 (subsequente ao desmame) e 90 dias de vida, foram submetidos ao jejum de 12 horas (*overnight*) e, em seguida, anestesiados com Ketamina (80mg/Kgi.p) e xilazina (10mg/Kgi.p) para coleta de amostras de sangue através do rompimento do plexo retro orbital. Após coagulação, o sangue foi centrifugado a 3500 RPM por 10 minutos para obtenção do soro, o qual foi transferido para um tubo Eppendorf e armazenado a -20°C até a realização das análises bioquímicas por meio do equipamento *Automatic Chemistry Analyzer* (ver. 4e) (Pioway Medical Lab Equimament Co., Ltd.). Foram analisados nas mães: a glicemia, triglicerídeos, proteínas totais, albumina e colesterol total. Na prole foram analisados: a glicemia, triglicerídeos, proteínas totais, albumina, alanina aminotransferase (ALT), aspartato aminotransferase (AST) e colesterol total aos 22 e 90 dias. A partir da dosagem de triglicerídeos foi obtido o valor de VLDL-colesterol pela fórmula de Friedewald = TG/5, conforme estabelecido na IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose: Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia (SPOSITO *et al.*, 2007).

5.5 Teste de tolerância à glicose (TTG)

Aos 90 dias de vida foi realizado o teste de tolerância à glicose (TTG) após um jejum de 6 horas, os animais foram separados em gaiolas individuais e o sangue foi coletado a partir de cortes nas extremidades da cauda do animal, sendo mensurado a glicemia basal no tempo 0 ou T0 (em duplicatas), e em seguida, foi administrada glicose a 20% (2mg/g de peso) através de gavagem e foi mensurada a glicose (em duplicata) após 15, 30, 60 e 120 minutos depois da administração da glicose (BARQUISSAU *et al.*, 2017).

5.6 Teste de tolerância ao piruvato (TTP)

Aos 90 dias de vida foi realizado o teste de tolerância ao piruvato (TTP) após um jejum de 12 horas *(overnight)*, os animais foram separados em gaiolas individuais. Em seguida, foi realizada a mensuração do nível basal de glicose no tempo 0 (T0), em duplicata, e injetado o piruvato de sódio a 50% (2g/kg de peso) por via intraperitoneal, sendo depois mensurada a glicemia (em duplicata) aos 15, 30, 45, 60 e 120 minutos após a administração do piruvato de sódio (Sigma-Aldrich Brasil Ltda, P2256) (BARQUISSAU *et al.*, 2017).

5.7 Teste de sensibilidade à insulina (TSI)

Aos 90 dias de vida foi realizado o teste de sensibilidade à insulina (TSI) após um jejum de 6 horas, os animais foram separados em gaiolas individuais. Em seguida, foi realizada a mensuração do nível basal de glicose no tempo 0 (T0), em duplicata, e injetada a insulina (1 mU/g de peso) por via intraperitoneal, sendo depois mensurada a glicemia (em duplicata) aos 15, 30, 45, 60 e 120 minutos após a administração de insulina humana regular (HUMULIN R, 100UI/ml) (BARQUISSAU *et al.*, 2017).

5.8 Avaliação do estresse oxidativo no tecido hepático

O estresse oxidativo foi avaliado no tecido hepático dos ratos, a partir de biomarcadores como malondialdeído (MDA), sulfidrila e sistemas enzimáticos antioxidantes: catalase, superóxido dismutase e glutationa transferase e através da razão GSH, GSSG e GSH / GSSG. Aos 90 dias, cinco animais de cada grupo foram sacrificados por guilhotina após jejum de 6 horas no período claro (6h às 12h) para coleta do fígado. Uma alíquota de aproximadamente 1 g do órgão foi armazenada em Eppendorf a -80ºC até o preparo da amostra. As análises de estresse oxidativo foram realizadas no Laboratório de bioquímica geral, molecular e do exercício do Centro Acadêmico de Vitória, Universidade Federal de Pernambuco.

5.8.1 Preparo do homogeneizado do tecido hepático

O tecido hepático foi homogeneizado em tampão de extração (Tris base 50 mM e EDTA 1mM, pH 7,4; com adição de ortovanadato de sódio 1 mM e PMSF 2 mM). Após a homogeneização, as amostras foram centrifugadas a 1180xg, a 4° C, por 10 minutos e os sobrenadantes submetidos à quantificação protéica.

5.8.2 Dosagem de proteína

A concentração de proteína foi determinada pelo método de Bradford (1976). O princípio do método baseia-se na determinação da concentração de ligações peptídicas através da medida da absorbância do complexo proteína-corante. Este complexo absorve em comprimento de onda de 595 nm. A absorbância é considerada diretamente proporcional à concentração de proteína na solução analisada, onde uma solução de BSA (2mg/ml) foi utilizada como padrão.

5.8.3 Avaliação dos níveis de peroxidação lipídica pela metodologia da substância Reativa ao Ácido Tiobarbitúrico

Para a dosagem de TBARS foi utilizada a técnica colorimétrica de Buege e Aust (1978), sendo uma técnica muito utilizada para avaliar a lipoperoxidação, pois o ácido tiobarbitúrico reage com os produtos da LPO, entre eles o malondialdeído e outros aldeídos. Adicionou-se a uma alíquota (300 μg proteína) do homogeneizado ao ácido tricloroacético (TCA) a 30%, o material foi centrifugado a 1180xg por 10 minutos, o sobrenadante retirado e adicionado ao ácido tiobarbitúrico a 0.73% que reage com os produtos da lipoperoxidação formando um composto de coloração rosada. A mistura foi incubada por 15 minutos a 100ºC e em seguida resfriada, utilizada para a leitura da absorbância a 535nm em espectrofotômetro (Biochrom Libra S12 Visible, USA), utilizando cubetas de quartzo. Os resultados foram expressos em μM/mg de proteína.

5.8.4 Avaliação do conteúdo de sulfidrila-SH

O conteúdo de sulfidrila foi determinado a partir da reação com o composto DTNB (5,5'- dithiobis (2 nitrobenzoic acid) (ELLMAN, 1959). A alíquota do homogenato (300 μg de proteína) foi incubada no escuro após a adição de DTNB 10mM e o volume final foi de 1 mL completado com tampão de extração pH 7.4 e realizada a leitura com absorbância (30°C) a 412nm em espectrofotômetro (Biochrom Libra S12 Visible, USA). Os resultados foram expressos em M/mg de proteína.

5.8.5 Avaliação da atividade enzimática da Superóxido dismutase (SOD)

A determinação da atividade de SOD foi feita de acordo com Misra e Fridovich (1972). O homogenato de fígado (80 μg de proteína) foi incubado no tampão carbonato de sódio (0,05 %, pH 10.2, 0.1 mM de EDTA) em banho-maria a 37°C, antes da avaliação da atividade enzimática. A reação teve início pela adição de 20 μM de epinefrina (150 mM), em ácido acético (0.05 %). A absorbância foi lida a 480nm por 3 min em espectrofotômetro (Biochrom Libra S12 Visible,USA). Os resultados foram expressos em U/mg de proteína. Uma unidade de SOD foi definida como a quantidade de proteína requerida para inibir a auto-oxidação de 1μmol de epinefrina por minuto.

5.8.6 Avaliação da atividade enzimática da Catalase

A atividade da catalase foi monitorada de acordo com AEBI (1984). O princípio do ensaio é baseado na determinação da constante k de decomposição de H_2O_2, que nas nossas condições de temperatura e pH foi definido como 4.6 x 10^7. Assim, 0,3M de H_2O_2 foi adicionado a amostra (80μg de proteína), seguido de adição do tampão fosfato 50mM, pH 7.0 a 20°C, a absorção de decaimento foi monitorada por 4 min a 240nm em espectrofotômetro (Biochrom Libra S12 Visible, USA). Os resultados foram expressos em U/mg de proteína (AEBI, 1984) . Uma unidade de catalase foi definida como quantidade de proteína requerida para converter 1μmol de H_2O_2 em H_2O por minuto.

5.8.7 Avaliação da atividade enzimática da Glutationa S-Transferase

A atividade da glutationa S- transferase é diretamente proporcional a taxa de formação do composto DNP-SG (dinitro fenil S glutationa), podendo desta forma ser medida por meio do monitoramento da taxa de formação do mesmo. Em uma cubeta de quartzo de 1 mL, adicionou-se a amostra (80μg de proteína) ao tampão fosfato (0.1M) e EDTA (1mM), GSH (1mM) e CDNB (1mM). A absorbância (340mm) foi registrada por um período de aproximadamente 3 min com controle da temperatura (30°C), em espectrofotômetro (Biochrom Libra S12 Visible, USA). Os resultados foram expressos em U/mg proteína. Uma unidade de atividade enzimática da GST foi definida como a quantidade necessária para catalisar a formação de 1μmol do composto DNP-SG por minuto (HABIG; PABST; JAKOBY, 1974).

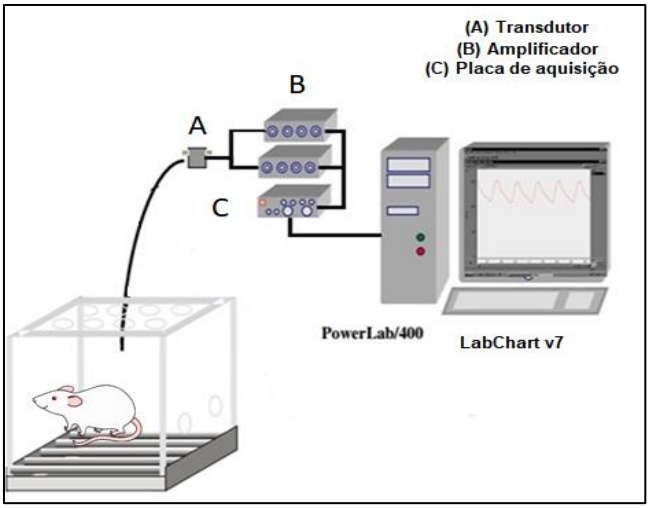
5.8.8 Quantificação do balanço redox (razão GSH/GSSG)

A razão GSH/GSSG foi avaliava através da quantificação dos níveis de GSH e GSSG segundo o método de HISSIN e HILF (1976). Em tampão 0,1M fosfato contendo 5mM de EDTA (pH 8.0) foi adicionado 100 μg do homogenato do figado e o fluorescente ortoftaldeido (1μM), e então incubado a temperatura ambiente, por 15 minutos e lido em espectrofluorímetro modelo FLUOstar Omega (BMG Labtech, USA), utilizando os comprimentos de onda de 350nm de excitação e 420nm de emissão. Para determinar os níveis de GSSG, a amostra foi incubada com 0.04M de N-ethylmaleimide por 30 minutos, seguido da adição de tampão NaOH a 0.1M. Quando os preparos estavam prontos, seguindo os mesmos passos de GSH foi executado para determinar GSSG. O estado redox foi determinado pela relação GSH/GSSG (HISSIN; HILF, 1976).

5.9 Avaliação dos parâmetros ventilatórios

As medidas de ventilação foram obtidas por pletismografia de corpo inteiro, em um sistema fechado (BARTLETT; TENNEY, 1970), no 90º dia de vida da prole. Durante a realização de cada medida de ventilação, o fluxo de ar foi interrompido e a câmara do animal permaneceu totalmente vedada por curtos períodos de tempo (~2 min). As oscilações causadas pela ventilação do animal foram captadas por um dispositivo conectado à câmara que contém o transdutor diferencial de pressão e o amplificador de sinais (ML141 spirometer, PowerLab, ADInstruments). O sinal foi então enviado para o sistema de aquisição e análise dos dados (LabChartTM Pro, PowerLab, ADInstruments). Três variáveis respiratórias foram medidas: a frequência respiratória (FR), o volume corrente (VT) e a ventilação (VE) (MALAN, 1973).

Figura 2 - Desenho esquemático da pletismografia de corpo inteiro de um animal, adaptado de Penintente *et al.*, 2007.



Fonte: Adapatado de Penintente *et al.* (2007).
Durante a realização de cada medida de ventilação, o fluxo de ar da caixa foi interrompido e a câmara do animal permaneceu totalmente vedada por curtos períodos de tempo (~2 min). As oscilações causadas pela ventilação do animal foram captadas por um dispositivo conectado à câmara que contém o transdutor diferencial de pressão e o amplificador de sinais. O sinal foi então enviado para a placa de aquisição e análise dos dados (LabChartTM Pro, PowerLab, ADInstruments).

5.10 Análise estatística

Os resultados foram expressos em média ± epm (erro padrão da média). A análise de normalidade da amostra foi realizada por meio do teste de *Kolmogorov-Sminorv*. A comparação entre os grupos foi realizada por meio do teste *ANOVA one-way* e *ANOVA two-way* (para os testes de tolerância à glicose, teste de tolerância ao piruvato e teste de sensibilidade à insulina), seguido do pós-teste de *Bonferroni*. Os dados foram analisados no

programa Graph Pad Prism (GraphPad Software Corporation, versão 5.0, 2007). O nível de significância considerado foi $p<0,05$.

6 RESULTADOS

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Effects of diet with high content saturated fatty acids enriched with omega-3 during gestation and lactation on the respiratory and metabolic parameters of rat' s offspring

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Abstract

Purpose: Maternal dietary intake rich in saturated fatty acids during gestation and lactation is associated with an increased risk of developing non-transmissible chronic diseases in adult offspring. In contrast, studies have shown that consumption of an omega-3-enriched diet during gestation and lactation reduces adiposity, improves insulin sensitivity, has an anti-inflammatory action and may play a hypotensive role in the offspring. In light of this evidence, in this study tested the hypothesis that rats from dams who were submitted to the diet rich in saturated fatty acids in the gestation and lactation period present alterations in the murinometry, dyslipidemia, hyperglycemia, glucose intolerance, decrease in insulin sensitivity, hepatic oxidative imbalance and impairment in ventilatory parameters. In addition, we tested the hypothesis that the enrichment with omega-3, from linseed oil-3, can interfere in these parameters and prevent the alterations resulting from the consumption of diet with high content of saturated fatty acids. **Methods**: Females wistar rats were mated and, after pregnancy detection, were placed in individual cages and randomly allocated in their respective diets with water and *ad libitum* ration: control diet group (GC, 19% of lipids and ratio ω6:ω3 = 12, 66), a diet group rich in saturated fatty acids (HL, 33% lipids and ratio ω6: ω3 = 21.22) and a high-saturated fatty acid diet group enriched with omega-3, from linseed oil-3 (HLW3, 33% lipids and ratio ω6: ω3 = 9.45). After weaning, the animals began to receive standard animal feed. Were evaluated: murinometric measurements; biochemical analyzes; glucose tolerance test (GTT), pyruvate tolerance test (PTT), insulin sensitivity test (IST), enzymes and markers related to oxidative stress in

hepatic tissue and measurement of pulmonary ventilation. **Results**: Our results demonstrate that dams who consumed a diet rich in saturated fatty acids had higher cholesterol levels when compared to the control and HLW3 groups. The maternal dietary intake rich in saturated fatty acids also is associated with a higher body weight at the age of 1, 7 and 14 days of life when compared to the control group. The HLW3 group presented lower body weight in relation to the HL group in the first day of life. But, on the 7th, 14th and 21st days of life, the HLW3 group also presented higher body weight when compared to the control group. However, we found no difference in weight among offspring at 30 days of life. At 90 days of life also no difference in body weight and Lee index was found between groups. Regarding the biochemical parameters evaluated, dams who consumed a diet rich in saturated fatty acids had higher cholesterol levels when compared to the control and HLW3 groups. HL offspring also presented dyslipidemia and hyperglycemia at 22 and 90 days of life, with the HLW3 group presenting better values in the lipid and glycemic profile. Animals from the HL group also had elevated ALT levels, whereas HLW3 offspring had lower ALT levels at 22 days of age. At 90 days of age, the HLW3 group had lower levels of AST than the control group and HL. An oxidative imbalance was observed in the HL group, with a reduction in the activity of the antioxidant enzymes GST and SOD, and no difference was found in relation to the control and HLW3 groups in these enzymes evaluated. However, a decrease in sulfhydryl levels and the GSH/GSSG ratio was found both in the HL group and in the HLW3 group. Regarding the ventilatory parameters, no differences were found between groups. **Conclusion**: Thus, the present study suggests that the maternal consumption of a diet rich in saturated acids is associated to impair in some murinometric parameters, biochemical and imbalances oxidative to hepatic level, with reduction of enzymes that participate in the antioxidant defense system. In addition, our findings confirm that consumption of omega-3 from flaxseed oil is able to attenuate these changes observed in animals that received a diet rich in saturated fatty acids.

Keywords: Phenotypic plasticity, hyperlipid diet, alpha-linolenic acid, chronic non transmissible diseases.

1 Introduction

In the rapid change of the morbimortality model, characterized by the epidemiological transition, the nutritional factor plays a major role. With the nutritional transition, prevalence is observed mainly among the mature population and the elderly, of non-transmissible chronic diseases: type 2 diabetes mellitus (DM2), obesity, systemic arterial hypertension, comorbidities associated with hypercaloric feeding, trans fats, saturated fatty acids , the exacerbated consumption of sugar and salt and other unhealthy practices related to Western lifestyle (1).

The high consumption of maternal diet rich in fats increases the levels of circulating free fatty acids and induces the activation of inflammatory pathways, increasing the risk of low-grade chronic inflammation in the offspring (2). Fatty acids act on macrophages to alter the cellular ratio M1 (pro-inflammatory) / M2 (anti-inflammatory). Saturated fatty acids stimulate the development of M1 cells, while unsaturated types help to elevate M2 cells in adipose tissue. While obesity develops, there is an increase in both adipose and systemic tissue of many proinflammatory cytokines such as interleukins (IL-1β, IL-6) and tumor necrosis factor (TNF-α) that are related to the progression of the obese phenotype metabolic syndrome. The establishment of the pro-inflammatory phenotype is characterized as the relationship between the development of obesity, its evolution towards insulin resistance, consequently DM2 and associated cardiovascular disease (3).

In response to inflammation, the body's defense cells, such as macrophages and lymphocytes, living in the injured area release pro-inflammatory cytokines into the circulation (IL-1β, IL-6 and TNF-α), which are able to increase sympathetic activity and promote increased blood pressure (4, 5). Unbalance of the autonomic nervous system, with predominance of sympathetic tone, is an important risk factor for the development of arterial hypertension in protein restriction models (6-8). Studies also demonstrate that the hyperlipidic diet may have a significant impact on the regulation of sympathetic activity in neurons in key regions of cardiorespiratory control, such as the solitary tract nucleus (NTS) (9). Rats from mothers who consumed a hyperlipidic diet (rich in saturated fatty acids) during pregnancy and lactation showed hemodynamic changes such as arterial hypertension, reduced baroreflex sensitivity, associated with autonomic imbalance and evident increase in sympathetic nervous system tone for the heart. Suggesting that the changes due to excessive lipid consumption through a diet rich in saturated fatty acids consumed by the mothers favor the installation of arterial hypertension in rats of this experimental model. Metabolic changes, such as hyperglycemia, hypertriglyceridemia, elevated LDL levels and decreased HDL were also observed in these animals (10).

It has been proposed that modifications in the generation and modulation of the basal respiratory rhythm (11, 12) and alterations in peripheral and central chemosensitivity to O2 and CO2 can trigger the hyperactivation of the sympathetic nervous system and, consequently, arterial hypertension (11-13), suggesting that respiratory mechanisms may also be involved in the process of establishing this pathology.

The ingestion of α-linolenic acid (ALA; 18: 3), also known as omega-3, a polyunsaturated fatty acid, is able to play a dose-dependent hypotensive role in hypertensive individuals (14) due mainly to its anti-inflammatory action by decreasing the synthesis of arachidonic acid derivatives: prostaglandin E2, thromboxane A2, prostacyclin and leukotriene B4 (15). In a meta-analysis, a significant reduction in blood pressure levels (between 2.0 and 3.4 mmHg) was found in hypertensive patients who consumed 5.6 g/day of omega-3 fatty acids (16). Similarly, other study (17) found that blood pressure in untreated hypertensive patients was reduced by 3.5-5.5 mmHg with a daily intake of 3g/day of omega-3 fatty acids. Consumption of an omega-3-enriched diet during gestation and lactation also reduces adiposity and improves insulin sensitivity in offspring (18, 19). The very long chain polyunsaturated fatty acids (LC-PUFA) through their binding to PPARs (peroxisome proliferator-activated receptor) can regulate lipid metabolism, acting in the modulation of the expression of genes related to the oxidation of fatty acid (20).

It is verified that omega-3 and its derivatives eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can act as cytoprotectors, through the positive regulation of the antioxidant enzymes and inhibition of the pro-inflammatory gene expression (21). The very long chain polyunsaturated fatty acids can regulate the expression of antioxidant enzymes (glutathione peroxidase, glutathione reductase, glutathione transferase and catalase) through the activation of NF-E2-related Factor 2 by means of their oxidation products (22).

Therefore, the objective of the present study was to evaluate the changes in the biochemical profile, murinometric, oxidative balance and respiratory parameters in the offspring of rats submitted to the diet with a high content of saturated fatty acids during gestation and lactation. In addition, it was assessed whether these changes are attenuated in the offspring of rats submitted to a diet high in saturated fatty acids enriched with omega-3.

2 Materials and methods

The experimental protocol was approved by the Ethical Committee (protocol 23076.049500/2016-37) of the Federal University of Pernambuco, Brazil. The Guidelines for the Care and Use of Laboratory Animals were followed and all efforts were made to minimize animal discomfort and the number of animals used.

2.1 Animals and experimental groups

Primparous albino rats (n = 20) from the Wistar line (Rattus norvegicus) were obtained from the biotery of the Academic Center of Vitória (CAV), Federal University of Pernambuco, Brazil. At 85 to 90 days of life and body weight comprised between 220 and 260 g, the rats were placed for mating in the ratio of 1: 3 (male: female). The determination of pregnancy was performed from the observation of the presence of spermatozoa in the vaginal smear, defining the first day of gestation. From the first day of gestation until the 21st day of lactation, the progenitor rats were separated and placed in individual cages and randomly allocated in their respective diets with water and chow *ad libitum*, the following groups were composed: control diet group (n = 35), diet group with high content of saturated fatty acids, HL (n = 27); and group with high saturated fatty acid content enriched with omega-3, HLW3 (n = 27).

Temperature and humidity were maintained within the range of 22 to 24 ° C and 55 to 65% respectively, with 12h light and dark cycle (lights on from 06:00 to 18:00h). Offspring from each female were reduced to 8 male rats per litter. In cases where the litter was composed of less than 8 male rats, female rats were used to standardize litter size until weaning.

2.2 Nutritional manipulation

For the nutritional manipulation the following diets were defined: control group received a standard diet whose composition was in accordance with AIN-93 (23) with 19% of the energy coming from the fats, 20% of the proteins and 61% of the carbohydrates; the HL group received a diet high in saturated fatty acids adapted from the composition of the diet used in the study of Ferro Cavalcante (24), with 33% of the energy coming from the fats, 20% of the proteins and 47% of the carbohydrates; and the HLW3 group received a diet high in saturated fatty acids, enriched with omega-3 with 33% energy from fats, 20% from proteins and 47% from carbohydrates (Table 1). The standard diet contained around 14,64 kJ/g and the hyperlipid diets around 18,82 kJ/g. Hyperlipidic diets had a higher content of saturated fatty acids and the diet enriched with omega-3 contained less omega 6 (table 2). After weaning, at 21 days of age, the animals received commercial standard diet for rats (Presence®, Neovia Group, São Paulo, Brazil), forming three groups: offspring dams fed with standard diet (control), offspring dams fed a diet high in saturated fatty acids (HL) and offspring dams fed a diet high in saturated fatty acids enriched with omega-3 (HLW3).

2.3 Evaluation of murinometric measurements

Were mensured the body weight, naso-anal length and abdominal circumference in the 1st, 7th, 14th, 21st, 30th and 90th days of life. After obtaining measurements, the Lee index was calculated on the 90th day of life from the relation between the cubic root of the body weight and the naso-anal length of the animal (25).

2.4 Biochemical analysis

Dams at 19 days of pregnancy and offspring at 22 (subsequent to weaning), 30 and 90 days of life, were submitted to a 12-hour fast (*overnight*), and then anesthetized with Ketamine (80mg / Kgi.p) and xylazine (10mg / Kgi.p) for collection of blood samples through the rupture of the retro orbital plexus. After coagulation, the blood was centrifuged at 3500 RPM for 10 minutes to obtain the serum, which was transferred to an Eppendorf tube and stored at -20 ° until the accomplishment of the biochemical analyzes by means of the equipment *Automatic Chemistry Analyzer* (ver. 4e) (Pioway Medical Lab Equimament Co., Ltd.). In the dams and offspring were analyzed: total proteins, albumin, glycemia, triglycerides, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST). From the triglyceride dosage the VLDL-cholesterol value was obtained by the Friedewald formula = TG / 5, as established in the IV Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis: Department of Atherosclerosis of the Brazilian Society of Cardiology (26).

2.5 Glucose tolerance test

At 90 days of age, the glucose tolerance test (GTT) was performed after a 6-hour fast, the animals were separated into individual cages and blood was collected from cuts at the tail ends of the animal, (in duplicates), being measured baseline glycemia at time 0 or T0 (in duplicates), and after that, 20% glucose (2mg / g by weight) was given through gavage and glucose was measured (in duplicate) at 15, 30, 60 and 120 minutes after administration of glucose (27).

2.6 Pyruvate tolerance test

At 90 days of life the sodium pyruvate tolerance test (PTT) was performed after a 12-hour fasting (overnight), the animals was separated into individual cages. Glucose measurement at time 0 (T0) was performed in duplicate, and then the sodium pyruvate to 50% (2g/kg of weight) was injected intraperitoneally, after this the blood glucose was measured (in duplicate) at 15, 30, 45, 60 and 120 minutes after administration sodium pyruvate (Sigma-Aldrich Brasil Ltda, P2256) (27).

2.7 Insulin sensitivity test

At 90 days of life the insulin sensitivity test (IST) was performed after a 6-hour fast, the animals were separated into individual cages. Glucose measurement at time 0 (T0) was performed in duplicate, and then the insulin (1 mU / g of weight) was injected intraperitoneally, after this the blood glucose was measured (in duplicate) at 15, 30, 45, 60 and 120 minutes after administration of regular human insulin (HUMULIN R, 100UI/ml) (27).

2.8 Evaluation of oxidative stress in hepatic tissue

At 90 days, five animals from each group were sacrificed by guillotine after 6 hours fasting in the clear period (6h at 12h) for collection of the liver. An aliquot of approximately 1 g of the organ was stored in Eppendorf at -80 ° C until sample preparation. The analyzes of oxidative stress were carried out at the

Laboratory of General Biochemistry, molecular and of the exercise of the Academic Center of Vitória, Federal University of Pernambuco.

2.8.1 Preparation of homogenate of liver tissue

Hepatic tissue was homogenized in extraction buffer (50 mM Tris base and 1 mM EDTA, pH 7.4, with addition of 1 mM sodium orthovanadate and 2 mM PMSF). After homogenization, the samples were centrifuged at 1180xg, at 4 ° C, for 10 minutes and the supernatants were submitted to protein quantification.

2.8.2 Protein dosage

The protein concentration was determined by the method of BRADFORD (1976) (28). The principle of the method is based on determining the concentration of peptide bonds by measuring the absorbance of the protein-dye complex. This complex absorbs at wavelength 595 nm. The absorbance is considered directly proportional to the protein concentration in the analyzed solution, where a solution of BSA (2mg / ml) was used as standard.

2.8.3 Evaluation of the levels of lipid peroxidation by the methodology of the substance Reactive to Thiobarbituric Acid

For the TBARS dosage, the colorimetric technique of BUEGE and AUST (1978) (29) was used, being a technique widely used to evaluate the lipoperoxidation, since the thiobarbituric acid reacts with the LPO products, among them malondialdehyde and other aldehydes. The aliquot (300μg protein) of the homogenate was added to 30% trichloroacetic acid (TCA), the material was centrifuged at 1180xg for 10 minutes, the supernatant removed and added to 0.73% thiobarbituric acid which reacted with the products of the lipoperoxidation forming a pink colored compound. The mixture was incubated for 15 minutes at 100 ° C and then cooled, used to read the absorbance at 535nm in a spectrophotometer (Biochrom Libra S12 Visible, USA), using quartz cuvettes. The results were expressed as μM / mg protein.

2.8.4 Evaluation of sulfhydryl-SH content

The sulfhydryl content was determined from the reaction with DTNB (5,5'-dithiobis (2-nitrobenzoic acid) (30). The aliquot of the homogenate (300 μg protein) was incubated in the dark after addition of 10 mM DTNB and the final volume was 1 mL completed with pH 7.4 extraction buffer and read with absorbance (30ºC) at 412 nm in spectrophotometer (Biochrom Libra S12 Visible, USA) The results were expressed as M / mg protein.

2.8.5 Evaluation of the enzymatic activity of Superoxide dismutase (SOD)

The determination of SOD activity was done according to MISRA and FRIDOVICH (1972) (31). Liver homogenate (80 μg protein) was incubated in sodium carbonate buffer (0.05%, pH 10.2, 0.1 mM EDTA) in a water bath at 37 ° C prior to evaluation of the enzymatic activity. The reaction was started by the addition of 20 μM epinephrine (150 mM) in acetic acid (0.05%). The absorbance was read at 480nm for 3 min in

spectrophotometer (Biochrom Libra S12 Visible, USA). The results were expressed as U / mg protein. One unit of SOD was defined as the amount of protein required to inhibit auto-oxidation of 1 μmol epinephrine per minute.

2.8.6 Evaluation of the enzymatic activity of Catalase

The catalase activity was monitored according to AEBI (1984). The principle of the assay is based on the determination of the decomposition constant k of H_2O_2, which in our temperature and pH conditions was set to 4.6 x 10^7. Thus, 0.3M H_2O_2 was added to the sample (80 μg protein), followed by addition of the 50 mM phosphate buffer, pH 7.0 at 20 °C, the decay absorption was monitored for 4 min at 240 nm in a spectrophotometer (Biochrom Libra S12 Visible, USA). The results were expressed as U / mg protein (32). A catalase unit was defined as the amount of protein required to convert 1μmol H_2O_2 to H_2O per minute.

2.8.7 Evaluation of the enzymatic activity of Glutathione S-Transferase

The activity of glutathione S-transferase (GST) is directly proportional to the rate of formation of the compound DNP-SG (dinitrophenyl S glutathione), which can be measured by monitoring the formation rate of the compound. In a 1 mL quartz cuvette, the sample (80 μg protein) was added to the phosphate buffer (0.1M) and EDTA (1mM), GSH (1mM) and CDNB (1mM). The absorbance (340mm) was recorded for a period of approximately 3 min with temperature control (30°C) in a spectrophotometer (Biochrom Libra S12 Visible, USA). The results were expressed as U / mg protein. One unit of GST enzymatic activity was defined as the amount required to catalyze the formation of 1 μmol of the DNP-SG compound per minute (33).

2.8.8 Quantification of redox balance (GSH / GSSG ratio)

The GSH / GSSG ratio was assessed by quantifying the levels of GSH (reduced glutathione) and GSSG (oxidized glutathione) according to the method of HISSIN and HILF (1976). In 0.1M phosphate buffer containing 5 mM EDTA (pH 8.0) was added 100 μg of the liver homogenate and the orthophthaldeide fluorescent (1 μM), and then incubated at room temperature for 15 minutes and read in a FLUOstar Omega spectrofluorimeter (BMG Labtech , USA), using the 350nm excitation and 420nm emission wavelengths. To determine GSSG levels, the sample was incubated with 0.04M N-ethylmaleimide for 30 minutes, followed by the addition of 0.1M NaOH buffer. When the preparations were ready, following the same steps GSH was run to determine GSSG. The redox state was determined by the GSH / GSSG ratio (34).

2.9 Evaluation of ventilatory parameters

Ventilation measurements were obtained by whole-body plethysmography, in a closed system (35), on the 90th day of the offspring life. During each ventilation measurement, airflow was interrupted and the chamber of the animal remained fully sealed for short periods (~ 2 min). The oscillations caused by the ventilation of the animal were captured by a device connected to the chamber containing the pressure differential transducer and the signal amplifier (ML141 spirometer, PowerLab, ADInstruments). The signal was then sent to

the acquisition system and data analysis (LabChartTM Pro, PowerLab, ADInstruments). Three respiratory variables were measured: respiratory frequency (RF), tidal volume (V_T) and ventilation (VE) (36).

2.10 Statistical analysis

The results were expressed as mean ± SEM (standard error of the mean). Normality analysis of the sample was performed using the Kolmogorov-Sminorv test. The comparison between the groups was performed using the one-way ANOVA and two-way ANOVA (for glucose tolerance tests, pyruvate tolerance test and insulin sensitivity test), followed by the Bonferroni post-test. Data were analyzed in the Graph Pad Prism program (GraphPad Software Corporation, version 5.0, 2007). The significance level considered was $p < 0.05$.

3. Results

3.1 Biochemical evaluation of dams s at 19 days of gestation

At 19 days of gestation (Table 3), no significant differences were found in total proteins, albumin, glycemia, triglycerides, cholesterol, VLDL, alanine aminotransferase (ALT), aspartate aminotransferase (AST). However, only the HL group presented increased serum levels of total cholesterol (Control: 73.29±1.74, HL: 90.01±3.27, HLW3: 64.34±4.73/ dL, n = 3-7, p = 0.0005) when compared to the other groups.

3.2 Evaluation of murinometric measurements

At the ages of 1, 7, 14, 21, 30 and 90 days of life were evaluated: body weight, abdominal circumference, naso-anal length and Lee index at 90 days of life. The HL offspring presented higher body mass in the 1st day of life (Control: 6.53 ± 0.15, HL: 7.41 ± 0.17, HLW3: 6.78 ± 0.11g, n = 25-35, p = 0.0003) (figure 1A) when compared to the group control and HLW3, and no difference was found between the control and HLW3 groups. On the 7th (Control: 18.06±0.265, HL: 20.83±0.184, HLW3: 20.08±0.516g, n=26-34; p<0.0001) (figura 1B) and 14th (Control: 32.22±0.396, HL: 34.72±0.522, HLW3: 36.17±0.611g, n=25-34; p<0.0001) (figure 1C) days of life, the HL and HLW3 groups presented higher body mass when compared to the control group. Already in the 21st (Control: 53.93±0.698, HL: 54.39±0.84, HLW3: 57.63±0.75g, n=23-31; p=0.0023) (figure 1D) day of life, the HLW3 group had higher body mass when compared to the other groups. However at 30 (Control: 93.31±2.36, HL: 97±2.29, HLW3: 100.2±2.58g, n=25-35; p=0.1316) (figure 1E) and 90 (Control: 370.1±6.62, HL: 358.1±4.35, HLW3: 380±7.07g, n=27-35; p=0.0680) (figure 1F) days of life, no differences were found between groups.

In the evaluation of the abdominal circumference, in the 1st day of life the offspring HL and HLW3 presented greater abdominal circumference than the control group (Control: 4.38±0.042, HL:4.58±0.04, HLW3: 4.54±0.044cm, n=27-35, p=0.0029) (figure 2A). Alderady at 7 (Control: 6.24±0.50, HL:6.56±0.060, HLW3: 6.38±0.10cm, n=26-35, p=0.0056) (figure 2B) and 14 (Control: 8.13±0.055, HL:8.47±0.075, HLW3: 8.316±0.057cm, n=25-34, p=0.0009) (figure 2C) days of life, only the HL group showed greater circumference when compared to the control group. On the 21st day of life (Control: 10.18±0.085, HL:10.18±0.129, HLW3: 10.14±0.11cm, n=27-35, p=0.9707) (figure 2D) no difference was found between groups. However at 30

(Control: 12.04±0.13, HL:12.67±0.209, HLW3: 11.83±0.191cm, n=27-35, p=0.0046) (figure 2E) days of life, the HL group had a greater abdominal circumference when compared to the other groups. And in the 90th (Control: 19.17±0.29, HL: 18.64±0.28, HLW3: 19.18±0.27cm, n=7-12, p=0.4271) (figure 2F) day of life, there was no difference between the groups.

In relation to the naso-anal length (figure 3), the animals of HL group were larger in relation to the control group and HLW3 in the 1st (Control: 5.14±0.052, HL: 5.43±0.074, HLW3: 5.19±0.068cm, n=25-34; p=0.0041) (figure 3A) and 7st (Control: 7.13±0.059, HL: 7.55±0.053, HLW3: 7.27±0.082cm, n=25-34; p<0.0001) (figure 3B) days of life, no difference was found between the control group and HLW3. In the 14th (Control: 8.685±0.093, HL: 8.81±0.072, HLW3: 8.53±0.065cm, n=25-34; p=0.0771) (figura 3C) day of life no difference was found between groups. Already at 21(Control: 10.58±0.12, n=34, HL: 11.75±0.111, n= 27, HLW3: 11.47±0.18cm, n=25-34; p<0.0001) (figura 3D) days of life, group HL and HLW3 were higher in relation to control. However, the animals of HL group were larger again at 30 days (Control: 13.37 ± 0.183, HL: 15.67 ± 0.341, HLW3: 13.79 ± 0.13cm, n = 27-34; p <0.0001) (figure 3E) when compared to the other groups, and no difference was found in relation to the control and HLW3 groups. Already at 90 days (Control: 22.17±0.54, HL: 22.83±0.38, HLW3: 22.80±0.43cm, n=7-12; p=0,5456) (figure 3F), the groups showed similar body growth, and no difference was found between the groups.

As for Lee's index (figure 4) at the age of 90 days, no difference was found between groups (Control: 0.32 ± 0.007, HL: 0.31 ± 0.005, HLW3: 0.32 ± 0.005cm, n = 7-12; p = 0.8768).

3.3 Biochemical evaluations of offspring at 22, 30 and 90 days of life

At 22 days of life, shown in table 4, no differences were found in serum levels of albumin, total proteins and AST / TGO between the groups. In relation to glucose, only the HL group presented higher levels (Control: 108.6 ± 3.34, HL: 119.3 ± 3.34, HLW3: 117 ± 1.59 mg / dL, n = 25-33, p = 0.0285) when compared to the control group. The HL group also had higher serum levels of total cholesterol (Control: 116.4 ± 1.66, HL: 126.8 ± 3.04, HLW3: 117.3 ± 2.18mg / dL, n = 23-33, p = 0.0030) ALT / TGP (Control: 17.37 ± 0.71, HL: 22.45 ± 1.81, HLW3: 16.99 ± 0.62U / L, n = 24-34, p = 0.0016) and lower levels relative to the ratio AST / ALT (Control: 8.56 ± 0.72, HL: 5.52 ± 0.85, HLW3: 8.63 ± 0.47U / L, n =24-32, p = 0.004) when compared to the other groups. Already the HLW3 group presented lower levels of triglycerides (Control: 138.5 ± 6.13, HL: 124.2 ± 6.88, HLW3: 116.3 ± 4.22mg / dL, n = 26-35, p = 0.0270) and VLDL (Control: 28.41 ± 1.45, HL: 24.85 ± 1.37, HLW3: 23.25 ± 0.84 mg / dL, n = 26, p = 0.0156) when compared to the control group.

At the age of 30 days, shown in table 4, no differences were found between groups in the biochemical parameters evaluated, albumin, glucose, total proteins, total cholesterol, triglycerides, VLDL, hepatic ALT transaminase, AST and AST / ALT ratio.

At 90 days of life, shown in table 5, no differences were found again in serum levels of albumin, total proteins and ALT/TGP between the groups. In relation to glucose, the HL group presented higher levels (Control: 120.8±3.12, HL: 142±3.64, HLW3: 126.3 ±2.94mg/dL, n=26-34, p<0.0001) when compared to the other groups. The HL group also presented higher serum levels of total cholesterol (Control: 80.89 ± 1.54, HL: 87.25 ±1.53, HLW3: 82.77 ±1.66mg/dL, n = 25-35, p = 0.0165), triglycerides (Control: 96.92 ±1.86, HL: 110.3 ±1.55, HLW3:108.5±4.45mg/dL, n = 26-34, p = 0.0294) and VLDL (Control: 19.38±0.37±1.45, HL:

22.07±1.11, HLW3: 21.71±0.88mg / dL, n = 26-34, p =0.0294) when compared to the control group, without difference between the control and HLW3 groups. Already the HLW3 group presented lower levels of AST / TGO (Control: 143 ±4.65, HL: 134.2 ±6.14, HLW3: 115±3.86U/L, n = 25-34, p =0.0007) and AST / ALT ratio (Control: 3.48±0.17, HL: 3.11±0.12, HLW3: 2.56±0.08mg / dL, n =23-34 , p = 0.0002) when compared to the control and HL groups.

3.4 Glucose tolerance test

In the glucose tolerance test, as shown in figure 5, no difference was found in glycemia levels between groups at time 0 (Control: 98.5±2.58, HL: 90.86±2.85, HLW3: 97.2±4.09mg/dL, n=10-18; p>0.05), prior to glucose administration and in the time 15 (Control: 177.70±8.15, HL: 190.93±5.87, HLW3: 195.55±11.41mg/dL, n=9-17; p>0.05), 30 (Control: 179.88±7.85, HL: 166.467±4.236, HLW3: 168.5±4.76mg/dL, n=10-18; p>0.05), 60 (Control: 166±6.90, HL: 160.6±7.20, HLW3: 170.90±9.34mg/dL, n=10-18; p>0.05) and 120 (Control: 105.50±3.41, HL: 96.73±3.65, HLW3: 105.2±4.73mg/dL, n=10-18; p>0.05) minutes after glucose administration.

3.5 Pyruvate tolerance test

In the glucose tolerance test, as shown in figure 6, no difference in glycemia was found between groups at time 0 (Control: 99.27±3.73, HL: 98.2±3.27, HLW3: 93±2.42mg/dL, n=10-18; p>0.05), prior to pyruvate administration and in the time 15 (Control: 119.16±5.44, HL: 116.93±6.05, HLW3: 134±8.11mg/dL, n=9-18; p>0.05), 30 (Control: 123.33±4.87, HL: 126.06±5.66, HLW3: 141.2±9.33mg/dL, n=10-18; p>0.05), 45 (Control: 113.94±3.98, HL: 111.2±5.76, HLW3: 124.1±7.20mg/dL, n=10-18; p>0.05), 60 (Control: 106.61±3.36, HL: 100.86±5.02, HLW3: 116±6.53mg/dL, n=10-18; p>0.05) and 120 (Control: 93.55±3.43, HL: 84.86±4.75, HLW3: 93±5.02mg/dL, n=10-18; p>0.05) minutes after administration of pyruvate.

3.6 Insulin sensitivity test

Regarding the insulin sensitivity test, as shown in figure 7, no difference in glycemia was found between groups at time 0 (Control: 79.50±2.32, HL: 85.64±3.68, HLW3: 91.50±4.03mg/dL, n=10-18; p<0.001), before administration of insulin and in the time 15 (Control: 85.83±3.46, HL: 81.14±5.61, HLW3: 88.8±5.28mg/dL, n=10-18; p>0.05), 30 (Control: 61.05±2.26, HL: 64.14±3.88, HLW3: 60.5±3.51mg/dL, n=10-18; p>0.05) , 45 (Control: 50.33±2.26, HL: 52.07±2.45, HLW3: 52.6±4.09mg/dL, n=10-18; p>0.05) , 60 (Control: 43.94±2.21, HL: 40.92±2.43, HLW3: 41.9±4.00mg/dL, n=10-18; p>0.05) and 120 (Controle: 36.11±2.25, HL: 39.071±2.702, HLW3: 38.1±4.023mg/dL, n=10-18; p>0.05) minutes after insulin administration.

3.7 Evaluation of oxidative stress in the hepatic tissue

Oxidative stress was evaluated in the hepatic tissue of the rats at 90 days of life, from biomarkers such as malondialdehyde (MDA), sulfhydryl and antioxidant enzyme systems: catalase, superoxide dismutase and glutathione transferase and through GSH, GSSG and GSH / GSSG ratio (figure 8). The HL group had higher levels of MDA when compared to the HLW3 group (Control: 38.40 ± 5.84, HL = 52.23 ± 5.61, HLW3 = 33.40 ± 4.22 μM / mg protein, n = 3-4, p = 0.0398) (Figure 8A), and no difference was found between the control and

HLW3 groups. Lower levels of sulfhydryl were found in the HL group when compared to the control group (Control = 0.0115 ± 0.001, HL = 0.0060 ± 0.00004M / mg protein, n = 4-5, p = 0.0034) (Figure 8B). The HLW3 group also had reduced levels of sulfhydryl in relation to the control group (Control = 0.0115 ± 0.001, HLW3 = 0.0066 ± 0.0007 M / mg protein, n = 4-5, p = 0.0114) (figure 8B).

As for the enzymatic systems, no difference was observed in the catalase activity among the offspring (Control = 8.305 ± 0.94, HL = 8.148 ± 1.25, HLW3 = 9.232 ± 1.67U / mg protein, n = 4, p> 0.05) (Figure 8C). However, the HL group presented a lower level of SOD in relation to the control group (Control = 0.853 ± 0.07, HL = 0.64 ± 0.01, HLW3 = 0.7405 ± 0.07U / mg protein, n = 5, p = 0.0225) and GST (Control = 27.94 ± 1.38, HL = 22.05 ± 0.741, HLW3 = 25.38 ± 1.21U / mg protein, n = 4, p = 0.0095) (Figure 8E), whereas the HLW3 group did not differ from the control group. In relation to GSH (Control: 1454±27.18, HL: 692.5±46.9, HLW3: 1018±32.18U/mg de protein, n=5; p<0.0001) (figure 8F) and GSSG (Control: 95.80±3.88, HL: 64.41±1.14, HLW3: 80.89±2.70U/mg de protein, n=5; p<0.0001) (figure 8G), the HL group presented lower levels when compared to the control group and HLW3 and the HLW3 group also presented lower levels when compared to the control group. In relation to the GSH / GSSG ratio, the HL and HLW3 groups presented lower values when compared to the control group (Control: 15.26±0.58, HL: 10.77±0.78, HLW3: 12.62±0.50, n=5; p=0.0011) (figure 8H).

3.8 Evaluation of ventilatory parameters

The ventilatory parameters of the offspring were analyzed at the 90th day of life regarding the respiratory frequency (figure 9), obtaining tidal volume values (figure 10) and ventilation (figure 11). In respiratory frequency no difference was found between groups on the 90th (Control: 98.12 ± 2.69, HL: 103.7 ± 1.71, HLW3: 93.58 ± 3.69cpm, n = 16-26, p = 0.0755) (figure 9) day of life.

In relation the V_T and VE analyzes, also no difference were found between groups in the V_T (Control: 8.33 ± 0.47, HL: 7.87 ± 0.46, HLW3: 7.72 ± 0.35mL / kg, n = 16-28, p = 0.6126) (figure 10) and VE (Control: 837.2 ± 48.69, HL: 801.2 ± 58.22, HLW3: 736.9 ± 49.41mL / kg / min, n = 16-28, p = 0.4313) (figure 11) at 90 days of life.

4 Discussion

In the present study, we observed an increase in body weight at the age of 1, 7 and 14 days of life in the group that received the diet rich in saturated fatty acids (HL) during gestation and lactation when compared to control, as also found in the study in Kojima et al. (2016) (37), already the HLW3 group presented lower body weight in relation to the HL group in the first day of life. It is verified that the ingestion of omega-3 during gestation and lactation can reduce the corporal adiposity in the offspring (38), even in the presence of a hyperlipidic diet (39). However, on the 7th and 14th days of life, the HLW3 group also presented higher body weight when compared to the control and on the 21st day of life the HLW3 group had higher body mass when compared to the other groups, demonstrating the predominant effect of the diet rich in saturated fatty acids, even with the presence of omega-3. However, we found no difference in weight between groups at 30 days and in adult life at 90 days of life. In relation to the abdominal circumference, we found an increase in the

circumference at the age of 1 day of life in the offspring submitted to the hyperlipidic diets when compared to the control, in the 7th and 14th days of life, the HL group had a larger circumference than the control, without difference between the HLW3 and control groups. At 21 days of life, no difference was found between the groups and at the 30th day of life, the HL group again presented a larger circumference when compared to the control and HLW3 groups. It has also been shown in animals that maternal supplementation of DHA during pregnancy and lactation reduces visceral and subcutaneous adipose tissue, which is associated with the risk for the development of obesity (40). The animals of the HL group also presented bigger, with a greater naso-anal length in the 1st and 7th days of life when compared to the other groups, on the 14th day no difference was found between the groups, already in the 21st day of life the groups submitted to the hyperlipidic diets were higher than the control and in the 30th day of life, again the offspring HL presented a longer length when compared to the other groups. However, at 90 days of age, in adulthood, no difference was found in naso-anal length, body weight, abdominal circumference and Lee index between groups, with normalization of anthropometric indicators of adiposity in adult life.

As regards the biochemical parameters evaluated, dams who consumed a diet rich in saturated fatty acids had higher cholesterol levels when compared to the control and HLW3 groups. The offspring of HL dams, at 22 days of age, also presented higher cholesterol levels when compared to the other groups. And the triglyceride levels of the HLW3 offspring were reduced in comparison to the control group. Regarding fasting blood glucose levels, only the HL group presented higher values when compared to the control, the values of the HLW3 group were similar to the control group. At 90 days of age, offspring HL also had higher cholesterol levels than the control group and higher levels of triglycerides and VLDL were found in these animals in relation to the control, without difference when compared to the control and HLW3 groups. At this age too, fasting blood glucose levels were higher in the HL group when compared to the other groups. Since the hyperlipidic diet during gestation and lactation can lead to metabolic alterations such as hyperglycemia, hypertriglyceridemia, elevated LDL-cholesterol and decreased HDL-cholesterol (10) the results found in the biochemical parameters evaluated in the HLW3 group can be justified by the action of the omega-3. Because omega-3 can improve lipid profile by lowering triglyceride levels, total cholesterol and LDL-C fraction (39). Among the mechanisms of action of omega 3, its effect on the decrease of hepatic lipogenesis by inhibition of SREBP (regulatory protein binding to the sterol element) and reduction in the expression of enzymes involved in the synthesis of cholesterol, fatty acids and triglycerides; inhibition of enzymes involved in the hepatic synthesis of triglycerides such as phosphatidic acid phosphatase and diacylglycerol acyltransferase and increased expression of lipoprotein lipase to capture triglycerides from circulating lipoproteins: VLDL and chylomicrons may justify their lipid-lowering action (41). In addition to its lipid-lowering role, omega-3 intake is associated with impaired insulin sensitivity, reduced with age, in the offspring of male rats (19). It was observed that the number of pancreatic islets was higher in the offspring of adult rats than the mothers received a diet rich in omega-3, without alteration in the volume of the pancreas (42).

The animals submitted to the diet rich in saturated fatty acids presented high levels of ALT, whereas animals that received a high-saturated fatty acids diet enriched with omega-3 presented better levels of transaminases at 22 days of life. Already at 90 days of age, the HLW3 group presented lower levels of AST than the control group and HL.The consumption of diet rich in saturated fats increases the gene expression of Scd1 and FadS2 that signal adipogenic pathways in the liver (43), besides inducing and / or aggravating liver injury

with subsequent increase in plasma levels of transaminases (44, 45). In contrast, omega-3 ingestion reduces levels of transaminases, mainly ALT in individuals with non-alcoholic fatty liver disease (46, 47).

The glucose tolerance test, pyruvate and the insulin sensitivity test were performed. The pyruvate tolerance test was performed to evaluate the hepatic production of fasting glucose from gluconeogenesis. Since pyruvate is a common metabolite of the major precursors used in gluconeogenesis (lactate, amino acids and gilcerol) (48). The pyruvate tolerance test also predicts the degree of hepatic insulin sensitivity. In the analyzes of the glucose tolerance, pyruvate and insulin sensitivity tests, no differences were found between the groups at the times evaluated. However, the progression of diabetes mellitus goes through intermediary stages, from altered fasting glycemia and glucose intolerance, and these stages are due to insulin resistance associated with pancreatic β-cell dysfunction. Although we found no difference in these tests, our study verified a tendency to develop diabetes mellitus in the group submitted to a diet rich in saturated fatty acids. Since fasting hyperglycemia at 22 and 90 days of life in these animals may provide evidence of both insulin resistance and beta cell dysfunction (49) and that the oxidative imbalance presented in the HL group at 90 days may be an event prior to the development of diabetes mellitus (50), it is possible that in a more advanced stage, the long term, a impaired of tolerance to glucose, pyruvate and insulin sensitivity can be found in these animals.

In the evaluation of the impact of the diet rich in saturated fatty acids on the parameters of oxidative stress in the liver and the effect of omega 3, we verified that the enrichment of the hyperlipid diet with omega-3, decreased levels of MDA, that being involved with fat visceral, since it is a predictor of lipid peroxidation (51), is also an indicator of cellular damage by the increase of reactive oxygen species (ROS) (52). Also, the enrichment of the omega 3 diet improved the antioxidant capacity, impaired by the diet rich in saturated fatty acids, with normalization of the levels of SOD and GST. No difference was found in relation to the control group and HLW3 in these evaluated enzymes, being these data according to the study of Ramaiyan et al (2016) (53) who also found an improvement in the antioxidant activity in the hepatic tissue of the offspring of dams who were submitted to a hyperlipidic diet with a higher amount of omega-3. In addition, GSH and GSSG levels were higher in the HL group when compared to the control and HLW3 groups. It was found that supplementation with EPA and DHA in mice submitted to the hyperlipid diet improved the levels of inflammatory cytokines (IL-6, IL-1β and TNF-α) and recovered levels of hepatic GSH and glutathione (22). Another study, found that the supplementation of EPA in mice submitted to a hyperlipid diet (32% of lipids) increased the activity of SOD and GSH, which was found reduced in the group that did not receive the supplementation, with decreased also levels of glucose, insulin, total cholesterol, transaminases, cholesterol deposition and hepatic triglycerides. The groups that were submitted to hyperlipidic diets (rich in saturated fatty acids) had a lower GSH / GSSG ratio in relation to the control group. A high proportion of GSH / GSSG protects the cell against oxidative damage, breaking that ratio leads to the activation of redox sensitive transcription factors such as NF-κB, which acts on the inflammatory response (50).

Little is known about the effects of the maternal hyperlipid diet and the omega-3 in the process of maturation of the respiratory system. It has already been verified that maternal protein malnutrition is related to an increase in respiratory rate at 30 and 90 days of life, as a mechanism underlying the installation of arterial hypertension (54). The study by Guimarães et al. (2017), like our findings, also found no differences in baseline respiratory rate, tidal volume, and pulmonary ventilation at 90 days of life of animals receiving a hyperlipidic diet during gestation and lactation. In contrast, when these same animals were exposed to the hypercapnia

condition (7% CO2), there was an increase in respiratory rate and pulmonary ventilation, suggesting a greater sensitization of chemoreceptors (55).

In conclusion, the present study demonstrates that maternal consumption of a diet rich in saturated acids is associated with losses in some murinometric parameters, biochemical and oxidative imbalance, with reduction of enzymes that participate in the antioxidant defense system. However, no changes were observed in the animals that were submitted to this diet, in the tests of tolerance to glucose, pyruvate, insulin sensitivity and in the respiratory parameters at 90 days of life, which could occur later, considering the risk factors found in these animals as: dyslipidemia, hyperglycemia and oxidative imbalance. In addition, our findings confirm that the consumption of omega 3 from flaxseed oil is able to attenuate some changes observed in animals that received a diet rich in saturated fatty acids during gestation and lactation, which are present in the diseases cardiovascular and metabolic. More studies are needed to evaluate the mechanisms related to metabolic and respiratory changes caused by the intake of a hyperlipidic diet, as well as the role of the omega-3 during the perinatal period. A complete understanding of these mechanisms and the role of omega-3 e do papel do ômega-3 sobre os mesmos may lead to the development of therapeutic strategies and government policies to improve public health.

References

1. Batista Filho M, Batista LV. Transição alimentar/ nutricional ou mutação antropológica? Ciência e Cultura. 2010;62:26-30.
2. Gruber L, Hemmerling J, Schuppel V, Muller M, Boekschoten MV, Haller D. Maternal High-fat Diet Accelerates Development of Crohn's Disease-like Ileitis in TNFDeltaARE/WT Offspring. Inflammatory bowel diseases. 2015;21(9):2016-25. Epub 2015/08/19.
3. Pirola L, Ferraz JC. Role of pro- and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity. World journal of biological chemistry. 2017;8(2):120-8. Epub 2017/06/08.
4. Paton JFR, Waki H. Is neurogenic hypertension related to vascular inflammation of the brainstem? Neuroscience & Biobehavioral Reviews. 2009;33(2):89-94.
5. Waki H, Gouraud SS, Maeda M, Paton JF. Evidence of specific inflammatory condition in nucleus tractus solitarii of spontaneously hypertensive rats. Experimental physiology. 2010;95(5):595-600. Epub 2009/11/20.
6. Barros MA, De Brito Alves JL, Nogueira VO, Wanderley AG, Costa-Silva JH. Maternal low-protein diet induces changes in the cardiovascular autonomic modulation in male rat offspring. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2015;25(1):123-30. Epub 2014/10/08.
7. de Brito Alves JL, Nogueira VO, Cavalcanti Neto MP, Leopoldino AM, Curti C, Colombari DS, et al. Maternal protein restriction increases respiratory and sympathetic activities and sensitizes peripheral chemoreflex in male rat offspring. The Journal of nutrition. 2015;145(5):907-14. Epub 2015/05/03.
8. Alves J, Maricelly Deodato de Oliveira J, Ferreira Dj, Assis V de Barros M, Nogueira V, Santos Alves D, et al. Maternal protein restriction induced-hypertension is associated to oxidative disruption at transcriptional and functional levels in the medulla oblongata2016.
9. Bardgett ME, Sharpe AL, Toney GM. Activation of corticotropin-releasing factor receptors in the rostral ventrolateral medulla is required for glucose-induced sympathoexcitation. American Journal of Physiology-Endocrinology and Metabolism. 2014;307(10):E944-E53.
10. Vidal-Santos R, Macedo FN, Santana MN, De Melo VU, de Brito Alves JL, Santos MR, et al. Western diet in the perinatal period promotes dysautonomia in the offspring of adult rats. Journal of developmental origins of health and disease. 2017;8(2):216-25. Epub 2016/12/10.
11. Moraes DJ, Bonagamba LG, Costa KM, Costa-Silva JH, Zoccal DB, Machado BH. Short-term sustained hypoxia induces changes in the coupling of sympathetic and respiratory activities in rats. The Journal of physiology. 2014;592(9):2013-33. Epub 2014/03/13.
12. Costa-Silva JH, de Brito-Alves JL, Barros MA, Nogueira VO, Paulino-Silva KM, de Oliveira-Lira A, et al. New Insights on the Maternal Diet Induced-Hypertension: Potential Role of the Phenotypic Plasticity and Sympathetic-Respiratory Overactivity. Frontiers in physiology. 2015;6:345. Epub 2015/12/05.

13. Costa-Silva JH, Zoccal DB, Machado BH. Chronic intermittent hypoxia alters glutamatergic control of sympathetic and respiratory activities in the commissural NTS of rats. American Journal of Physiology : Regulatory, Integrative and Comparative Physiology. 2012;302(6):R785-R93.
14. Howe PRC. Dietary Fats and Hypertension Focus on Fish Oil. Annals of the New York Academy of Sciences. 1997;827(1):339-52.
15. Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. The American journal of clinical nutrition. 2000;71(1 Suppl):349S-51S. Epub 2000/01/05.
16. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation. 1993;88(2):523-33.
17. Appel LJ, Miller ER, Iii, Seidler AJ, Whelton PK. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. Archives of Internal Medicine. 1993;153(12):1429-38.
18. Korotkova M, Gabrielsson B, Lonn M, Hanson LA, Strandvik B. Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet. Journal of lipid research. 2002;43(10):1743-9. Epub 2002/10/05.
19. Sardinha FLC, Fernandes FS, Tavares do Carmo MG, Herrera E. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2012;304(4):R313-R20.
20. Khaire AA, Kale AA, Joshi SR. Maternal omega-3 fatty acids and micronutrients modulate fetal lipid metabolism: A review. Prostaglandins, leukotrienes, and essential fatty acids. 2015;98:49-55. Epub 2015/05/11.
21. Dossi CG, Tapia GS, Espinosa A, Videla LA, D'Espessailles A. Reversal of high-fat diet-induced hepatic steatosis by n-3 LCPUFA: role of PPAR-α and SREBP-1c. The Journal of nutritional biochemistry. 2014;25(9):977-84.
22. Valenzuela R, Espinosa A, González-Mañán D, D'Espessailles A, Fernández V, Videla LA, et al. N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. PloS one. 2012;7(10):e46400-e.
23. Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of nutrition. 1993;123(11):1939-51. Epub 1993/11/01.
24. Ferro Cavalcante TC, Lima da Silva JM, da Marcelino da Silva AA, Muniz GS, da Luz Neto LM, Lopes de Souza S, et al. Effects of a westernized diet on the reflexes and physical maturation of male rat offspring during the perinatal period. Lipids. 2013;48(11):1157-68. Epub 2013/09/21.
25. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. Laboratory animals. 2007;41(1):111-9. Epub 2007/01/20.
26. Sposito AC, Caramelli B, Fonseca FAH, Bertolami MC, Afiune Neto A, Souza AD, et al. IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose: Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. Arquivos brasileiros de cardiologia. 2007;88:2-19.
27. Barquissau V, Capel F, Dardevet D, Feillet-Coudray C, Gallinier A, Chauvin M-A, et al. Reactive oxygen species enhance mitochondrial function, insulin sensitivity and glucose uptake in skeletal muscle of senescence accelerated prone mice SAMP8. Free Radical Biology and Medicine. 2017;113:267-79.
28. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976;72(1):248-54.
29. Buege JA, Aust SD. [30] Microsomal lipid peroxidation. In: Fleischer S, Packer L, editors. Methods in enzymology: Academic Press; 1978. p. 302-10.
30. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959;82(1):70-7.
31. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of biological chemistry. 1972;247(10):3170-5.
32. Aebi H. [13] Catalase in vitro. Methods in enzymology: Academic Press; 1984. p. 121-6.
33. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-Transferases: THE FIRST ENZYMATIC STEP IN MERCAPTURIC ACID FORMATION. Journal of Biological Chemistry. 1974;249(22):7130-9.
34. Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Analytical biochemistry. 1976;74(1):214-26.
35. Bartlett D, Tenney SM. Control of breathing in experimental anemia. Respiration Physiology. 1970;10(3):384-95.
36. Malan A. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. Respir Physiol. 1973;17(1):32-44. Epub 1973/01/01.
37. Kojima S, Catavero C, Rinaman L. Maternal high-fat diet increases independent feeding in pre-weanling rat pups2016.

38. Muhlhausler BS, Gibson RA, Makrides M. The effect of maternal omega-3 long-chain polyunsaturated fatty acid (n-3 LCPUFA) supplementation during pregnancy and/or lactation on body fat mass in the offspring: A systematic review of animal studies. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2011;85(2):83-8.
39. Waddell BJ, Zulkafli IS, Mark PJ. Postnatal Dietary Omega-3 Fatty Acid Supplementation Rescues Glucocorticoid-Programmed Adiposity, Hypertension, and Hyperlipidemia in Male Rat Offspring Raised on a High-Fat Diet. Endocrinology. 2013;154(9):3110-7.
40. Bagley HN, Wang Y, Campbell MS, Yu X, Lane RH, Joss-Moore LA. Maternal docosahexaenoic acid increases adiponectin and normalizes IUGR-induced changes in rat adipose deposition. Journal of obesity. 2013;2013:312153. Epub 2013/03/28.
41. Backes J, Anzalone D, Hilleman D, Catini J. The clinical relevance of omega-3 fatty acids in the management of hypertriglyceridemia. Lipids in health and disease. 2016;15(1):118-.
42. Siemelink M, Verhoef A, Dormans JA, Span PN, Piersma AH. Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. Diabetologia. 2002;45(10):1397-403. Epub 2002/10/16.
43. Leikin-Frenkel A, Shomonov-Wagner L, Juknat A, Pasmanik-Chor M. Maternal Diet Enriched with α-Linolenic or Saturated Fatty Acids Differentially Regulates Gene Expression in the Liver of Mouse Offspring. Lifestyle Genomics. 2015;8(4-6):185-94.
44. Tain Y-L, Lin Y-J, Sheen J-M, Yu H-R, Tiao M-M, Chen C-C, et al. High Fat Diets Sex-Specifically Affect the Renal Transcriptome and Program Obesity, Kidney Injury, and Hypertension in the Offspring. Nutrients. 2017;9(4):357.
45. Kai M, Miyoshi M, Fujiwara M, Nishiyama Y, Inoue T, Maeshige N, et al. A lard-rich high-fat diet increases hepatic peroxisome proliferator–activated receptors in endotoxemic rats. Journal of Surgical Research. 2017;212:22-32.
46. He X-X, Wu X-L, Chen R-P, Chen C, Liu X-G, Wu B-J, et al. Effectiveness of Omega-3 Polyunsaturated Fatty Acids in Non-Alcoholic Fatty Liver Disease: A Meta-Analysis of Randomized Controlled Trials. PloS one. 2016;11(10):e0162368-e.
47. Yu L, Yuan M, Wang L. The effect of omega-3 unsaturated fatty acids on non-alcoholic fatty liver disease: A systematic review and meta-analysis of RCTs. Pakistan journal of medical sciences. 2017;33(4):1022-8.
48. Viollet B, Guigas B, Leclerc J, Hébrard S, Lantier L, Mounier R, et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. Acta physiologica (Oxford, England). 2009;196(1):81-98. Epub 2009/02/19.
49. Davies MJ, Raymond NT, Day JL, Hales CN, Burden AC. Impaired glucose tolerance and fasting hyperglycaemia have different characteristics. Diabetic Medicine. 2000;17(6):433-40.
50. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. The World Allergy Organization journal. 2012;5(1):9-19.
51. Lee SM, Cho YH, Lee SY, Jeong DW, Cho AR, Jeon JS, et al. Urinary Malondialdehyde Is Associated with Visceral Abdominal Obesity in Middle-Aged Men. Mediators of inflammation. 2015;2015:6.
52. França BK, Melo Alves MR, Silveira Souto FM, Tiziane L, Freire Boaventura R, Guimarães A, et al. Peroxidação lipídica e obesidade: Métodos para aferição do estresse oxidativo em obesos. GE Jornal Português de Gastrenterologia. 2013;20(5):199-206.
53. Ramaiyan B, Bettadahalli S, Talahalli RR. Dietary omega-3 but not omega-6 fatty acids down-regulate maternal dyslipidemia induced oxidative stress: A three generation study in rats. Biochemical and biophysical research communications. 2016;477(4):887-94.
54. de Brito Alves JL, Nogueira VO, de Oliveira GB, da Silva GSF, Wanderley AG, Leandro CG, et al. Short- and long-term effects of a maternal low-protein diet on ventilation, O2/CO2 chemoreception and arterial blood pressure in male rat offspring. British Journal of Nutrition. 2014;111(4):606-15. Epub 2013/09/23.
55. Guimarães KSL, de Araújo EV, Aquino JS, Gadelha DA, Balarini CM, Costa-Silva JH, et al. Effect of maternal dyslipidaemia on the cardiorespiratory physiology and biochemical parameters in male rat offspring. British Journal of Nutrition. 2017;118(11):930-41. Epub 2017/11/27.

Figure captions

Figure 1. Body mass on the 1st, 7th, 14th, 21st, 30th and 90th days of life of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-way ANOVA test and Bonferroni post-test for the evaluation periods of 1 day of life (A), 7 days of life (B), 14 days of life (C), 21 days of life (D), 30 days of life (E) and 90 days of life (F). (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n=23-35).

Figure 2. Abdominal circumference on the 1st, 7th, 14th, 21st, 30th and 90th days of life of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-way ANOVA test and Bonferroni post-test for the evaluation periods of 1 day of life (A), 7 days of life (B), 14 days of life (C), 21 days of life (D), 30 days of life (E) and 90 days of life (F). (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n=7-35).

Figure 3. Naso-anal length on the 1st, 7th, 14th, 21st, 30th and 90th days of life of male offspring whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-way ANOVA test and Bonferroni post-test for the evaluation periods of the 1st day of life (A), 7th day of life (B), 14th day of life (C), 21st day of life (D), 30th day of life (E) and 90th day of life (F). (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 7-34).

Figure 4. Lee index on the 90th day of life of male offspring whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 7-12).

Figure 5. Glucose tolerance test at 90 days of offspring of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty

acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. Glucose was measured at time 0 before glucose administration (after 6 hours of fasting) at time 15, 30, 60 and 120 minutes after glucose administration. The black line represents the control group, the dashed line the HL group and the gray line the HLW3 group.The graphs were expressed as mean ± SEM, after the two-way ANOVA test and Bonferroni post-test.(* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 10-18).

Figure 6. Pyruvate tolerance test at 90 days of offspring of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. Glucose was measured at time 0 before glucose administration (after 6 hours of fasting) at time 15, 30, 45, 60 and 120 minutes after pyruvate administration. The black line represents the control group, the dashed line the HL group and the gray line the HLW3 group.The graphs were expressed as mean ± SEM, after the two-Way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 10-18).

Figure 7. Insulin sensitivity test at 90 days of offspring of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. Glucose was measured at time 0 before glucose administration (after 6 hours of fasting) at time 15, 30, 45, 60 and 120 minutes after insulin administration. The black line represents the control group, the dashed line the HL group and the gray line the HLW3 group.The graphs were expressed as mean ± SEM, after the two-Way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 10-18).

Figure 8. Evaluation of oxidative stress in hepatic tissue as to the concentration of malondialdehyde, sulfhydryl, catalase, superoxide dismutase, glutathione transferase, reduced glutathione, oxidized glutathione and reduced and oxidized glutathione ratio at 90 days of offspring whose dams were submitted to the control diet, HL and HLW3. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-way ANOVA test and Bonferroni post-test. A = Malondialdehyde (MDA); B = Sulfhydryl; C = Catalase; D = Superoxide dismutase; E = Glutathione S-Transferase (GST); F = reduced glutathione (GSH); G= oxidized glutathione (GSSG) e and GSH/GSSG ratio (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 3-5).

Figure 9. Evaluation of the respiratory rate on the 90th day of life of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group.

The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-Way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 16-28).

Figura 10. Assessment of tidal volume on the 90th day of life of male offspring whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-Way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 16-28).

Figure 11. Evaluation of pulmonary ventilation on the 90th day of life of males whose dams were submitted to control, HL and HLW3 diets during gestation and lactation. The rats were submitted during gestation and lactation to control diet (19% lipids) or high-fat diet (rich in saturated fatty acids - 33% lipids), HL, or high in saturated fatty acids enriched with omega-3 with 3.5% linseed oil, HLW3, according to the experimental group. The white bars represent the control group, the black group HL and the gray group HLW3. The graphs were expressed as mean ± SEM, after the one-Way ANOVA test and Bonferroni post-test (* $p<0.05$ *vs.* Control, # $p<0.05$ *vs.* HL; n= 16-28).

Fig. 1

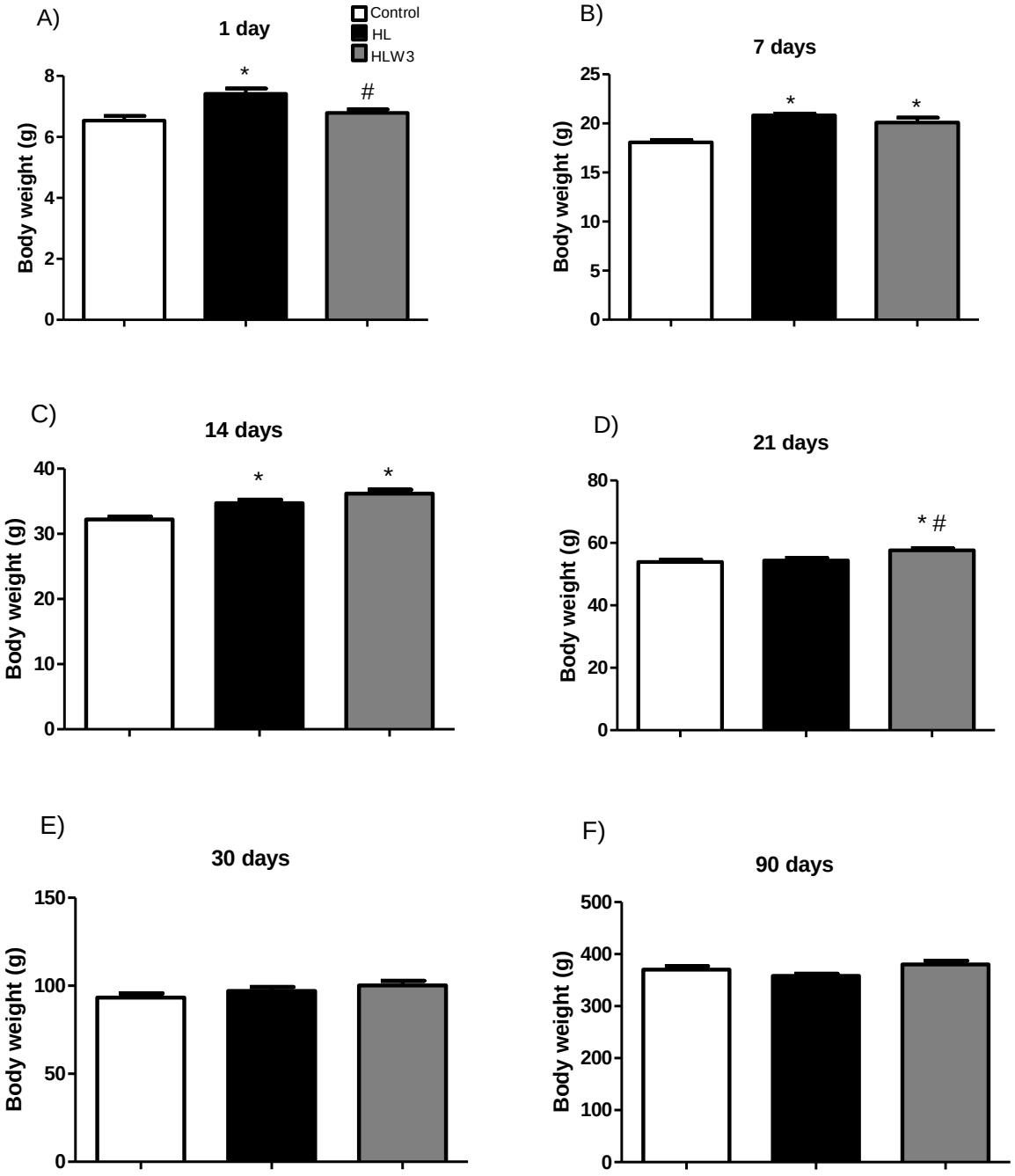

A)
1 day
Control
HL
HLW3
Body weight (g)
0
2
4
6
8
*
#
B)
7 days
Body weight (g)
0
5
10
15
20
25
*
*
C)
14 days
Body weight (g)
0
10
20
30
40
*
*
D)
21 days
Body weight (g)
0
20
40
60
80
* #
E)
30 days
Body weight (g)
0
50
100
150
F)
90 days
Body weight (g)
0
100
200
300
400
500

Fig. 2

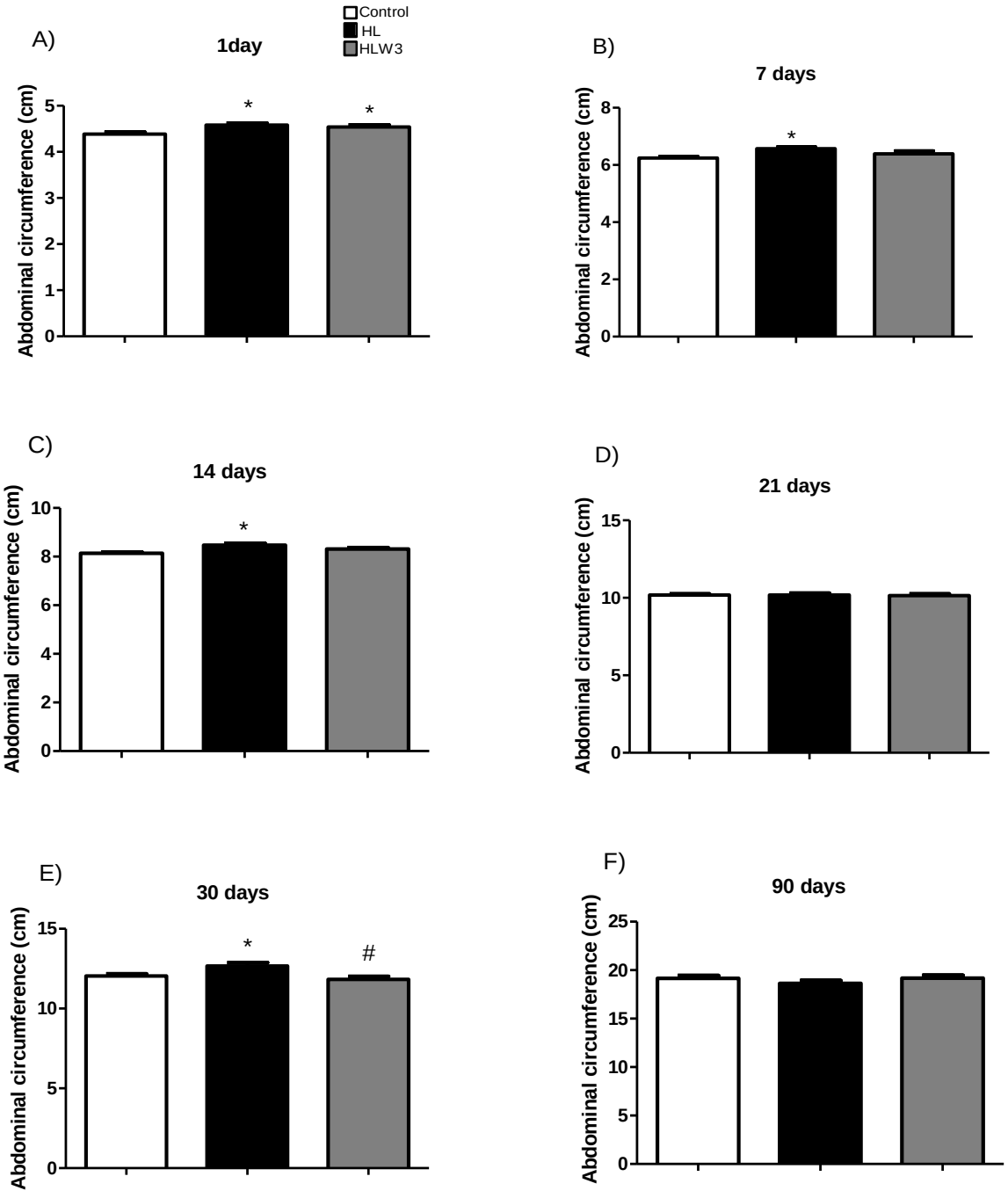

A)
1day
Control
HL
HLW3
Abdominal circumference (cm)
0
1
2
3
4
5
*
*
B)
7 days
Abdominal circumference (cm)
0
2
4
6
8
*
C)
14 days
Abdominal circumference (cm)
0
2
4
6
8
10
*
D)
21 days
Abdominal circumference (cm)
0
5
10
15
E)
30 days
Abdominal circumference (cm)
0
5
10
15
*
#
F)
90 days
Abdominal circumference (cm)
0
5
10
15
20
25

Fig. 3

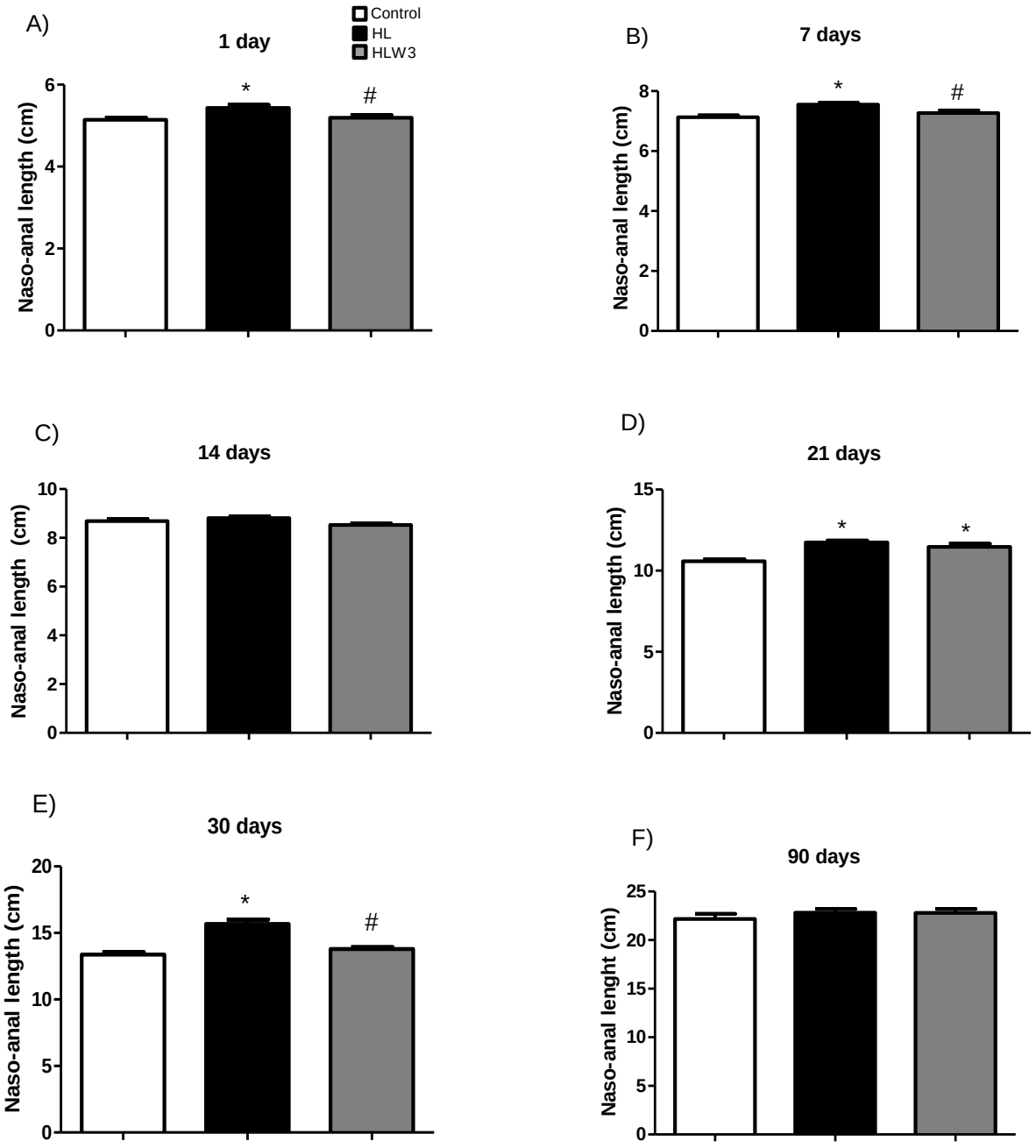

A)
1 day
Control
HL
HLW3
Naso-anal length (cm)
0
2
4
6
*
#
B)
7 days
Naso-anal length (cm)
0
2
4
6
8
*
#
C)
14 days
Naso-anal length (cm)
0
2
4
6
8
10
D)
21 days
Naso-anal length (cm)
0
5
10
15
*
*
E)
30 days
Naso-anal length (cm)
0
5
10
15
20
*
#
F)
90 days
Naso-anal lenght (cm)
0
5
10
15
20
25

Fig. 4

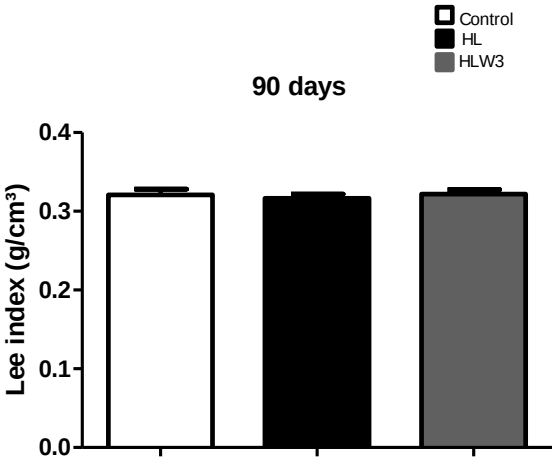


Fig. 5

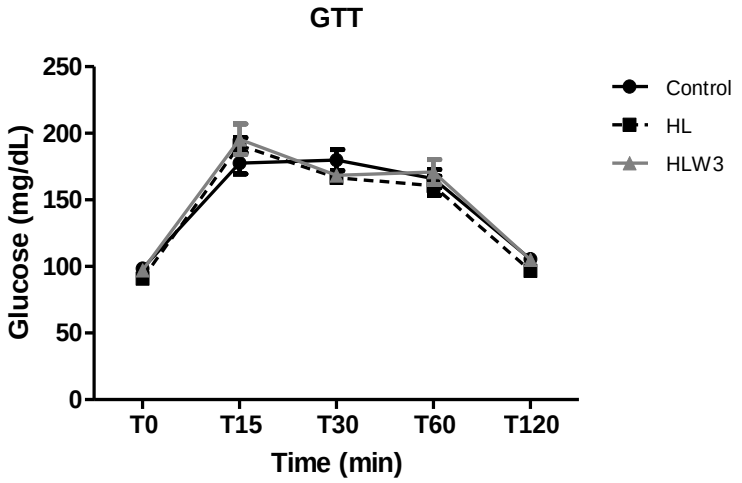


Fig. 6

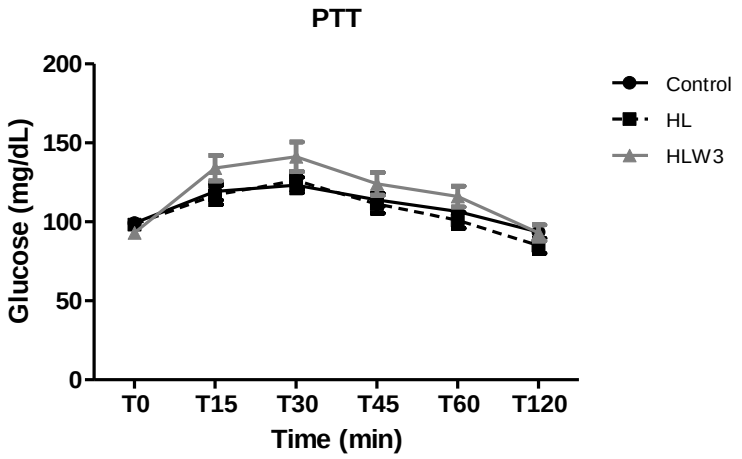


Fig. 7

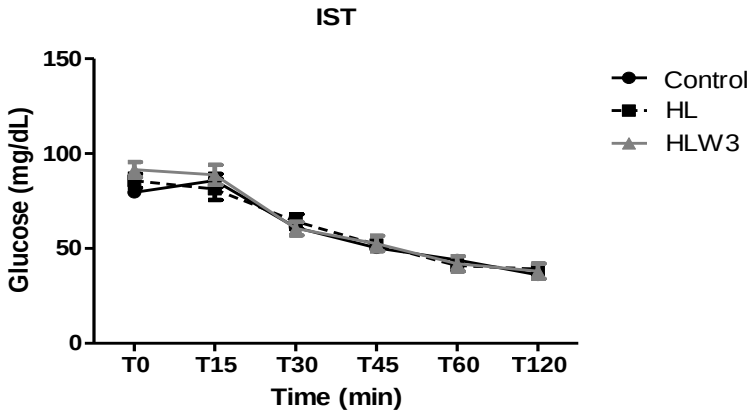
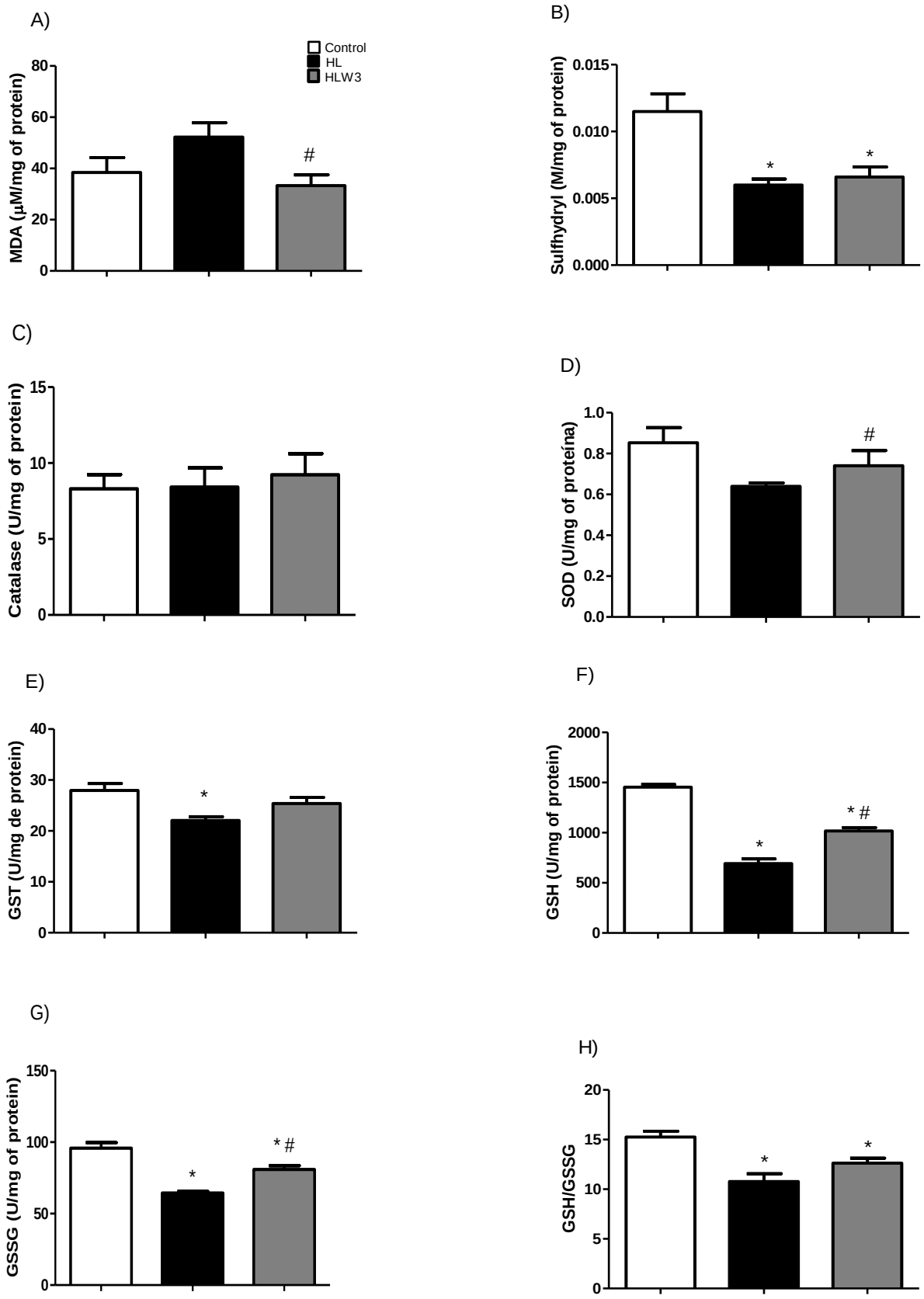


Fig. 8


A)
Control
HL
HLW3
MDA (µM/mg of protein)
80
60
40
20
0
#
B)
Sulfhydryl (M/mg of protein)
0.015
0.010
0.005
0.000
*
*
C)
Catalase (U/mg of protein)
15
10
5
0
D)
SOD (U/mg of proteína)
1.0
0.8
0.6
0.4
0.2
0.0
#
E)
GST (U/mg de protein)
40
30
20
10
0
*
F)
GSH (U/mg of protein)
2000
1500
1000
500
0
*
* #
G)
GSSG (U/mg of protein)
150
100
50
0
*
* #
H)
GSH/GSSG
20
15
10
5
0
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*

Fig. 9

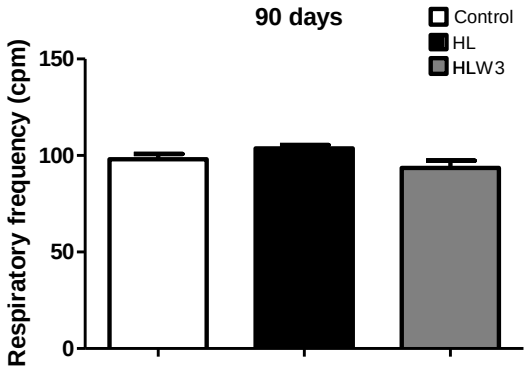

Fig. 10

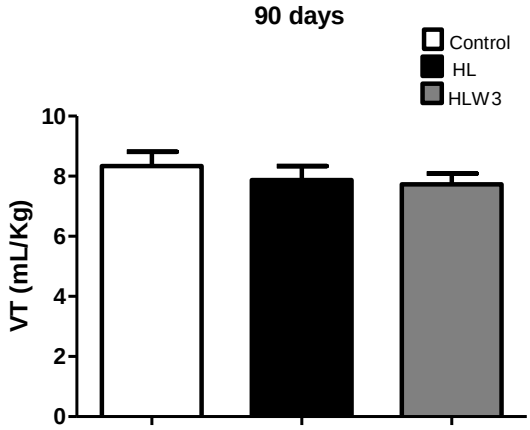

Fig. 11

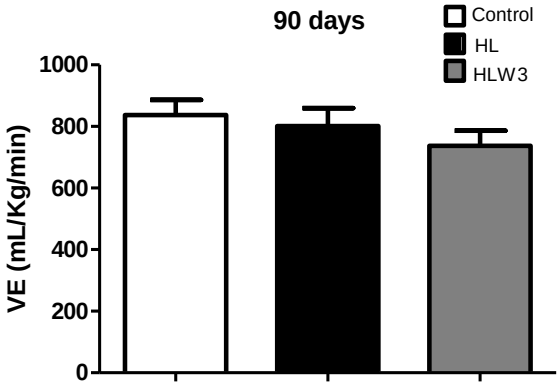

Tabela 1. Composition regarding the ingredients of experimental diets

Ingredient / quantity per 100g of diet	AIN-93 G	Hyperlipid diet	Hyperlipidic diet enriched with omega 3
Cornstarch	39,7	15	15
Dextrinized cornstarch	13,2	-	-
Wheat flour	-	12	12
Cornflour biscuit	-	7	7
Soy flour	-	6	6
Lard	-	2	2
Butter	-	8	8
Casein (>85%)	20	20	20
Guar gum	-	0,5	0,5
Sucrose	10	18	18
Linseed oil	-	-	3,5
Soybean oil	7	7	3,5
Fiber (cellulose)	5	0,3	0,3
Vitamin mix	1	0,7	0,7
Mineral mix	3,5	2,5	2,5
DL-methionine	0,3	0,25	0,25
Choline bitartrate	0,25	0,25	0,25
BTH	0,0014	0,014	0,014
Monosodium glutamate (12,3%)	-	0,2	0,2
Sodium Chloride	-	0,3	0,3
Total (g)	100	100	100
kJ /100g	15,43	18,91	18,86
% Total fat	18,6	33,6	32,3
% Proteins	20,2	19,6	18,2
% Carbohydrates	61	46,8	49, 4

The AIN-93G diet was adapted as recommended by Reeves et al., 1993; The hyperlipid diet was adapted from the study of Ferro Cavalcante et al., 2013; The nutritional composition of calories and caloric percentage of fats, proteins and carbohydrates was determined from the centesimal analysis of the diets carried out in the Laboratory of Bromatology of the Academic Center of Vitória of the Federal University of Pernambuco.

Tabela 2. Percent composition of fatty acids as for presence of double bond in the carbonic chain of experimental diets

	Percent composition of fatty acids Diets		
Fatty acids	AIN-93G	Hyperlipidic	Hyperlipidic with omega 3
Saturated			
Octanoic acid (C8:0)	0	0	0
Decanoic acid (C10:0)	0	0,85	0,74
Lauric acid (C12:0)	0	1,16	1,15
Myristic acid (C14:0)	0	4,22	4,37
Pentadecanoate acid (C15:0)	0	0,47	0
Palmitic acid (C16:0)	11,97	22,78	21,73
Heptadecanoate acid (C17:0)	0	0	0
Stearic acid (C18:0)	4,62	7,53	8,07
Arachidic acid (C20:0)	0,36	0	0
Behenic acid (C22:0)	0,37	0	0
Total	17,32	37,01	36,06
Monounsaturated			
Myristoleic acid (C14:1)	0	0,46	0
Palmitoleic acid (C16:1)	0	0,95	0,87
Heptadecanoic acid (C17:1)	0	0	0
Oleic acid (C18:1)	29,93	28,32	38,47
Total	29,93	29,73	39,34
Polyunsaturated			
Linolenic acid (C18:3)	3,89	1,64	2,32
Linoleic acid (C18:2)	48,87	31,63	22,28
Total	52,76	33,27	24,6
Omega 3 (g/100g)	0,27	0,27	0,4
Ômega 6 (g/100g)	3,42	5,73	3,78
Ômega 6:3 ratio	**12,66**	**21,22**	**9,45**

The fatty acids were identified according to external standard (FAME Supelco ™ mix C4-C24, Bellefonte, PA, USA) and the percentage (%) calculated according to the normalization of the areas of the peaks by the gas chromatography method in the Laboratory of Phytochemicals and processes of the Center of Technologies and Strategies of the Northeast. From the percentage determination of linolenic (omega 3) and linoleic acid (omega 6) the amounts (g/100g of diet) of these fatty acids were calculated and the omega 6/3 ratio was obtained. The nomenclature of fatty acids followed the classification determined by the I Guideline on the consumption of fats and cardiovascular health (Santos et al., 2013).

Table 3. Fasting biochemical profile at 19 days of gestation of the dams who were submitted to the control diets, with a high content of saturated fatty acids and with a high content of saturated fatty acids enriched with ômega-3

Age / Variables	Group			P
	Control	HL	HLW3	
19 days of gestation				
Albumin (g/dL)	4.82±0.11	4.76±0.13	4.58±0.19	0.5708
Total protein (g/dL)	6.56±0.27	6.27±0.11	5.98±0.12	0.1138
Cholesterol (mg/dL)	73.29±1.74	90.01±3.27*	34.64±4.73#	**0.0005**
Triglycerides (mg/dL)	145.7±21.31	121.5±17.61	119±17.58	0.6519
VLDL (mgL)	34.36±6.17	24.30±3.52	23.80±3.51	0.2193
Glucose (mg/dL)	120.8±4.11	129.4±6.79	109±10.58	0.1943
ALT	49.33 ±18.87	54.53 ±10.69	37.82±6.24	0.5626
AST	94.89±24.34	131.3±8.03	97.23±12.41	0.0688
AST/ALT	2.03±0.28	2.86±0.50	2.15±0.72	0.6212

The rats were submitted during gestation to a control diet (19% lipids) or with a high content of saturated fatty acids, HL, with 33% lipids or with a high content of saturated fatty acids enriched with omega-3 with 3.5 % flaxseed oil, HLW3, according to the experimental group..Values were expressed as mean ± SEM. There was no statistical difference after the application of One-Way ANOVA and Bonferroni post-test (N = 2-9).* p<0.05 *vs.* Control; # p<0.05 *vs.* HL.

Table 4. Fasting biochemical profile at 22 and 30 days of the offspring of males whose mothers were submitted to control diets, with a high content of saturated fatty acids and with a high content of saturated fatty acids enriched with omega-3 during gestation and lactation.

Age/ Variables	Group			P
	Control	HL	HLW3	
22 days of life				
Albumin (g/dL)	4.06±0.02	4.1 ±0.00	4.16± 0.02	0.0660
Total protein (g/dL)	5.26 ±0.02	5.27± 0.03	5.29± 0.03	0.7678
Cholesterol (mg/dL)	116.4 ± 1.66	126.8 ±3.04*	117.3 ±2.18#	**0.0030**
Triglycerides (mg/dL)	138.5 ±6.13	124.2 ±6.88	116.3 ±4.22*	**0.0270**
VLDL (mg/dL)	28.41±1.45	24.85±1.37	23.25±0.84*	**0.0156**
Glucose (mg/dL)	108.6±3.34	119.3±3.34*	117 ±1.59	**0.0285**
ALT	17.37 ±0.71	22.45 ±1.81*	16.99±0.62#	**0.0016**
AST	152.2 ±4.75	137.8 ±3.87	142.4±4.40	0.0755
AST/ALT	8.56±0.72	5.52±0.85*	8.63±0.47#	**0.0040**
30 days of life				
Albumin (g/dL)	4.26±0.02	4.19±0.03	4.29±0.03	0.0547
Total protein (g/dL)	5.32± 0.03	5.33± 0.03	5.42±0.05	0.1597
Cholesterol (mg/dL)	119.9 ±2.12	123.4±2.64	125.5±2.98	0.2828
Triglycerides (mg/dL)	162.5 ±11.46	151.6 ±10.08	183.5 ±15.17	0.2251
VLDL (mg/dL)	28.79±2.69	22.3±2.76	24.04±3.84	0.2872
Glucose (mg/dL)	90.58 ±5.12	88.41±3.06	100.5±4.36	0.1623
ALT	30.83 ±1.38	34.08 ±2.70	38.11 ±2.48	0.0615
AST	149± 4.44	143.3 ±6.72	141.7 ±0.04	0.5114
AST/ALT	4.96 ±0.37	4.45 ±0.62	3.88 ±0.41	0.2569

The rats were submitted during gestation to a control diet (19% lipids) or with a high content of saturated fatty acids, HL, with 33% lipids or with a high content of saturated fatty acids enriched with omega-3 with 3.5 % flaxseed oil, HLW3, according to the experimental group. Values were expressed as mean ± SEM. There was no statistical difference after the application of One-Way ANOVA and Bonferroni post-test (N = 19-35). * p<0.05 *vs.* Control; # p<0.05 *vs.* HL.

Table 5. A fasting biochemical profile at 90 days of offspring of males whose mothers were submitted to the control diets, with a high content of saturated fatty acids and with a high content of saturated fatty acids enriched with omega-3 during gestation and lactation.

Age/ Variables	Group			P
	Control	HL	HLW3	
90 days of life				
Albumin (g/dL)	4.43±0.03	4.52 ±0.04	4.41± 0.04	0.1679
Total protein (g/dL)	5.39 ±0.04	5.13± 0.11	5.25± 0.09	0.1864
Cholesterol (mg/dL)	80.89 ± 1.54	87.25 ±1.53*	82.77 ±1.66	**0.0165**
Triglycerides (mg/dL)	96.92 ±1.86	110.3 ±1.55*	108.5 ±4.45	**0.0294**
VLDL (mg/dL)	19.38±0.37	22.07±1.11*	21.71±0.88	**0.0294**
Glucose (mg/dL)	120.8±3.12	142±3.64*	126.3 ±2.94#	**<0.0001**
ALT	43.95±1.86	44.02 ±1.88	45.28±1.10	0.8465
AST	143 ±4.65	134.2 ±6.14	115±3.86*#	**0.0007**
AST/ALT	3.48±0.17	3.11±0.12	2.56±0.08*#	**0.0002**

The rats were submitted during gestation to a control diet (19% lipids) or with a high content of saturated fatty acids, HL, with 33% lipids or with a high content of saturated fatty acids enriched with omega-3 with 3.5 % flaxseed oil, HLW3, according to the experimental group. Values were expressed as mean ± SEM. There was no statistical difference after the application of One-Way ANOVA and Bonferroni post-test (N = 23-35). * $p<0.05$ *vs.* Control; # $p<0.05$ *vs.* HL.

7 SUMÁRIO DOS RESULTADOS

Nossos resultados demonstram que o consumo materno de dieta rica em ácidos graxos saturados está associado a um maior peso corporal na idade de 1, 7 e 14 dias de vida quando comparado ao grupo controle. Já o grupo HLW3 apresentou menor peso corporal em relação ao grupo HL no 1º dia de vida. Porém no 7º, 14º, 21º dia de vida, o grupo HLW3 também apresentou maior peso corporal quando comparado ao controle e aos 21 dias de idade apresentou maior peso corporal quando comparado aos demais grupos, demonstrando o efeito sobrepujante da dieta rica em ácidos graxos saturados, mesmo com a presença do ômega-3. No entanto, não encontramos diferença de peso entre as proles aos 30 dias de vida. Na vida adulta, aos 90 dias de vida também não foi encontrada diferença de peso coroporal e do índice de Lee entre os grupos.

Quanto aos parâmetros bioquímicos avaliados, as mães que consumiram dieta rica em ácidos graxos saturados apresentaram maiores níveis de colesterol quando comparado ao grupo controle e HLW3. A prole das mães HL, aos 22 dias de vida, também apresentaram maiores níveis de colesterol, quando comparado aos demais grupos. E os níveis de triglicerídeos da prole HLW3 foram reduzidos em comparação ao grupo controle. Em relação aos níveis de glicemia em jejum, apenas o grupo HL apresentou maiores valores quando comparado ao controle, os valores do grupo HLW3 equipararam-se ao controle. Aos 90 dias de vida, a prole HL também apresentou maiores níveis de colesterol que o grupo controle e foram encontrados nesses animais maiores níveis de triglicerídeos e VLDL em relação ao controle, sem diferença quando comparado o grupo controle e HLW3. Nesta mesma idade, os níveis de glicemia em jejum foram maiores no grupo HL quando comparado aos demais grupos. Demonstrando o papel do ômega-3 na melhora do perfil glicêmico e lipídico. Os animais submetidos também à dieta rica em ácidos graxos saturados apresentaram níveis elevados de ALT, indicando possível injúria hepatocelular, enquanto que os animais que receberam uma dieta com alto teor de ácidos graxos saturados enriquecida com ômega-3 apresentaram melhores níveis de ALT aos 22 dias de vida. E aos 90 dias de vida, o grupo HLW3 apresentou menores níveis de AST em relação ao grupo controle e HL.

O enriquecimento da dieta com ômega-3 também melhorou a capacidade antioxidante a nível hepático, prejudicada pela dieta rica em ácidos graxos saturados, com normalização dos níveis de SOD e GST, não sendo encontrada diferença em relação ao grupo controle e HLW3 nestas enzimas avaliadas. Além disso, o grupo HL apresentou redução dos níveis de

GSH em relação ao grupo controle e HLW3. No entanto, foi encontrada uma diminuição dos níveis de sulfidrilas e da razão GSH/GSSG, considerada um indicador de estresse oxidativo, tanto no grupo HL, como no grupo HLW3, observando o efeito da dieta rica em ácidos graxos saturados, mesmo na presença do ômega-3.

8 CONCLUSÃO

Desta forma, o presente estudo sugere que o consumo materno de dieta rica em ácidos saturados está associado a prejuízos em alguns parâmetros murinométricos, bioquímicos e ao desequilíbrio oxidativo a nível hepático, com redução de enzimas que participam do sistema de defesa antioxidante. No entanto, não foram encontradas alterações nos animais que foram submetidos a esta dieta, nos testes de tolerância à glicose, piruvato, sensibilidade à insulina e nos parâmetros respiratórios aos 90 dias de vida, o que poderia ocorrer mais tardiamente, considerando os fatores de riscos encontrados nesses animais como: a dislipidemia, hiperglicemia e desbalanço oxidativo. Além disso, nossos achados confirmam que o consumo de ômega-3, a partir do óleo de linhaça é capaz de atenuar algumas alterações observadas nos animais que receberam dieta rica em ácidos graxos saturados durante o período de gestação e lactação, às quais estão presentes nas doenças cardiovasculares e metabólicas. Mais estudos são necessários para avaliar os mecanismos relacionados às alterações metabólicas e respiratórias ocasionadas pela ingestão de dieta hiperlipidica, além do papel do ômega-3 durante o periodo perinatal. A compreensão completa destes mecanismos relacionados e do papel do ômega-3 sobre os mesmos poderá levar ao desenvolvimento de estratégias terapêuticas e políticas governamentais para melhorar a saúde pública.

REFERÊNCIAS

ADLAN, A. M.; LIP, G. Y.; PATON, J. F.; KITAS, G. D.; FISHER, J. P. Autonomic function and rheumatoid arthritis: a systematic review. **Semin Arthritis Rheum,** New York: Stratton, v. 44, n. 3, p. 283-304, Dec 2014.

AEBI, H. Catalase in vitro. *In:* PACKER, L. (Ed.). **Methods Enzymol.** New York: Academic Press, 1984. cap. 13, p.121-126.

AKERELE, O. A.; CHEEMA, S. K. A balance of omega-3 and omega-6 polyunsaturated fatty acids is important in pregnancy. **Journal of Nutrition & Intermediary Metabolism,** Amsterdam: Elsevier, v. 5, p. 23-33, 2016.

ALFARADHI, M. Z.; OZANNE, S. E. Developmental programming in response to maternal overnutrition. **Front Genet,** Lausanne: Frontiers Research Foundation,v. 2, p. 27, 2011.

ALVES, J.; MARICELLY DEODATO DE OLIVEIRA, J.; FERREIRA, D. J.; ASSIS V DE BARROS, M.; NOGUEIRA, V.; SANTOS ALVES, D.; VIDAL, H.; GOIS LEANDRO, C.; LAGRANHA, C.; PIROLA, L.; COSTA-SILVA, J. Maternal protein restriction induced-hypertension is associated to oxidative disruption at transcriptional and functional levels in the medulla oblongata. **Clin Exp Pharmacol Physiol.**, Oxford, v. 43, n. 12, p. 1177-1184, 2016.

APPEL, L. J.; MILLER, E. R.; III; SEIDLER, A. J.; WHELTON, P. K. Does supplementation of diet with "fish oil" reduce blood pressure? A meta-analysis of controlled clinical trials. **Archives of Internal Medicine,** Chicago: American Medical Assn, v. 153, n. 12, p. 1429-1438, 1993.

ARMITAGE, J. A.; POSTON, L.; TAYLOR, P. D. Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. **Front Horm Res,** Basel; New York, Karger, v. 36, p. 73-84, 2008.

ASTRUP, A.; DYERBERG, J.; ELWOOD, P.; HERMANSEN, K.; B HU, F.; JAKOBSEN, M.; KOK, F. J.; KRAUSS, R.; LECERF, J.-M.; LEGRAND, P.; NESTEL, P.; RISÉRUS, U.; SANDERS, T.; SINCLAIR, A.; STENDER, S.; THOLSTRUP, T.; C WILLETT, W. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? **Am J Clin Nutr.**, Bethesda, v. 93, n. 4, p. 684-688, 2011.

BAGLEY, H. N.; WANG, Y.; CAMPBELL, M. S.; YU, X.; LANE, R. H.; JOSS-MOORE, L. A. Maternal docosahexaenoic acid increases adiponectin and normalizes IUGR-induced changes in rat adipose deposition. **J Obes,** New York; Cairo, Egypt: Hindawi Publishing Corporation, v. 2013, p. 312153, 2013.

BANNENBERG, G.; SERHAN, C. N. Specialized pro-resolving lipid mediators in the inflammatory response: An update. **Biochim Biophys Acta,** Amsterdam: Elsevier Pub, v. 1801, n. 12, p. 1260-73, Dec 2010.

BARDGETT, M. E.; SHARPE, A. L.; TONEY, G. M. Activation of corticotropin-releasing factor receptors in the rostral ventrolateral medulla is required for glucose-induced sympathoexcitation. **American Journal of Physiology-Endocrinology and Metabolism,** Bethesda, MD.: American Physiological Society, v. 307, n. 10, p. E944-E953, 2014.

BARKER, D. J.; OSMOND, C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. **Lancet,** London: J. Onwhyn, v. 1, n. 8489, p. 1077-81, May 1986.

BARQUISSAU, V.; CAPEL, F.; DARDEVET, D.; FEILLET-COUDRAY, C.; GALLINIER, A.; CHAUVIN, M.-A.; RIEUSSET, J.; MORIO, B. Reactive oxygen species enhance mitochondrial function, insulin sensitivity and glucose uptake in skeletal muscle of senescence accelerated prone mice SAMP8. **Free Radical Biology and Medicine,** New York: Pergamon, v. 113, p. 267-279, 2017.

BARROS, M. A.; DE BRITO ALVES, J. L.; NOGUEIRA, V. O.; WANDERLEY, A. G.; COSTA-SILVA, J. H. Maternal low-protein diet induces changes in the cardiovascular autonomic modulation in male rat offspring. **Nutr Metab Cardiovasc Dis,** Heidelberg: Springer International, v. 25, n. 1, p. 123-30, Jan 2015.

BARTLETT, D.; TENNEY, S. M. Control of breathing in experimental anemia. **Respiration Physiology,** Amsterdam, v. 10, n. 3, p. 384-395, 1970.

BATISTA FILHO, M.; BATISTA, L. V. Transição alimentar/ nutricional ou mutação antropológica? **Ciência e Cultura,** São Paulo, v. 62, p. 26-30, 2010.

BELKINA, A. C.; DENIS, G. V. Obesity genes and insulin resistance. **Current Opinion in Endocrinology, Diabetes and Obesity,** London: Lippincott, Williams & Wilkins, v. 17, n. 5, p. 472-477, 2010.

BETTERIDGE, D. J. What is oxidative stress? **Metabolism - Clinical and Experimental,** New York, Grune & Stratton, v. 49, n. 2, p. 3-8, 2000.

BIRBEN, E.; SAHINER, U. M.; SACKESEN, C.; ERZURUM, S.; KALAYCI, O. Oxidative stress and antioxidant defense. **World Allergy Organ J,** Philadelphia: Lippincott Williams & Wilkins, v. 5, n. 1, p. 9-19, 2012.

BOGDARINA, I.; WELHAM, S.; KING, P. J.; BURNS, S. P.; CLARK, A. J. Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. **Circ Res,** Baltimore: Grune & Stratton. v. 100, n. 4, p. 520-6, Mar 2 2007.

BONEVA NADEZHDA, B.; YAMASHIMA, T. New insights into "GPR40-CREB interaction inadult neurogenesis" specific for primates. **Hippocampus,** New York: Churchill Livingstone, v. 22, n. 4, p. 896-905, 2011.

BORING, L.; GOSLING, J.; CLEARY, M.; CHARO, I. F. Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis. **Nature,** London: Macmillan Journals, v. 394, p. 894, 1998.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal Biochem,** Orlando: Academic Press, v. 72, n. 1, p. 248-254, 1976/05/07/ 1976.

BRITO ALVES, J. L.; OLIVEIRA, J. M. D.; FERREIRA, D. J. S.; BARROS, M. A. D. V.; NOGUEIRA, V. O.; ALVES, D. S.; VIDAL, H.; LEANDRO, C. G.; LAGRANHA, C. J.; PIROLA, L.; COSTA-SILVA, J. H. Maternal protein restriction induced-hypertension is associated to oxidative disruption at transcriptional and functional levels in the medulla oblongata. **Clinical and Experimental Pharmacology and Physiology,** Oxford: Blackwell Scientific Publications, v. 43, n. 12, p. 1177-1184, 2016.

BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. *In:* FLEISCHER, S.; PACKER, L. (Eds.). **Methods Enzymol**. New York: Academic Press, 1978. cap. 30, p. 302-310.

CARLSON, S. E.; COLOMBO, J.; GAJEWSKI, B. J.; GUSTAFSON, K. M.; MUNDY, D.; YEAST, J.; GEORGIEFF, M. K.; MARKLEY, L. A.; KERLING, E. H.; SHADDY, D. J. DHA supplementation and pregnancy outcomes. **Am J Clin Nutr,** Bethesda, v. 97, n. 4, p. 808-15, Apr 2013.

CAROCHO, M.; FERREIRA, I. C. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. **Food Chem Toxicol,** Oxford; New York: Pergamon Press, v. 51, p. 15-25, Jan 2013.

CASTOLDI, A.; ANDRADE-OLIVEIRA, V.; AGUIAR, C. F.; AMANO, M. T.; LEE, J.; MIYAGI, M. T.; LATANCIA, M. T.; BRAGA, T. T.; DA SILVA, M. B.; IGNACIO, A.; CAROLA CORREIA LIMA, J. D.; LOURES, F. V.; ALBUQUERQUE, J. A. T.; MACEDO, M. B.; ALMEIDA, R. R.; GAIARSA, J. W.; LUEVANO-MARTINEZ, L. A.; BELCHIOR, T.; HIYANE, M. I.; BROWN, G. D.; MORI, M. A.; HOFFMANN, C.; SEELAENDER, M.; FESTUCCIA, W. T.; MORAES-VIEIRA, P. M.; CAMARA, N. O. S. Dectin-1 Activation Exacerbates Obesity and Insulin Resistance in the Absence of MyD88. **Cell Rep,** Cambridge: Cell Press, v. 19, n. 11, p. 2272-2288, Jun 2017.

CATALANO, P.; EHRENBERG, H. Review article: The short- and long-term implications of maternal obesity on the mother and her offspring. **BJOG: An International Journal of Obstetrics & Gynaecology,** Oxford: Blackwell Science, v. 113, n. 10, p. 1126-1133, 2006.

CHAVATTE-PALMER, P.; TARRADE, A.; ROUSSEAU-RALLIARD, D. Diet before and during Pregnancy and Offspring Health: The Importance of Animal Models and What Can Be Learned from Them. **International Journal of Environmental Research and Public Health,** Basel: MDPI, v. 13, n. 6, p. 586, 2016.

COSTA-SILVA, J. H.; DE BRITO-ALVES, J. L.; BARROS, M. A.; NOGUEIRA, V. O.; PAULINO-SILVA, K. M.; DE OLIVEIRA-LIRA, A.; NOBRE, I. G.; FRAGOSO, J.; LEANDRO, C. G. New Insights on the Maternal Diet Induced-Hypertension: Potential Role of the Phenotypic Plasticity and Sympathetic-Respiratory Overactivity. **Front Physiol,** Lausanne: Frontiers Research Foundation, v. 6, p. 345, 2015.

COSTA-SILVA, J. H.; SIMOES-ALVES, A. C.; FERNANDES, M. P. Developmental Origins of Cardiometabolic Diseases: Role of the Maternal Diet. **Front Physiol,** Lausanne: Frontiers Research Foundation, v. 7, p. 504, 2016.

COSTA-SILVA, J. H.; ZOCCAL, D. B.; MACHADO, B. H. Chronic intermittent hypoxia alters glutamatergic control of sympathetic and respiratory activities in the commissural NTS of rats. **American Journal of Physiology : Regulatory, Integrative and Comparative Physiology,** Lausanne: Frontiers Research Foundation, v. 302, n. 6, p. R785-R793, 2012.

CRAWFORD, M. Placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of preterm infants. **Am J Clin Nutr,** Bethesda, v. 71, n. 1 Suppl, p. 275S-84S, Jan 2000.

DE ALMEIDA, C. A.; RIBAS FILHO, D.; MELLO, E.; BERTOLUCCI, P.; CÍCERO FALCÃO, M. I Consenso da Associação Brasileira de Nutrologia sobre recomendações de DHA durante gestação, lactação e infância. **International Journal of Nutrology,** São Paulo, ano 7, n. 3, p. 4-13, 2014. Trabalho apresentado no 18º Congresso Brasileiro de Nutrologia, 2014, São Paulo.

DE BRITO ALVES, J. L.; NOGUEIRA, V. O.; CAVALCANTI NETO, M. P.; LEOPOLDINO, A. M.; CURTI, C.; COLOMBARI, D. S.; COLOMBARI, E.; WANDERLEY, A. G.; LEANDRO, C. G.; ZOCCAL, D. B.; COSTA-SILVA, J. H. Maternal protein restriction increases respiratory and sympathetic activities and sensitizes peripheral chemoreflex in male rat offspring. **J Nutr,** Springfield: C. C. Thomas, v. 145, n. 5, p. 907-14, May 2015.

DE BRITO ALVES, J. L.; NOGUEIRA, V. O.; DE OLIVEIRA, G. B.; DA SILVA, G. S. F.; WANDERLEY, A. G.; LEANDRO, C. G.; COSTA-SILVA, J. H. Short- and long-term effects of a maternal low-protein diet on ventilation, O2/CO2 chemoreception and arterial blood pressure in male rat offspring. **British Journal of Nutrition,** Cambridge: Cambridge University Press, v. 111, n. 4, p. 606-615, 2014.

DESAI, M.; JELLYMAN, J. K.; HAN, G.; BEALL, M.; LANE, R. H.; ROSS, M. G. Maternal obesity and high-fat diet program offspring metabolic syndrome. **Am J Obstet Gynecol,** St. Louis, v. 211, n. 3, p. 237 e1-237 e13, Sep 2014.

DOSSI, C. G.; TAPIA, G. S.; ESPINOSA, A.; VIDELA, L. A.; D'ESPESSAILLES, A. Reversal of high-fat diet-induced hepatic steatosis by n-3 LCPUFA: role of PPAR-α and SREBP-1c. **J Nutr Biochem,** Stoneham: Butterworths, v. 25, n. 9, p. 977-984, 2014.

DUTTAROY, A. K. Transport of fatty acids across the human placenta: a review. **Prog Lipid Res,** Oxford; Elmsford, N. Y.: Pergamon Press, v. 48, n. 1, p. 52-61, Jan 2009.

ELLMAN, G. L. Tissue sulfhydryl groups. **Arch Biochem Biophys,** v. 82, n. 1, p. 70-77, 1959.

ESTADELLA, D.; DA PENHA OLLER DO NASCIMENTO, C. M.; OYAMA, L. M.; RIBEIRO, E. B.; DAMASO, A. R.; DE PIANO, A. Lipotoxicity: effects of dietary saturated

and transfatty acids. **Mediators Inflamm,** Oxford: Rapid Communications of Oxford Ltd., v. 2013, p. 137579, 2013.

ESTEVE, E.; RICART, W.; FERNÁNDEZ-REAL, J. M. Dyslipidemia and inflammation: an evolutionary conserved mechanism. **Clin Nutr,** Edinburgh; New York: Churchill Livingstone, v. 24, n. 1, p. 16-31, 2005.

FEOLI, A. M.; ROEHRIG, C.; ROTTA, L. N.; KRUGER, A. H.; SOUZA, K. B.; KESSLER, A. M.; RENZ, S. V.; BRUSQUE, A. M.; SOUZA, D. O.; PERRY, M. L. S. Serum and liver lipids in rats and chicks fed with diets containing different oils. **Nutrition,** Burbank, v. 19, n. 9, p. 789-793, 2003.

FERRO CAVALCANTE, T. C.; LIMA DA SILVA, J. M.; DA MARCELINO DA SILVA, A. A.; MUNIZ, G. S.; DA LUZ NETO, L. M.; LOPES DE SOUZA, S.; MANHAES DE CASTRO, R.; FERRAZ, K. M.; DO NASCIMENTO, E. Effects of a westernized diet on the reflexes and physical maturation of male rat offspring during the perinatal period. **Lipids,** Chicago: American Oil Chemists' Society, v. 48, n. 11, p. 1157-68, Nov 2013.

FURUKAWA, S.; FUJITA, T.; SHIMABUKURO, M.; IWAKI, M.; YAMADA, Y.; NAKAJIMA, Y.; NAKAYAMA, O.; MAKISHIMA, M.; MATSUDA, M.; SHIMOMURA, I. Increased oxidative stress in obesity and its impact on metabolic syndrome. **J Clin Invest,** New Haven: American Society for Clinical Investigation, v. 114, n. 12, p. 1752-61, Dec 2004.

GLUCKMAN, P. D.; HANSON, M. A.; BATESON, P.; BEEDLE, A. S.; LAW, C. M.; BHUTTA, Z. A.; ANOKHIN, K. V.; BOUGNERES, P.; CHANDAK, G. R.; DASGUPTA, P.; SMITH, G. D.; ELLISON, P. T.; FORRESTER, T. E.; GILBERT, S. F.; JABLONKA, E.; KAPLAN, H.; PRENTICE, A. M.; SIMPSON, S. J.; UAUY, R.; WEST-EBERHARD, M. J. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. **Lancet,** London: J. Onwhyn, v. 373, n. 9675, p. 1654-7, May 9 2009.

GRAYSON, B. E.; LEVASSEUR, P. R.; WILLIAMS, S. M.; SMITH, M. S.; MARKS, D. L.; GROVE, K. L. Changes in melanocortin expression and inflammatory pathways in fetal offspring of nonhuman primates fed a high-fat diet. **Endocrinology,** Los Angeles: Association for the Study of Internal Secretions, v. 151, n. 4, p. 1622-32, Apr 2010.

GRUBER, L.; HEMMERLING, J.; SCHUPPEL, V.; MULLER, M.; BOEKSCHOTEN, M. V.; HALLER, D. Maternal High-fat Diet Accelerates Development of Crohn's Disease-like Ileitis in TNFDeltaARE/WT Offspring. **Inflamm Bowel Dis,** New York: Raven Press, v. 21, n. 9, p. 2016-25, Sep 2015.

HABIG, W. H.; PABST, M. J.; JAKOBY, W. B. Glutathione S-Transferases: THE FIRST ENZYMATIC STEP IN MERCAPTURIC ACID FORMATION. **Journal of Biological Chemistry,** Baltimore: American Society for Biochemistry and Molecular Biology, v. 249, n. 22, p. 7130-7139, Nov. 1974.

HALES, C. N.; BARKER, D. J. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. **Diabetologia,** Berlin: Springer Verlag, v. 35, n. 7, p. 595-601, Jul 1992.

_____. The thrifty phenotype hypothesis. **Br Med Bull,** London, v. 60, p. 5-20, 2001.

HALES, C. N.; BARKER, D. J. P.; CLARK, P. M. S.; COX, L. J.; FALL, C.; OSMOND, C.; WINTER, P. D. Fetal And Infant Growth And Impaired Glucose Tolerance At Age 64. **BMJ: British Medical Journal,** London : British Medical Association, v. 303, n. 6809, p. 1019-1022, 1991.

HALLIWELL, B.; WHITEMAN, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? **Br J Pharmacol,** London: Macmillian Journals, v. 142, n. 2, p. 231-255, 2004.

HANSON, M.; GODFREY, K. M.; LILLYCROP, K. A.; BURDGE, G. C.; GLUCKMAN, P. D. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. **Prog Biophys Mol Biol,** New York, v. 106, n. 1, p. 272-80, Jul 2011.

HARRIS, W. S.; CONNOR, W. E.; LINDSEY, S. Will dietary omega-3 fatty acids change the composition of human milk? **Am J Clin Nutr,** Bethesda, v. 40, n. 4, p. 780-5, Oct 1984.

HEERWAGEN, M. J. R.; STEWART, M. S.; DE LA HOUSSAYE, B. A.; JANSSEN, R. C.; FRIEDMAN, J. E. Transgenic Increase in N-3/N-6 Fatty Acid Ratio Reduces Maternal Obesity-Associated Inflammation and Limits Adverse Developmental Programming in Mice. **PLoS One,** San Francisco: Public Library of Science, v. 8, n. 6, p. e67791, 2013.

HIROTANI, Y.; OZAKI, N.; TSUJI, Y.; URASHIMA, Y.; MYOTOKU, M. Effects of eicosapentaenoic acid on hepatic dyslipidemia and oxidative stress in high fat diet-induced steatosis. **Int J Food Sci Nutr,** Basingstoke: Scientific and Medical Division, Macmillan Press, v. 66, n. 5, p. 569-73, 2015.

HISSIN, P. J.; HILF, R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. **Anal Biochem,** Orlando: Academic Press, v. 74, n. 1, p. 214-226, 1976.

HOTAMISLIGIL, G. S.; ARNER, P.; CARO, J. F.; ATKINSON, R. L.; SPIEGELMAN, B. M. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. **J Clin Invest,** New Haven: American Society for Clinical Investigation, v. 95, n. 5, p. 2409-15, May 1995.

HOWE, P. R. C. Dietary Fats and Hypertension Focus on Fish Oil. **Ann N Y Acad Sci,** New York: The Academy, v. 827, n. 1, p. 339-352, 1997.

IM, D.-S. Functions of omega-3 fatty acids and FFA4 (GPR120) in macrophages. **Eur J Pharmacol,** Amsterdam: North Holland Pub. Co., v. 785, p. 36-43, 2016.

INOUE, T.; TANAKA, M.; MASUDA, S.; OHUE-KITANO, R.; YAMAKAGE, H.; MURANAKA, K.; WADA, H.; KUSAKABE, T.; SHIMATSU, A.; HASEGAWA, K.; SATOH-ASAHARA, N. Omega-3 polyunsaturated fatty acids suppress the inflammatory responses of lipopolysaccharide-stimulated mouse microglia by activating SIRT1 pathways. **Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids,** Amsterdam: Elsevier, v. 1862, n. 5, p. 552-560, 2017.

JAKOBSEN, M. U.; O'REILLY, E. J.; HEITMANN, B. L.; PEREIRA, M. A.; BALTER, K.; FRASER, G. E.; GOLDBOURT, U.; HALLMANS, G.; KNEKT, P.; LIU, S.; PIETINEN, P.; SPIEGELMAN, D.; STEVENS, J.; VIRTAMO, J.; WILLETT, W. C.; ASCHERIO, A. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. **Am J Clin Nutr,** Bethesda, v. 89, n. 5, p. 1425-32, May 2009.

KANDA, H.; TATEYA, S.; TAMORI, Y.; KOTANI, K.; HIASA, K.-I.; KITAZAWA, R.; KITAZAWA, S.; MIYACHI, H.; MAEDA, S.; EGASHIRA, K.; KASUGA, M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. **J Clin Invest,** New Haven: American Society for Clinical Investigation, v. 116, n. 6, p. 1494-1505, 2006.

KĘPCZYŃSKA, M. A.; WARGENT, E. T.; CAWTHORNE, M. A.; ARCH, J. R. S.; O'DOWD, J. F.; STOCKER, C. J. Circulating levels of the cytokines IL10, IFNγ and resistin in an obese mouse model of developmental programming. **J Dev Orig Health Dis,** Cambridge: Cambridge University Press, v. 4, n. 6, p. 491-498, 2013.

KHAIRE, A. A.; KALE, A. A.; JOSHI, S. R. Maternal omega-3 fatty acids and micronutrients modulate fetal lipid metabolism: A review. **Prostaglandins Leukot Essent Fatty Acids,** Edinburgh; New York: Churchill Livingstone, v. 98, p. 49-55, Jul 2015.

KHAN, I. Y.; DEKOU, V.; DOUGLAS, G.; JENSEN, R.; HANSON, M. A.; POSTON, L.; TAYLOR, P. D. A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,** Basel; New York: Karger, v. 288, n. 1, p. R127-R133, 2005.

KHATIB, S. A.; ROSSI, E. L.; BOWERS, L. W.; HURSTING, S. D. Reducing the burden of obesity-associated cancers with anti-inflammatory long-chain omega-3 polyunsaturated fatty acids. **Prostaglandins Other Lipid Mediat,** New York: Elsevier Science, v. 125, p. 100-7, Sep 2016.

KIRK, S. L.; SAMUELSSON, A. M.; ARGENTON, M.; DHONYE, H.; KALAMATIANOS, T.; POSTON, L.; TAYLOR, P. D.; COEN, C. W. Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. **PLoS One,** San Francisco: Public Library of Science, v. 4, n. 6, p. e5870, Jun 2009.

KLEINRIDDERS, A.; KONNER, A. C.; BRUNING, J. C. CNS-targets in control of energy and glucose homeostasis. **Curr Opin Pharmacol,** Oxford: Elsevier Science Ltd., v. 9, n. 6, p. 794-804, Dec 2009.

KOJIMA, S.; CATAVERO, C.; RINAMAN, L. Maternal high-fat diet increases independent feeding in pre-weanling rat pups. **Physiol Behav.,** New York, v. 1, n. 157, p. 237-245, 2016.

KOLETZKO, B.; CETIN, I.; BRENNA, J. T.; PERINATAL LIPID INTAKE WORKING, G.; CHILD HEALTH, F.; DIABETIC PREGNANCY STUDY, G.; EUROPEAN ASSOCIATION OF PERINATAL, M.; EUROPEAN ASSOCIATION OF PERINATAL, M.; EUROPEAN SOCIETY FOR CLINICAL, N.; METABOLISM; EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY, H.; NUTRITION, C. O. N.;

INTERNATIONAL FEDERATION OF PLACENTA, A.; INTERNATIONAL SOCIETY FOR THE STUDY OF FATTY, A.; LIPIDS. Dietary fat intakes for pregnant and lactating women. **Br J Nutr,** Cambridge, New York: Cambridge University Press, v. 98, n. 5, p. 873-7, Nov 2007.

KOLETZKO, B.; LIEN, E.; AGOSTONI, C.; BOHLES, H.; CAMPOY, C.; CETIN, I.; DECSI, T.; DUDENHAUSEN, J. W.; DUPONT, C.; FORSYTH, S.; HOESLI, I.; HOLZGREVE, W.; LAPILLONNE, A.; PUTET, G.; SECHER, N. J.; SYMONDS, M.; SZAJEWSKA, H.; WILLATTS, P.; UAUY, R.; WORLD ASSOCIATION OF PERINATAL MEDICINE DIETARY GUIDELINES WORKING, G. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. **J Perinat Med,** Berlin, New York: Walter de Gruyter, v. 36, n. 1, p. 5-14, 2008.

KOROTKOVA, M.; GABRIELSSON, B.; LONN, M.; HANSON, L. A.; STRANDVIK, B. Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet. **J Lipid Res,** Memphis: Lipid Research, inc., v. 43, n. 10, p. 1743-9, Oct 2002.

KREMER, J. M. n-3 fatty acid supplements in rheumatoid arthritis. **Am J Clin Nutr,** Bethesda, v. 71, n. 1 Suppl, p. 349S-51S, Jan 2000.

KRIS-ETHERTON, P. M.; HARRIS, W. S.; APPEL, L. J.; AMERICAN HEART ASSOCIATION. NUTRITION, C. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Circulation,** Dallas: American Heart Association, v. 106, n. 21, p. 2747-57, Nov 19 2002.

MAGDER, S. Reactive oxygen species: toxic molecules or spark of life? **Crit Care,** London: Current Science Ltd., v. 10, n. 1, p. 208, Feb 2006.

MALAN, A. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. **Respir Physiol,** Amsterdam, v. 17, n. 1, p. 32-44, Jan 1973.

MARCON, R.; BENTO, A. F.; DUTRA, R. C.; BICCA, M. A.; LEITE, D. F.; CALIXTO, J. B. Maresin 1, a proresolving lipid mediator derived from omega-3 polyunsaturated fatty acids, exerts protective actions in murine models of colitis. **J Immunol,** Baltimore: Williams & Wilkins, v. 191, n. 8, p. 4288-98, Oct 15 2013.

MARTIN, C. A.; ALMEIDA, V. V. D.; RUIZ, M. R.; VISENTAINER, J. E. L.; MATSHUSHITA, M.; SOUZA, N. E. D.; VISENTAINER, J. V. Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e ocorrência em alimentos. **Revista de Nutrição,** Campinas, v. 19, p. 761-770, 2006.

MEDICINE, I. O. **Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids**. Washington, DC: The National Academies Press, 2005. 1358 p.

MICHA, R.; MOZAFFARIAN, D. Saturated Fat and Cardiometabolic Risk Factors, Coronary Heart Disease, Stroke, and Diabetes: a Fresh Look at the Evidence. **Lipids,** Chicago: American Oil Chemists' Society, v. 45, n. 10, p. 893-905, 2010.

MISRA, H. P.; FRIDOVICH, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. **J Biol Chem,** Baltimore, MD: American Society for Biochemistry and Molecular Biology, v. 247, n. 10, p. 3170-3175, 1972.

MOKDAD, A. H.; FORD, E. S.; BOWMAN, B. A.; ET AL. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. **JAMA,** Chicago: American Medical Association, v. 289, n. 1, p. 76-79, 2003.

MORAES, D. J.; BONAGAMBA, L. G.; COSTA, K. M.; COSTA-SILVA, J. H.; ZOCCAL, D. B.; MACHADO, B. H. Short-term sustained hypoxia induces changes in the coupling of sympathetic and respiratory activities in rats. **J Physiol,** London: Cambridge Univ. Press, v. 592, n. 9, p. 2013-33, May 2014.

MORI, T. A.; WATTS, G. F.; BURKE, V.; HILME, E.; PUDDEY, I. B.; BEILIN, L. J. Differential Effects of Eicosapentaenoic Acid and Docosahexaenoic Acid on Vascular Reactivity of the Forearm Microcirculation in Hyperlipidemic, Overweight Men. **Circulation,** Dallas: American Heart Association, v. 102, n. 11, p. 1264-1269, 2000.

MORRIS, M. C.; SACKS, F.; ROSNER, B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. **Circulation,** Dallas: American Heart Association, v. 88, n. 2, p. 523-533, 1993.

MURABAYASHI, N.; SUGIYAMA, T.; ZHANG, L.; KAMIMOTO, Y.; UMEKAWA, T.; MA, N.; SAGAWA, N. Maternal high-fat diets cause insulin resistance through inflammatory changes in fetal adipose tissue. **European Journal of Obstetrics & Gynecology and Reproductive Biology,** Amsterdam: Excerpta Medica, v. 169, n. 1, p. 39-44, 2013.

NASCIMENTO, L. F.; SOUZA, G. F.; MORARI, J.; BARBOSA, G. O.; SOLON, C.; MOURA, R. F.; VICTORIO, S. C.; IGNACIO-SOUZA, L. M.; RAZOLLI, D. S.; CARVALHO, H. F.; VELLOSO, L. A. n-3 Fatty Acids Induce Neurogenesis of Predominantly POMC-Expressing Cells in the Hypothalamus. **Diabetes,** New York: American Diabetes Association, v. 65, n. 3, p. 673-86, Mar 2016.

NOVELLI, E. L.; DINIZ, Y. S.; GALHARDI, C. M.; EBAID, G. M.; RODRIGUES, H. G.; MANI, F.; FERNANDES, A. A.; CICOGNA, A. C.; NOVELLI FILHO, J. L. Anthropometrical parameters and markers of obesity in rats. **Lab Anim,** London: Laboratory Animals Ltd., v. 41, n. 1, p. 111-9, Jan 2007.

O'DONNELL, V. B.; MASKREY, B.; TAYLOR, G. W. Eicosanoids: generation and detection in mammalian cells. **Methods Mol Biol,** Clifton: Humana Press, v. 462, p. 5-23, 2009.

OLLIVER, M.; VEYSEY, M.; LUCOCK, M.; NIBLETT, S.; KING, K.; MACDONALD-WICKS, L.; GARG, M. L. Erythrocyte omega-3 polyunsaturated fatty acid levels are associated with biomarkers of inflammation in older Australians. **Journal of Nutrition & Intermediary Metabolism,** Amsterdam: Elsevier, v. 5, p. 61-69, 2016.

PASCOAL, L. B.; BOMBASSARO, B.; RAMALHO, A. F.; COOPE, A.; MOURA, R. F.; CORREA-DA-SILVA, F.; IGNACIO-SOUZA, L.; RAZOLLI, D.; DE OLIVEIRA, D.; CATHARINO, R.; VELLOSO, L. A. Resolvin RvD2 reduces hypothalamic inflammation and rescues mice from diet-induced obesity. **J Neuroinflammation,** London: BioMed Central, v. 14, n. 1, p. 5, Jan 5 2017.

PATON, J. F. R.; WAKI, H. Is neurogenic hypertension related to vascular inflammation of the brainstem? **Neuroscience & Biobehavioral Reviews,** New York: Pergamon Press, v. 33, n. 2, p. 89-94, 2009/02/01/ 2009.

PELUSO, I.; MORABITO, G.; URBAN, L.; IOANNONE, F.; SERAFINI, M. Oxidative stress in atherosclerosis development: the central role of LDL and oxidative burst. **Endocr Metab Immune Disord Drug Targets,** Saif Zone, Sharjah, U.A.E.; San Francisco, CA: Bentham Science Publishers, v. 12, n. 4, p. 351-60, Dec 2012.

PENITENTE, A. R.; FERNANDES, L. G.; CARDOSO, L. M.; SILVA, M. E.; PEDROSA, M. L.; SILVA, A. L.; HAIBARA, A. S.; MORAES, M. F. D.; CHIANCA, D. A. Malnutrition enhances cardiovascular responses to chemoreflex activation in awake rats. **Life Sciences,** Oxford; Elmsford, N. Y.: Pergamon Press, v. 81, n. 7, p. 609-614, 2007.

PIEPOLI, M. F.; HOES, A. W.; AGEWALL, S.; ALBUS, C.; BROTONS, C.; CATAPANO, A. L.; COONEY, M. T.; CORRA, U.; COSYNS, B.; DEATON, C.; GRAHAM, I.; HALL, M. S.; HOBBS, F. D. R.; LOCHEN, M. L.; LOLLGEN, H.; MARQUES-VIDAL, P.; PERK, J.; PRESCOTT, E.; REDON, J.; RICHTER, D. J.; SATTAR, N.; SMULDERS, Y.; TIBERI, M.; VAN DER WORP, H. B.; VAN DIS, I.; VERSCHUREN, W. M. M.; BINNO, S.; GROUP, E. S. C. S. D. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). **Eur Heart J,** London: Saunders, v. 37, n. 29, p. 2315-2381, Aug. 2016.

PIETTA, P.-G. Flavonoids as Antioxidants. **J Nat Prod,** Cincinnati: American Society of Pharmacognosy, v. 63, n. 7, p. 1035-1042, 2000.

PIMENTEL, G. D.; GANESHAN, K.; CARVALHEIRA, J. B. Hypothalamic inflammation and the central nervous system control of energy homeostasis. **Mol Cell Endocrinol,** Amsterdam, v. 397, n. 1-2, p. 15-22, Nov. 2014.

PIMENTEL, G. D.; LIRA, F. S.; ROSA, J. C.; OLIVEIRA, J. L.; LOSINSKAS-HACHUL, A. C.; SOUZA, G. I.; DAS GRACAS, T. D. C. M.; SANTOS, R. V.; DE MELLO, M. T.; TUFIK, S.; SEELAENDER, M.; OYAMA, L. M.; OLLER DO NASCIMENTO, C. M.; WATANABE, R. H.; RIBEIRO, E. B.; PISANI, L. P. Intake of trans fatty acids during gestation and lactation leads to hypothalamic inflammation via TLR4/NFkappaBp65 signaling in adult offspring. **J Nutr Biochem,** Stoneham, MA, USA: Butterworths, v. 23, n. 3, p. 265-71, Mar 2012.

PIROLA, L.; FERRAZ, J. C. Role of pro- and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity. **World J Biol Chem,** Hong Kong: Baishideng Publishing Group, v. 8, n. 2, p. 120-128, May 26 2017.

POPKIN, B. M.; ADAIR, L. S.; NG, S. W. Global nutrition transition and the pandemic of obesity in developing countries. **Nutr Rev,** Washington, D.C.: International Life Sciences Institute, v. 70, n. 1, p. 3-21, 2012.

POPKIN, B. M.; GORDON-LARSEN, P. The nutrition transition: worldwide obesity dynamics and their determinants. **International Journal Of Obesity,** Hampshire, UK: Macmillan Press, v. 28, p. S2, 2004.

PRUCHNIAK, M. P.; ARAZNA, M.; DEMKOW, U. Biochemistry of Oxidative Stress. **Adv Exp Med Biol,** New York: Plenum Press, v. 878, p. 9-19, 2016.

REEVES, P. G.; NIELSEN, F. H.; FAHEY, G. C., JR. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. **J Nutr,** Springfield: C. C. Thomas, v. 123, n. 11, p. 1939-51, Nov 1993.

SACKS, F. M.; KATAN, M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. **The American Journal of Medicine,** New York, Donnelly, v. 113, n. 9, p. 13-24, 2002.

SAKAI, C.; ISHIDA, M.; OHBA, H.; YAMASHITA, H.; UCHIDA, H.; YOSHIZUMI, M.; ISHIDA, T. Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. **PLoS One,** San Francisco, CA: Public Library of Science, v. 12, n. 11, p. e0187934, 2017.

SALTIEL, A. R. Fishing out a sensor for anti-inflammatory oils. **Cell,** Cambridge: MIT Press, v. 142, n. 5, p. 672-4, Sep. 2010.

SARDINHA, F. L. C.; FERNANDES, F. S.; TAVARES DO CARMO, M. G.; HERRERA, E. Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,** Bethesda, Md.: American Physiological Society, v. 304, n. 4, p. R313-R320, 2012.

SCHERER, P. E. Adipose tissue: from lipid storage compartment to endocrine organ. **Diabetes,** New York: American Diabetes Association, v. 55, n. 6, p. 1537-45, Jun. 2006.

SIEMELINK, M.; VERHOEF, A.; DORMANS, J. A.; SPAN, P. N.; PIERSMA, A. H. Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. **Diabetologia,** Berlin: Springer Verlag, v. 45, n. 10, p. 1397-403, Oct 2002.

SILVA, D. R. B. D.; MIRANDA JÚNIOR, P. F.; SOARES, E. D. A. A importância dos ácidos graxos poliinsaturados de cadeia longa na gestação e lactação. **Revista Brasileira de Saúde Materno Infantil,** Recife, v. 7, p. 123-133, 2007.

SMITH, S. C., JR.; BENJAMIN, E. J.; BONOW, R. O.; BRAUN, L. T.; CREAGER, M. A.; FRANKLIN, B. A.; GIBBONS, R. J.; GRUNDY, S. M.; HIRATZKA, L. F.; JONES, D. W.; LLOYD-JONES, D. M.; MINISSIAN, M.; MOSCA, L.; PETERSON, E. D.; SACCO, R. L.; SPERTUS, J.; STEIN, J. H.; TAUBERT, K. A.; WORLD HEART, F.; THE PREVENTIVE CARDIOVASCULAR NURSES, A. AHA/ACCF Secondary Prevention and Risk Reduction Therapy for Patients with Coronary and other Atherosclerotic Vascular Disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation. **Circulation,** Dallas: American Heart Association, v. 124, n. 22, p. 2458-73, Nov 29 2011.

SPERETTA, G.; BASSI, M.; RUCHAYA, P.; SUMNERS, C.; MENANI, J.; COLOMBARI, E.; COLOMBARI, D. Arterial pressure and gene expression in the nucleus of the solitary tract in rats fed with high-fat diet (874.4). **The FASEB Journal,** Bethesda, Md.: The Federation, v. 28, n. 1_supplement, p. 874, 2014.

SPOSITO, A. C.; CARAMELLI, B.; FONSECA, F. A. H.; BERTOLAMI, M. C.; AFIUNE NETO, A.; SOUZA, A. D.; LOTTENBERG, A. M. P.; CHACRA, A. P.; FALUDI, A. A.; LOURES-VALE, A. A.; CARVALHO, A. C.; DUNCAN, B.; GELONESE, B.; POLANCZYK, C.; RODRIGUES SOBRINHO, C. R. M.; SCHERR, C.; KARLA, C.; ARMAGANIJAN, D.; MORIGUCHI, E.; SARAIVA, F.; PICHETTI, G.; XAVIER, H. T.; CHAVES, H.; BORGES, J. L.; DIAMENT, J.; GUIMARÃES, J. I.; NICOLAU, J. C.; SANTOS, J. E. D.; LIMA, J. J. G. D.; VIEIRA, J. L.; NOVAZZI, J. P.; FARIA NETO, J. R.; TORRES, K. P.; PINTO, L. D. A.; BRICARELLO, L.; BODANESE, L. C.; INTROCASO, L.; MALACHIAS, M. V. B.; IZAR, M. C.; MAGALHÃES, M. E. C.; SCHMIDT, M. I.; SCARTEZINI, M.; NOBRE, M.; FOPPA, M.; FORTI, N. A.; BERWANGER, O.; GEBARA, O. C. E.; COELHO, O. R.; MARANHÃO, R. C.; SANTOS Fº, R. D. D.; COSTA, R. P.; BARRETO, S.; KAISER, S.; IHARA, S.; CARVALHO, T. D.; MARTINEZ, T. L. R.; RELVAS, W. G. M.; SALGADO, W. IV Diretriz Brasileira sobre Dislipidemias e Prevenção da Aterosclerose: Departamento de Aterosclerose da Sociedade Brasileira de Cardiologia. **Arq Bras Cardiol,** São Paulo, v. 88, p. 2-19, 2007.

SUGANAMI, T.; NISHIDA, J.; OGAWA, Y. A Paracrine Loop Between Adipocytes and Macrophages Aggravates Inflammatory Changes. **Arterioscler Thromb Vasc Biol,** Dallas, TX: American Heart Association, v. 25, n. 10, p. 2062, 2005.

THALER, J.; YI, C.-X.; SCHUR, E. A.; GUYENET, S. J.; HWANG, B.; DIETRICH, M.; ZHAO, X.; SARRUF, D. A.; IZGUR, V.; MARAVILLA, K. R.; NGUYEN, H. T.; FISCHER, J. D.; MATSEN, M.; WISSE, B. E.; MORTON, G. J.; HORVATH, T.; BASKIN, D.; TSCHÖP, M.; SCHWARTZ, M. W. Obesity is associated with hypothalamic injury in rodents and humans. **The Journal of Clinical Investigation,** New Haven: American Society for Clinical Investigation, v. 122, n. 1, p. 153-162, 2012.

TIMPER, K.; BRUNING, J. C. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. **Dis Model Mech,** Cambridge: Company of Biologists Ltd., v. 10, n. 6, p. 679-689, Jun 1 2017.

TITOS, E.; RIUS, B.; GONZÁLEZ-PÉRIZ, A.; LÓPEZ-VICARIO, C.; MORÁN-SALVADOR, E.; MARTÍNEZ-CLEMENTE, M.; ARROYO, V.; CLÀRIA, J. Resolvin D1

and Its Precursor Docosahexaenoic Acid Promote Resolution of Adipose Tissue Inflammation by Eliciting Macrophage Polarization toward an M2-Like Phenotype. **The Journal of Immunology,** Baltimore: Williams & Wilkins, v. 187, n. 10, p. 5408-5418, 2011.

TSAOUSIDOU, E.; PAEGER, L.; BELGARDT, BENGT F.; PAL, M.; WUNDERLICH, CLAUDIA M.; BRÖNNEKE, H.; COLLIENNE, U.; HAMPEL, B.; WUNDERLICH, F. T.; SCHMIDT-SUPPRIAN, M.; KLOPPENBURG, P.; BRÜNING, JENS C. Distinct Roles for JNK and IKK Activation in Agouti-Related Peptide Neurons in the Development of Obesity and Insulin Resistance. **Cell Rep,** Cambridge, MA: Cell Press, v. 9, n. 4, p. 1495-1506, 2014.

TUNCER, S.; BANERJEE, S. Eicosanoid pathway in colorectal cancer: Recent updates. **World J Gastroenterol,** Beijing: WJG Press, v. 21, n. 41, p. 11748-66, Nov. 2015.

VALENZUELA, R.; ESPINOSA, A.; GONZÁLEZ-MAÑÁN, D.; D'ESPESSAILLES, A.; FERNÁNDEZ, V.; VIDELA, L. A.; TAPIA, G. N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. **PLoS One,** San Francisco, CA: Public Library of Science, v. 7, n. 10, p. e46400-e46400, 2012.

VICKERS, M. H. Early life nutrition, epigenetics and programming of later life disease. **Nutrients,** Basel: MDPI Publishing, v. 6, n. 6, p. 2165-78, Jun. 2014.

VIDAL-SANTOS, R.; MACEDO, F. N.; SANTANA, M. N.; DE MELO, V. U.; DE BRITO ALVES, J. L.; SANTOS, M. R.; BRITO, L. C.; NASCIMENTO, E.; COSTA-SILVA, J. H.; SANTANA-FILHO, V. J. Western diet in the perinatal period promotes dysautonomia in the offspring of adult rats. **J Dev Orig Health Dis,** Cambridge: Cambridge University Press, v. 8, n. 2, p. 216-225, Apr 2017.

VOGT, M. C.; PAEGER, L.; HESS, S.; STECULORUM, S. M.; AWAZAWA, M.; HAMPEL, B.; NEUPERT, S.; NICHOLLS, H. T.; MAUER, J.; HAUSEN, A. C.; PREDEL, R.; KLOPPENBURG, P.; HORVATH, T. L.; BRUNING, J. C. Neonatal insulin action impairs hypothalamic neurocircuit formation in response to maternal high-fat feeding. **Cell,** Cambridge: MIT Press, v. 156, n. 3, p. 495-509, Jan. 2014.

WAKI, H.; GOURAUD, S. S.; MAEDA, M.; PATON, J. F. Evidence of specific inflammatory condition in nucleus tractus solitarii of spontaneously hypertensive rats. **Exp Physiol,** Cambridge; New York, NY, USA : Published for the Physiological Society by Cambridge University Press, v. 95, n. 5, p. 595-600, May 2010.

WANG, G.; ZHOU, P.; REPUCCI, M. A.; GOLANOV, E. V.; REIS, D. J. Specific actions of cyanide on membrane potential and voltage-gated ion currents in rostral ventrolateral medulla neurons in rat brainstem slices. **Neuroscience Letters,** Amsterdam: Elsevier/North-Holland, v. 309, n. 2, p. 125-129, 2001.

WEN-JU LIN, C.; JAMES, W. A.; DARRELL, J. Propionate May Mediate the Hypocholesterolemic Effects of Certain Soluble Plant Fibers in Cholesterol-Fed Rats. **Proceedings of the Society for Experimental Biology and Medicine,** Malden, MA: Blackwell Science, v. 175, n. 2, p. 215-218, 1984.

WEST-EBERHARD, M. J. Alternative adaptations, speciation, and phylogeny (A Review). **Proc Natl Acad Sci U S A,** Washington, DC: National Academy of Sciences, v. 83, n.5, p. 1388-92, Mar 1986.

WEST-EBERHARD, M. J. Phenotypic Plasticity and the Origins of Diversity. **Annual Review of Ecology and Systematics,** Palo Alto: Annual Reviews inc.,v. 20, n. 1, p. 249-278, 1989.

WEST-EBERHARD, M. J. Phenotypic accommodation: adaptive innovation due to developmental plasticity. **Journal of Experimental Zoology Part B: Molecular and Developmental Evolution,** Hoboken, NJ: Wiley-Liss, v. 304B, n. 6, p. 610-618, 2005.

XUE, B.; THUNHORST, R.; YU, Y.; GUO, F.; BELTZ, T.; B FELDER, R.; JOHNSON, A. Central Renin-Angiotensin System Activation and Inflammation Induced by High-Fat Diet Sensitize Angiotensin II-Elicited Hypertension. **Hypertension**, Hagerstown, v. 67, n. 1, p. 163-170, jan. 2016.

YOON, A.; TAMMEN, S. A.; PARK, S.; HAN, S. N.; CHOI, S. W. Genome-wide hepatic DNA methylation changes in high-fat diet-induced obese mice. **Nutr Res Pract,** Seoul, v. 11, n. 2, p. 105-113, Apr 2017.

YU, B. P. Cellular defenses against damage from reactive oxygen species. **Physiological Reviews,** Washington: American Physiological Society, v. 74, n. 1, p. 139-162, 1994.

ANEXO A - APROVAÇÃO DO COMITÊ DE ÉTICA NO USO DE ANIMAIS

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Recife, 09 de agosto de 2017.

Ofício nº 67/17

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Prof. João Henrique da Costa Silva**
Centro Acadêmico de Vitória
Universidade Federal de Pernambuco
Processo nº **23076.049500/2016-37**

Certificamos que a proposta intitulada **"Efeitos da suplementação de ômega-3 sobre os parâmetros ventilatórios, de pressão arterial, composição corporal e metabolismo na prole de machos de ratas submetidoas à dieta hiperlipídica durante o período perinatal"**, registrada com o nº **23076.049500/2016-37** sob a responsabilidade de **Prof. João Henrique da Costa Silva** que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 07/06/2017.

Finalidade	() Ensino (X) Pesquisa Científica
Vigência da autorização	Até 08/2019
Espécie/ linhagem/raça	Ratos isogênicos/Wistar
Nº de animais	32
Peso/Idade	200-250g / 90 dias
Sexo	9 machos e 18 fêmeas
Origem	Bioterio do departamento de Fisiologia e Farmacologia – CB/UFPE.

Atenciosamente,

Prof. Sebastião R. F. Silva
Vice-Presidente CEUA/UFPE
SIAPE 2345691

ANEXO B – ARTIGO DE REVISÃO

ARTIGO 2- Artigo a ser submetido à Revista Frontiers in Phisiology

Fator de impacto: 3,394 **Qualis:** A1 (Nutrição)

Cardiometabolic impacts of saturated fatty acids: Are they all comparable ?

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Abbreviation list+++

AMPK- AMP-activated protein kinase

ApoE - Apolipoprotein E

APPL1- Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1

CCR2- Chemokine receptor 2

CD14- Cluster of differentiation 14

CNS- Central nervous system

COX- Cyclooxygenase

CR-MCT - Diet rich in cholesterol and supplemented with medium chain triglycerides

CR-LCT- Diet rich in cholesterol and supplemented with long chain triglycerides

EFSA - European Food Safety Authority

ER- Endoplasmic reticulum

FFA- Free fatty acids

FAS- Fatty acid synthase

GLP1- Glucagon-like peptide 1GLUT-4- Glucose transporter type 4

GPCR- G protein-coupled receptors

HDL- C- High-density lipoprotein cholesterol

I-BABP - Ileal bile acid binding protein

ICAM-1 - Intracellular adhesion molecules

IKK - Inhibitor of κB kinase

IL-6 - Interleukin-6

iNOS - Nitric oxide synthase

IRS- Insulin receptor substrates on tyrosine

JNK- c-Jun N-terminal kinase

K_{ATP} - ATP-sensitive potassium channels

LC SFA - Long chain fatty acidsLDL-C- Low-density lipoprotein cholesterol

LPS- Lipopolysaccharide

MCP-1 - Monocyte chemotactic protein-1

MC SFA - Medium chain fatty acids

MCTs - Medium-chain triglycerides

MCTs - Medium-chain triglycerides

mRNA- Messenger RNA

MUFAs - Monounsaturated fatty acids

NAFLD - Non-alcoholic fatty liver diseases

NF-kB - Nuclear factor kappa B

NO- Nitric oxide

PUFAs - Polyunsaturated fatty acids

PYY- Peptide YY

SC SFA - Short chain fatty acids

SFA - Saturated fatty acids

TC- Total cholesterol

T2DM- Type 2 diabetes mellitus

T3- Triiodothyronine

VCAM-1- Vascular cell adhesion molecules

VLC - Very long chain fatty acids

Abstract: In last decades, a phenomenon named nutrition transition has been observed in many countries around the world. It has been characterized by increased consumption of fat-rich diets, predisposing to cardiometabolic diseases and high prevalence of the obesity. In the dietary recommendations cited to prevent metabolic diseases, there is a consensus to decrease intake of saturated fatty acids (SFA) to less than 10% of total energy intake, as recommended by the European Food Safety Authority (EFSA). However, fatty acids of different chain lengths may exhibit different cardiometabolic effects.. Thus, our major aim was to review the cardiometabolic effects of different classes of SFA according to carbon chain length, i.e. short-, medium- and long-chains. The review emphasizes that not all SFA may have harmful cardiometabolic effects since short- and medium-chain SFA can provide beneficial health effects and participate to the prevention of metabolic disorders.

Keywords: Obesity; metabolic changes; cardiovascular diseases; dyslipidemia

Introduction

In the rapid change of the morbimortality model, the nutritional factor plays a major role. With the nutritional transition, the prevalence of non-transmissible chronic diseases associated with hypercaloric diets, trans fats, saturated fatty acids, and exacerbated consumption of sugar and salt, lack of physical activity and other unhealthy practices related to western lifestyle are observed worldwide (1). Characteristics of western diets include high levels of saturated fats, sodium, simple sugars and low levels of fiber (2, 3).

In the dietary recommendations cited to prevent metabolic diseases, there is a consensus to decrease intake of saturated fatty acids (SFA) to less than 10% of total energy intake (4). However, fatty acids of different chain lengths may exhibit different cardiometabolic effects. (5). As for chain size, fatty acids can be classified into short chain fatty acids (containing from 2 to 6 carbon atoms, SC), medium chain (with 8 to 12 carbon atoms, MC), long chain (with 14 to 18 carbon atoms, LC) and very long chain (having 20 or more carbon atoms, VLC) (6). According to the origin, saturated fatty acids (SFA) can be divided into animal or vegetable origin. Good examples are vegetable oils sources of SFA, such as palm and coconut oils. However, there is a considerable variety in this category. Coconut oil is rich in lauric acid (12: 0) and also in caprylic (8:0), decanoic (10:0) and myristic (14:0) acids. Palm oil consists mainly of palmitic acid (16:0) and oleic acid (18: 1n-9), the last one being a monounsaturated fattyd acid. In general, the most common dietary SFA are palmitic acid (16:0) and stearic acid (18:0) found in animal fats and also in plants. Palmitic acid (16:0) is one of the major fatty acids in plasma and human tissues, reflecting the consumption of moderately high fat diets (5). Milk fat, from humans (7) as well as from farm animals (cow, goat) (8), has a very characteristic composition, since it contains SC, MC and LC SFA, as well as monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Still, in most dairy products, the main fatty acid is palmitic acid (16: 0) (5).

The aim of this study was to review the main cardiometabolic effects of different classes of SFA according to carbon chain length, i.e. short-, medium- and long-chains. Highlighting harmful and beneficial health effects of SFA according to carbon chain length.

Physiological and cardiometabolic effects of short-chain SFA

The consumption of SC SFA is uncommon in the human diet, with the exception of dairy products (5). Fat from goat milk has approximately a percentage of 20% SC SFA and

that from cow milk on average 11% (8). Dietary carbohydrates, specifically resistant starches and dietary fibers, are substrates for fermentation by the intestinal microbiota that produce SC SFA, such as acetic acid (2:0), propionic acid (3:0) and butyric acid (4:0). It is assumed that most SC SFA are products of colonic fermentation in humans (5, 9). SC SFA are used by enterocytes as an energy source or are transported through the intestinal epithelium into the bloodstream where they can interact with peripheral cells and tissues as signaling molecules. The concentrations of SC SFA in the peripheral circulation are very low, consequently due to hepatic metabolism (10, 11). The daily production of SC SFA in humans is approximately 100-200 mM (12, 13). The concentrations of SC SFA estimated in the proximal colon varies from 70 to 140 mM (12, 13) and reduce to 20 to 70 mM in the distal colon (13). These concentrations of SC SFA are influenced by diet, portion of the intestine and type of microbiota present (14). The predominant SC SFA found in plasma is acetic acid (2:0) in concentrations ranging from 58 to 230 μM. Serum concentrations of 3 to 15 μM and 1 to 28 μM have been reported for propionic acid (3:0) and butyric acid (4:0), respectively (15-17). It has been proposed that serum levels of SC FAs may be positively associated with the quantity of fiber habitually consumed (18).

In general, SC SFAs can activate G protein-coupled receptors (GPCR41 and GPCR43) in colon epithelial and enteroendocrine cells, leading to the release of the peptide YY (PYY) and glucagon-like peptide 1 (GLP1) which are intestinal anorectic hormones (i.e. they decrease the appetite) (19). This could contribute to reducing food intake and protecting individuals against obesity and related metabolic disorders.

Acetic acid (2:0) is easily absorbed and transported to the liver, so it is less metabolized in the colon and penetrates the peripheral circulation. The presence of acetyl-CoA synthetase in the adipose cells and mammary glands allows the use of acetic acid (2:0) for lipogenesis, and in the muscle cells to generate energy from its oxidation. Acetic acid also increase cholesterol synthesis (20). Lactulose, a dietary fiber that is rapidly fermented in the colon, caused higher serum cholesterol levels when compared to the control diet. (21). However, polyfructans fibers (inulin and oligofructose) in combination with viscous fibers (oat bran) can lower serum cholesterol (20) although acetic acid production is enhanced. This is due to the fact that the parallel increase in propionic acid (3:0) production from polyfructans is able to counteract the impact of acetic acid, thus reducing the risk of cardiovascular diseases (20).

Indeed, propionic acid (3: 0) has been suggested to have anti-inflammatory actions and beneficial effects for prevention of obesity, type 2 diabetes (T2DM) and cardiocvascular diseases (5, 22). First, propionic acid (3: 0) has moderate inhibitory activity on cyclooxygenase (COX), an important enzyme in the production of pro-inflammatory eicosanoids (23). Although inflammation acts in the defense of the host, chronic inflammatory condition is a pathological characteristic observed in the metabolic syndrome, non-alcoholic fatty liver disease, T2DM and cardiovascular diseases (24, 25). Second, propionic acid is extensively absorbed by the liver and has been shown to inhibit cholesterol synthesis (26). Studies in rodents also suggest that propionic acid (3:0) has effects on the reduction of fatty acid concentration in the liver (27, 28) and plasma (29, 30). Finally, propionic acid (3:0) is probably able to favour adipogenesis in white adipose tissue (31, 32). Indeed, propionic acid (3:0) was found to inhibit in a dose-dependent manner the isoproterenol-induced lipolysis in differentiated adipocytes 3T3-L1 (31, 32). Furthermore, propionic acid was shown to dose-dependently induce the expression of the glucose transporter type 4 (GLUT-4) in human adipose tissue (22) thus enhancing glucose captation for favouring triglycerides synthesis. The mechanism apointed for the induction of adipogenesis includes the binding of propionic acid (3:0) to the G protein-coupled receptor (GPCR43), which is an important receptor in cellular function (31). GPR43 belongs to a subfamily of GPCRs, including GPR40 and GPR41, which have been identified as receptors for fatty acids (33). GPCR43 is induced during the differentiation process of adipocytes and its expression is increased in white adipose tissue of rodents fed a high-fat diet (31), suggesting that GPCR43 participates to the regulation of adipocyte functions. A greater expression of the GPCR43 receptor was found *in vitro* in four white adipose tissues (perirenal, mesenteric, epididimal and parametrial) of mice after administration of propionic acid (3:0) (31).

In the context of control of cellular lipid content, some studies have suggested that propionic acid (3:0) may modulate an important hormone secreted primarily by white adipose tissue, i.e. leptin (34). Leptin levels correlate positively with adiposity and serve as an index of body fat stores (35). Fasting decreases leptin level, whereas food intake restores its level (34). This anorectic hormone penetrates the blood-brain barrier and exerts an effect on receptors located in the hypothalamus, causing the release of mediators leading to anorexic action and inhibiting appetite (36). Leptin expression in the white adipose tissue of rats (34) and humans (37) was induced after treatment with propionic acid (3:0) potentially through activation of GPCR41 (37, 38). The inhibitory effects of propionic acid (3:0) on inflammation

and free fatty acid metabolism suggest that propionic acid may play a role as a therapeutic agent to improve insulin sensitivity (22). Indeed, elevation of plasma free fatty acid concentration has been shown to cause inflammation (39, 40) and vice versa (41, 42), and both lead to insulin resistance (42-44).

Butyric acid (4:0), which is found in milk (5), is the primordial source of energy of the colonocytes (20). It plays major role in colonic mucosa nutrition and in the prevention of colon cancer (45, 46). The mechanisms include inhibition of the nuclear factor kappa B (NF-kB) and of the histone deacetylase enzyme (47), which has been shown to induce apoptosis in tumor cells (48). Butyric acid (4: 0) has also been shown to play a role in preventing intestinal inflammation, through binding to the GPR43 receptor (also present in cells of the colon, neutrophils and eosinophils) which blunts the inflammatory responses. Hence, rats deficient in GPCR43 are models of exacerbated inflammation associated to colitis, arthritis and asthma (49). Interestingly, germ-free mice, which are devoided of bacteria, evidence little or no circulating SC SFA (49). They also demonstrated similar exacerbation in certain inflammatory responses (49) as in GPR43 KO animals. Butyric acid (4:0) may help preventing atherosclerosis and heart failure through inhibition of the NF-kB pathway (50). Hence, dietary supplementation with butyric acid (4:0) suppressed atherosclerotic lesions in apolipoprotein E (apoE) knockout mice (50). Butyric acid (4:0) reduces monocyte chemotactic protein-1 (CCL2 / MCP-1), the vascular cell adhesion molecule 1 and the matrix metalloproteinase 2 production at the lesion site, leading to decreased macrophage migration and increased collagen deposition and atherosclerotic plaque stability (50, 51). In a co-culture system with adipocytes and macrophages, 0.1 to 1.0 mM of butyric acid (4:0) significantly reduced production of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), and release of glycerol and free fatty acids. This suggested that butyric acid (4:0) is able to blunt inflammatory and to inhibit triglyceride lipolysis through its binding to GPCR43 and GPCR41 present in adipocytes (51, 52).

Physiological and cardiometabolic effects of medium Chain SFA

The MC SFA are found in dairy products and coconut and palm kernel oil (5). MC SFA are also found in human milk and colostrum, and are suggested to provide antimicrobial protection on the mucosal surfaces of children (53). Medium-chain triglycerides (MCTs) are a family of triglycerides, composed of caproic (6:0), caprylic (8:0), capric fatty acids (10:0),

and lauric (12:0) (54). MCTs are partially hydrolyzed by gastric lipase in the stomach and thereafter by pancreatic lipase within the intestinal lumen, allowing the direct absorption of MC SFA through the portal vein to the liver, instead of being absorbed through the lymphatic system, which is the conventional pathway for the absorption of LC SFA (54, 55). Compared to LC SFA, MC SFA are more favorable to β oxidation in the liver and are not stored as fat in the same proportion as LC SFA (4, 61). Few studies showed that they raise the basal metabolic rate and thermogenesis (5, 56). As previously mentioned, MC SFA are more easily absorbed and transported to the liver through the portal circulation, which leaves them adequate in states of intestinal malabsorption (condition that occurs when inflammation, disease or injury reduces the ability of absorption of nutrients by the small intestine), pancreatic insufficiency, impaired chylomicron lymphatic transport, total parenteral nutrition. MC SFA are also used in premature infant formula (57).

In chicken embryo hepatocytes, caproic acid (6:0) and caprylic acid (8:0) have been shown to significantly decrease the expression and activity of fatty acid synthase (FAS) induced by insulin and T3 (triiodothyronine) (58). FAS is a key enzyme of *de novo* lipogenesis. It is positively regulated in the postprandial state and is suspected to contribute to obesity and non-alcoholic fatty liver diseases (NAFLD) (59, 60). In cultured hepatocytes, caprylic acid (8:0) and capric acid (10:0) inhibited the induction of cell steatosis induced by oleic acid (18:1) and palmitic acid (16:0) (61).

It has been shown that MC SFA can prevent endotoxic lipopolysaccharide (LPS)-mediated endotoxemia. The rats were fed either MC SFA or corn oil (source of LC fatty acids) by daily gavage during 1 week before they received an acute intravenous dose of LPS. Injection of LPS caused mortality in animals fed corn oil, but no mortality occurred in MC SFA fed rats. In LPS treated groups, liver lesions and markers of inflammation (CD14, cluster of differentiation 14, cellular receptor involved in inflammation; TNF-α, tumor necrosis factor alpha) were lower in MC SFA fed rats than in those which received corn oil (62). Furthermore, intestinal permeability was improved in MC SFA/LPS animals compared to those treated with corn oil/LPS (62). The MC SFA gavage also prevents intestinal atrophy (a problem that occurs during parenteral nutrition) (62). This study thus demonstrated that MC SFA supplementation was able to limit endotoxemia-induced inflammation and lesions, which are associated with obesity and the metabolic syndrome.

In humans, a meta-analysis of randomized clinical trials (nine parallel studies) showed that compared to LC SFA, MC SFA administered for 3 weeks or more significantly

reduced body weight, waist circumference, hip circumference, total body fat, total subcutaneous fat and visceral fat (63). Small reductions in adiposity were confirmed after ingestion of 12 weeks of caprylic acid (8:0) and capric (10:0) in humans compared to the group that consumed common edible oil, blended rapeseed oil and soybean oil used as source LC SFA. The test diet containing LCT or MCT oil was offered in bread preparation, during breakfast, the daily intake of the test oil was set at 10 g. (64). In mice fed a diet rich in cholesterol and supplemented with medium chain triglycerides [capillary acids (C8:0) and capric acids (C10:0)] (CR-MCT) of long chain triglycerides (CR-LCT) for 16 weeks, MCT supplementation decreased body weight and serum levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and increased serum high-density lipoprotein cholesterol (HDL-C) / LDL-C ratio (65). An increase in primary bile acid and secondary bile acid concentrations in feces and secondary bile acids in bile was found with reduction of ileal bile acid binding protein (I-BABP) gene expression in the small intestine in the CR-MCT group. This suggests that caprylic acid (C8:0) and capric acid (C10:0)-induced reduction in serum cholesterol levels may be mediated through increased bile acid excretion, probably thanks to the reduction of bile acid reabsorption in the small intestine (65).

By contrast with other MC SFA, lauric acid (12:0) is classified in the literature as MC SFA, and sometimes as LC SFA (5). It can be absorbed through both the portal and lymphatic system. MC SFA monoglycerides, and specifically lauric acid monolaurin (12:0), demonstrated antimicrobial effects and protection against *Helicobacter pylori* (66). Lauric acid (12:0) is found in coconut oil, palm kernel oil, dairy products and some margarines. It was shown to increase the risk of cardiovascular diseases, by increasing TC and LDL-C, suggesting the oils sources of lauric acid would be very damaging to cardiovascular health. Ingestion of lauric acid (12:0) elevates HDL levels to a higher degree than LDL, thus improving the TC: HDL-C ratio when compared to carbohydrates (67, 68). However in rabbits, an isocaloric diet rich in coconut oil (at either 15% or 26% of total energy) in comparison with standard feeding (4.2% fat derived from soybean) induced the development of atherosclerosis and marked metabolic disorders, even without body weight gain (69). In humans, the consumption of a meal rich in coconut oil (composition of fatty acids: 89.6% saturated fats, 5.8% monounsaturated and 1.9% polyunsaturated) compared to a meal rich in safflower oil (composition of fatty acids: 75% polyunsaturated fats, 13.6% monounsaturated and 8.8% saturated) decreased the anti-inflammatory action of HDL-C and promoted atherogenic factors (70). Furthermore, expression of the intracellular adhesion molecules

(ICAM)-1 and vascular cell adhesion molecules (VCAM)-1 was increased in human umbilical vein endothelial cells incubated with HDL-C from individuals who had consumed a meal rich in coconut oil, whereas it was decreased following incubation with HDL from individuals who had consumed a meal rich in safflower oil (71). These data suggest that lauric acid (12:0) despite of its impact on increased plasma HDL-C, is not favourable to cardiovascular protection. This conclusion is supported by a review study which evidenced that the consumption of coconut oil, compared to the consumption of unsaturated oils and other saturated fats, does not help improving plasma lipid and lipoprotein profiles, and does not help reducing the risk of cardiovascular diseases (72).

Physiological and cardiometabolic effects of long chain SFA

The human plasma of healthy subjects after an overnight fast contains on average 214 mM of free fatty acids, with approximately 63.8 mM of palmitic acid (16:0) and 22.1mM of stearic acid (18:0) (73). In humans, palmitic acid (16:0) consumption increases serum TC, LDL-C, and HDL-C concentrations in comparision to diets rich in carbohydrate and it is accepted that this SFA increases the risks of cardiovascular diseases (5). By contrast, involvement of stearic acid (18:0) in the increased risk of cardiovascular diseases are less consensual (74). In a systematic review and meta-analysis involving 51 dietary intervention trials, it was found that substitution of palmitic acid (16:0) for stearic acid (18:0) induced higher concentrations of various biomarkers of coronary heart disease and cardiovascular disease, both unfavorable (TC, LDL-C, apolipoprotein B and LDL-C/ HDL-C) and favorable (HDL-C), whereas whereas most of the biomarkers were significantly lower when compared with diets rich in myristic (14:0)/lauric (12:0) acid (75). Postprandial lipemia was highly increased after consumption of stearic acid (18:0) in comparision to SC SFA and MC SFA (76), but plasma LDL levels were lower in comparison to other LC SFA such as palmitic acid (16:0) (77). Furthermore, stearic acid (18:0) consumption had similar impacts on plasma lipoproteins (LDL e HDL) compared to a diet rich in carbohydrates (77). However when compared to a high-fat milk diet (for 5 weeks), characterized by high content of palmitic acid (16:0), a diet rich in stearic acid (18:0) (33% of energy from fat [9.3% of energy as stearic acid], has been shown to induce levels of lipoprotein A (+ 10%), a risk factor for cardiovascular disease (78). In addition in men, stearic acid (18:0) intake for 5 weeks was shown to increase levels of plasma fibrinogen compared to high-carbohydrate diets, other

SFA (palmitic acid (16:0) and lauric acid (12:0)), trans fatty acids and oleic acid (18:1n-9) (79). However, other studies failed to evidence the prothrombotic effect of stearic acid compared to palmitic acid (16:0) (80) or even showed beneficial effects in relation to thrombogenic risk profiles (81). Stearic acid (18:0) has been shown to influence further death and inflammation of endothelial cells in humans. It induces apoptosis of human coronary artery endothelial cells by activating the inhibitor of κB kinase (IKK) and NF-kB. The role of stearic acid in the activation of NF-KB was also demonstrated in comparison to other LC SFAs in an *in vitro* study (82).

Palmitic acid (16:0) induces lipotoxicity. When the absorption of free fatty acids (FFA) more active than the metabolism of FFA occurs an accumulation of fatty acids and deposition of lipids in peripheral tissues such as liver, muscle and pâncreas (83). This abnormal ectopic lipid accumulation (lipotoxicity) is enough associated with insulin resistance (84, 85). It has been established that lipotoxicity plays an important role in the development of heart failure in obesity and diabetes (86, 87). A large increase in free fatty acids can lead to apoptosis and dysfunction of pancreatic β-cells (83). Insulin secretion is related to sequential ionic events following glucose stimulation. ATP-sensitive potassium channels (K_{ATP}) serve as glucose sensors and initiate insulin secretion through the stimulation of glucose (88). A study of 8 week old mice injected intraperitoneally with 100 mg / kg palmitic acid (16: 0) five times a day for 2 weeks and after 2 weeks were injected with 200 mg / kg palmitic acid (16:0) twice daily for another 2 weeks, found in these mice T2DM characteristics. Pancreatic sections of these mice showed a decrease in the expression of K_{ATP} channels and pancreatic β-cell apoptosis (83). It has been shown that FFAs increase endoplasmic reticulum (ER) stress in β-cells, inducing to dysregulation of protein folding, processing, trafficking, and calcium buffering. After exposure to saturated FFAs, such as palmitic acid (16:0), a sustained depletion of ER Ca^{2+}, followed by mitochondria dysfunction and the production of nitric oxide (NO) free radicals through the increased activity of inducible nitric oxide synthase (iNOS), causing to β-cell apoptosis (89). Lipotoxicity leads to insulin resistance in the liver and skeletal muscle, reducing normal responses to insulin, decreasing glucose uptake by skeletal muscle, increasing gluconeogenesis and providing glucose from the liver to the circulation (90). The mechanisms pointing to reduction of insulin insulin signaling include reduction of insulin receptor expression and activity, reduction of phosphorylation of insulin receptor substrates on tyrosine (IRS) and activation of kinases such as c-Jun N-terminal kinase (JNK), which phosphorylate the IRS in serine residues, inhibiting

its activity (90, 91) Lipotoxicity is also related to insulin resistance through mechanisms of mitochondrial dysfunction (91).

Study *in vitro* with H9c2 cells (embryonic cardiac myoblasts) treated with palmitic acid (16:0) (100 mM) induced a lipotoxicity, a gradual increase in intracellular lipid accumulation, as well as apoptotic cell death. An increase in stress in the endoplasmic reticulum (ER) was observed in these mice in response to palmitic acid (16:0), which also induced resistance to adiponectin, assessed via AMP-activated protein kinase (AMPK) phosphorylation, by decreasing the expression of adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1). Cardiomyocyte apoptosis is a component of cardiac remodeling leading to heart failure (92). Adiponectin is considered an important cardioprotective adipokine, with effects anti-apoptotic, anti-inflammatory, anti-fibrotic and beneficial metabolic effects (93) . A targeted mechanism that may lead to adiponectin resistance includes decreased expression of the components of the adiponectin signaling pathway such as APPL1. APPL1 is vital for many actions of adiponectin, involving metabolic and antiapoptotic effects (94, 95).

There is an increasing concepts that LC SFAs act on macrophages to alter the cellular ratio M1 (pro-inflammatory)/ M2 (anti-inflammatory). These fatty acids stimulate the development of M1 cells, while unsaturated types help to elevate M2 cells. During the development of obesity and adipocyte hypertrophy, infiltration of proinflammatory macrophages M1 and subsets of T cells occurs in the adipose tissue (96). Indeed, hypertrophied adipocytes release monocyte chemotactic protein-1 (MCP-1) (97), this activates the blood monocytes through the chemokine receptor 2 (CCR2) and induces the migration of monocytes into adipose tissue and differentiation into macrophages. Macrophages in adipose tissue are activated in response to SFA, such as palmitic acid (16:0), released from hypertrophied adipocytes, thus producing larger amounts of inflammatory mediators, such as TNF-α, interleukin-6, MCP-1 (40). *In vitro* studies have also demonstrated that exposure of adipocytes to high concentrations of palmitic acid (16:0) increased TNF-α and IL-6 messenger RNA (mRNA) and protein expressions (98).

To finish, a maternal diet rich in LC SFA (with 15.7% palmitic acid and 13.8% stearic acid from total fatty acids), also containing 12% trans fatty acids, with a PUFA: SFA ratio of 0.86, in the period of gestation and lactation leads to hypothalamic inflammation in rodent offspring (99). Maternal fat-rich dietary intake (35% of calories from fat) has also been shown to induce inflammation in offspring in non-human primates (100). Within a few days,

by eating a diet rich in calories and fat (60% of calories from fat), an increased amount of SFA from the periphery crosses the blood-brain barrier and induces an inflammatory response in hypothalamic neurons, being an early event in the development of obesity (101), involving the activation of microglia, tissue macrophages resident in the central nervous system (CNS) (102).

Conclusion

We observed that the different types of saturated fatty acids, classified according to carbon chain length, exhibit different cardiometabolic effects, from very beneficial to harmful. Dietary recommendations that suggest a general decrease of SFA consumption need to be weighed and new conceptions may be discussed by as some SFA, according to the length of their carbon chain, have beneficial effects on cardiometabolic parameters, acting in the prevention of metabolic, cardiovascular diseases and anti-inflammatory action.

Author contributions

AMNLGB, BM and JHCS drafted the work and revised critically for the important intellectual content; wrote the paper; final review of the manuscript.

Conflict of Interest Statement:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Batista Filho M, Batista LV. Transição alimentar/ nutricional ou mutação antropológica? Ciência e Cultura. 2010;62:26-30.
2. Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. International Journal Of Obesity. 2004;28:S2.
3. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. Nutrition reviews. 2012;70(1):3-21.
4. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal. 2010;8(3):1461.
5. Iggman D, Risérus U. Role of different dietary saturated fatty acids for cardiometabolic risk. Clinical Lipidology: Taylor & Francis; 2011. p. 209-23.
6. Silva SMCSd, Mura JDAP. Tratado de Alimentação, Nutrição e Dietoterapia 1° ed2007.
7. Garwolińska D, Namieśnik J, Kot-Wasik A, Hewelt-Belka W. Chemistry of human breast milk – a comprehensive review of the composition and role of milk metabolites in child development. Journal of agricultural and food chemistry. 2018.
8. Vieitez I, Irigaray B, Callejas N, González V, Gimenez S, Arechavaleta A, et al. Composition of fatty acids and triglycerides in goat cheeses and study of the triglyceride composition of goat milk and cow milk blends. Journal of Food Composition and Analysis. 2016;48:95-101.
9. Cummings JH. Short chain fatty acids in the human colon. Gut. 1981;22(9):763-79. Epub 1981/09/01.
10. Peters SG, Pomare EW, Fisher CA. Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. Gut. 1992;33(9):1249-52.
11. Li M, van Esch BCAM, Henricks PAJ, Garssen J, Folkerts G. Time and Concentration Dependent Effects of Short Chain Fatty Acids on Lipopolysaccharide- or Tumor Necrosis Factor α-Induced Endothelial Activation. Frontiers in Pharmacology. 2018;9(233).
12. Cook, Sellin. Review article: short chain fatty acids in health and disease. Alimentary pharmacology & therapeutics. 1998;12(6):499-507.
13. Topping DL, Clifton PM. Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. Physiological Reviews. 2001;81(3):1031-64.
14. Hooper LV, Midtvedt T, Gordon JI. HOW HOST-MICROBIAL INTERACTIONS SHAPE THE NUTRIENT ENVIRONMENT OF THE MAMMALIAN INTESTINE. Annual Review of Nutrition. 2002;22(1):283-307.
15. Wolever TMS, Josse RG, Leiter LA, Chiasson J-L. Time of day and glucose tolerance status affect serum short-chain fatty concentrations in humans. Metabolism - Clinical and Experimental. 1997;46(7):805-11.
16. Wolever TMS, Schrade KB, Vogt JA, Tsihlias EB, McBurney MI. Do colonic short-chain fatty acids contribute to the long-term adaptation of blood lipids in subjects with type 2 diabetes consuming a high-fiber diet? The American journal of clinical nutrition. 2002;75(6):1023-30.
17. Vogt JA, Pencharz PB, Wolever TMS. l-Rhamnose increases serum propionate in humans. The American journal of clinical nutrition. 2004;80(1):89-94.
18. Hashemi Z, Fouhse J, Im HS, Chan CB, Willing BP. Dietary Pea Fiber Supplementation Improves Glycemia and Induces Changes in the Composition of Gut Microbiota, Serum Short Chain Fatty Acid Profile and Expression of Mucins in Glucose Intolerant Rats. Nutrients. 2017;9(11).
19. Everard A, Cani PD. Gut microbiota and GLP-1. Reviews in endocrine & metabolic disorders. 2014;15(3):189-96. Epub 2014/05/03.
20. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. Journal of clinical gastroenterology. 2006;40(3):235-43. Epub 2006/04/25.
21. Jenkins DJ, Wolever TM, Jenkins A, Brighenti F, Vuksan V, Rao AV, et al. Specific types of colonic fermentation may raise low-density-lipoprotein-cholesterol concentrations. The American journal of clinical nutrition. 1991;54(1):141-7.
22. Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, Venema K. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochimica et biophysica acta. 2010;1801(11):1175-83. Epub 2010/08/10.
23. Bos CL, Richel DJ, Ritsema T, Peppelenbosch MP, Versteeg HH. Prostanoids and prostanoid receptors in signal transduction. The international journal of biochemistry & cell biology. 2004;36(7):1187-205. Epub 2004/04/28.

24. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135-43. Epub 2002/03/06.
25. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7. Epub 2006/12/15.
26. Galisteo M, Duarte J, Zarzuelo A. Effects of dietary fibers on disturbances clustered in the metabolic syndrome. The Journal of nutritional biochemistry. 2008;19(2):71-84. Epub 2007/07/10.
27. Nishina PM, Freedland RA. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. The Journal of nutrition. 1990;120(7):668-73. Epub 1990/07/01.
28. Demigne C, Morand C, Levrat MA, Besson C, Moundras C, Remesy C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. The British journal of nutrition. 1995;74(2):209-19. Epub 1995/08/01.
29. DiCostanzo A, Williams JE, Keisler DH. Effects of short- or long-term infusions of acetate or propionate on luteinizing hormone, insulin, and metabolite concentrations in beef heifers. Journal of animal science. 1999;77(11):3050-6. Epub 1999/11/24.
30. Wen-Ju Lin C, James WA, Darrell J. Propionate May Mediate the Hypocholesterolemic Effects of Certain Soluble Plant Fibers in Cholesterol-Fed Rats. Proceedings of the Society for Experimental Biology and Medicine. 1984;175(2):215-8.
31. Hong YH, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, et al. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology. 2005;146(12):5092-9. Epub 2005/08/27.
32. Ge H, Li X, Weiszmann J, Wang P, Baribault H, Chen J-L, et al. Activation of G Protein-Coupled Receptor 43 in Adipocytes Leads to Inhibition of Lipolysis and Suppression of Plasma Free Fatty Acids. Endocrinology. 2008;149(9):4519-26.
33. Covington DK, Briscoe CA, Brown AJ, Jayawickreme CK. The G-protein-coupled receptor 40 family (GPR40–GPR43) and its role in nutrient sensing. Biochemical Society transactions. 2006;34(5):770-3.
34. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, et al. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(4):1045-50.
35. Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nature Medicine. 1995;1:1311.
36. Maciejewska-Stelmach J, Śliwińska-Stańczyk P, Łącki JK. Review paper
The role of leptin in inflammatory connective tissue diseases. Reumatologia/Rheumatology. 2007;45(4):219-24.
37. Al-Lahham SH, Roelofsen H, Priebe M, Weening D, Dijkstra M, Hoek A, et al. Regulation of adipokine production in human adipose tissue by propionic acid. European journal of clinical investigation. 2010;40(5):401-7. Epub 2010/04/01.
38. Thomsen W, Leonard J, Behan DP. Orphan GPCR target validation. Current opinion in molecular therapeutics. 2004;6(6):640-56. Epub 2005/01/25.
39. Håversen L, Danielsson KN, Fogelstrand L, Wiklund O. Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages. Atherosclerosis. 2009;202(2):382-93.
40. Suganami T, Nishida J, Ogawa Y. A Paracrine Loop Between Adipocytes and Macrophages Aggravates Inflammatory Changes. Arteriosclerosis, thrombosis, and vascular biology. 2005;25(10):2062.
41. Green A, Dobias SB, Walters DJ, Brasier AR. Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. Endocrinology. 1994;134(6):2581-8.
42. Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochemical and biophysical research communications. 2006;341(2):507-14. Epub 2006/01/24.
43. Kennedy A, Martinez K, Chuang C-C, LaPoint K, McIntosh M. Saturated Fatty Acid-Mediated Inflammation and Insulin Resistance in Adipose Tissue: Mechanisms of Action and Implications. The Journal of nutrition. 2009;139(1):1-4.
44. Micha R, Mozaffarian D. Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. Nature reviews Endocrinology. 2009;5(6):335-44. Epub 2009/04/29.
45. Young GP, Hu Y, Le Leu RK, Nyskohus L. Dietary fibre and colorectal cancer: a model for environment--gene interactions. Molecular nutrition & food research. 2005;49(6):571-84. Epub 2005/05/03.
46. Sengupta S, Muir JG, Gibson PR. Does butyrate protect from colorectal cancer? Journal of gastroenterology and hepatology. 2006;21(1):209-18.
47. Chen J, Ghazawi FM, Bakkar W, Li Q. Valproic acid and butyrate induce apoptosis in human cancer cells through inhibition of gene expression of Akt/protein kinase B. Molecular Cancer. 2006;5(1):71.

48. Belakavadi M, Prabhakar BT, Salimath BP. Purification and characterization of butyrate-induced protein phosphatase involved in apoptosis of Ehrlich ascites tumor cells. Biochimica et biophysica acta. 2007;1770(1):39-47. Epub 2006/10/13.
49. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282-6.
50. Aguilar EC, Leonel AJ, Teixeira LG, Silva AR, Silva JF, Pelaez JMN, et al. Butyrate impairs atherogenesis by reducing plaque inflammation and vulnerability and decreasing NFκB activation. Nutrition, Metabolism and Cardiovascular Diseases.24(6):606-13.
51. Ohira H, Tsutsui W, Fujioka Y. Are Short Chain Fatty Acids in Gut Microbiota Defensive Players for Inflammation and Atherosclerosis? Journal of Atherosclerosis and Thrombosis. 2017;24(7):660-72.
52. Ohira H, Fujioka Y, Katagiri C, Mamoto R, Aoyama-Ishikawa M, Amako K, et al. Butyrate attenuates inflammation and lipolysis generated by the interaction of adipocytes and macrophages. J Atheroscler Thromb. 2013;20(5):425-42. Epub 2013/03/09.
53. Isaacs CE. Human milk inactivates pathogens individually, additively, and synergistically. The Journal of nutrition. 2005;135(5):1286-8. Epub 2005/05/04.
54. Bach AC, Babayan VK. Medium-chain triglycerides: an update. The American journal of clinical nutrition. 1982;36(5):950-62.
55. Traul KA, Driedger A, Ingle DL, Nakhasi D. Review of the toxicologic properties of medium-chain triglycerides. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2000;38(1):79-98. Epub 2000/02/24.
56. Seaton TB, Welle SL, Warenko MK, Campbell RG. Thermic effect of medium-chain and long-chain triglycerides in man. The American journal of clinical nutrition. 1986;44(5):630-4. Epub 1986/11/01.
57. Marten B, Pfeuffer M, Schrezenmeir J. Medium-chain triglycerides. International Dairy Journal. 2006;16(11):1374-82.
58. M. AM, Floriane P, Sabine S, Anne R, Catherine M. Inhibition of Insulin and T3-Induced Fatty Acid Synthase by Hexanoate. Lipids. 2010;45(11):997-1009.
59. Sanders FWB, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biological Reviews of the Cambridge Philosophical Society. 2016;91(2):452-68.
60. Berlanga A, Guiu-Jurado E, Porras JA, Auguet T. Molecular pathways in non-alcoholic fatty liver disease. Clinical and Experimental Gastroenterology. 2014;7:221-39.
61. Wang B, Fu J, Li L, Gong D, Wen X, Yu P, et al. Medium-chain fatty acid reduces lipid accumulation by regulating expression of lipid-sensing genes in human liver cells with steatosis. International journal of food sciences and nutrition. 2016;67(3):288-97.
62. Kono H, Fujii H, Asakawa M, Yamamoto M, Matsuda M, Maki A, et al. Protective Effects of Medium-Chain Triglycerides on the Liver and Gut in Rats Administered Endotoxin. Annals of surgery. 2003;237(2):246-55.
63. Mumme K, Stonehouse W. Effects of Medium-Chain Triglycerides on Weight Loss and Body Composition: A Meta-Analysis of Randomized Controlled Trials. Journal of the Academy of Nutrition and Dietetics. 2015;115(2):249-63.
64. Tsuji H, Kasai M, Takeuchi H, Nakamura M, Okazaki M, Kondo K. Dietary Medium-Chain Triacylglycerols Suppress Accumulation of Body Fat in a Double-Blind, Controlled Trial in Healthy Men and Women. The Journal of nutrition. 2001;131(11):2853-9.
65. Li H, Liu Y, Zhang X, Xu Q, Zhang Y, Xue C, et al. Medium-chain fatty acids decrease serum cholesterol via reduction of intestinal bile acid reabsorption in C57BL/6J mice. Nutrition & metabolism. 2018;15:37.
66. Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, et al. Glycerol monolaurate prevents mucosal SIV transmission. Nature. 2009;458(7241):1034-8. Epub 2009/03/06.
67. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. The American journal of clinical nutrition. 2003;77(5):1146-55. Epub 2003/04/30.
68. Micha R, Mozaffarian D. Saturated Fat and Cardiometabolic Risk Factors, Coronary Heart Disease, Stroke, and Diabetes: a Fresh Look at the Evidence. Lipids. 2010;45(10):893-905.
69. Waqar AB, Koike T, Yu Y, Inoue T, Aoki T, Liu E, et al. High-fat diet without excess calories induces metabolic disorders and enhances atherosclerosis in rabbits. Atherosclerosis. 2010;213(1):148-55.
70. Nicholls SJ, Lundman P, Harmer JA, Cutri B, Griffiths KA, Rye KA, et al. Consumption of saturated fat impairs the anti-inflammatory properties of high-density lipoproteins and endothelial function. Journal of the American College of Cardiology. 2006;48(4):715-20. Epub 2006/08/15.
71. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths: Elsevier. 1829-39 p.

72. Eyres L, Eyres MF, Chisholm A, Brown RC. Coconut oil consumption and cardiovascular risk factors in humans. Nutrition reviews. 2016;74(4):267-80.
73. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. Journal of lipid research. 2010;51(11):3299-305. Epub 2010/07/31.
74. Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. The American journal of clinical nutrition. 2010;91(1):46-63.
75. Fattore E, Bosetti C, Brighenti F, Agostoni C, Fattore G. Palm oil and blood lipid–related markers of cardiovascular disease: a systematic review and meta-analysis of dietary intervention trials. The American journal of clinical nutrition. 2014;99(6):1331-50.
76. Sanders TA. Fat and fatty acid intake and metabolic effects in the human body. Annals of nutrition & metabolism. 2009;55(1-3):162-72. Epub 2009/09/16.
77. Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. The American journal of clinical nutrition. 1997;65(5 Suppl):1628S-44S. Epub 1997/05/01.
78. Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M. Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. The American journal of clinical nutrition. 1997;65(5):1419-26.
79. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. The American journal of clinical nutrition. 2004;79(6):969-73. Epub 2004/05/26.
80. Thijssen MAMA, Hornstra G, Mensink RP. Stearic, Oleic, and Linoleic Acids Have Comparable Effects on Markers of Thrombotic Tendency in Healthy Human Subjects. The Journal of nutrition. 2005;135(12):2805-11.
81. Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Abedin L, Li D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. European journal of clinical nutrition. 2001;55(2):88-96. Epub 2001/04/18.
82. Harvey KA, Walker CL, Pavlina TM, Xu Z, Zaloga GP, Siddiqui RA. Long-chain saturated fatty acids induce pro-inflammatory responses and impact endothelial cell growth. Clinical nutrition.29(4):492-500.
83. Ruan J-S, Lin J-K, Kuo Y-Y, Chen Y-W, Chen P-C. Chronic palmitic acid-induced lipotoxicity correlates with defective trafficking of ATP sensitive potassium channels in pancreatic β cells. The Journal of nutritional biochemistry. 2018;59:37-48.
84. Consitt LA, Bell JA, Houmard JA. Intramuscular lipid metabolism, insulin action, and obesity. IUBMB life. 2009;61(1):47-55.
85. Samuel VT. Fructose induced lipogenesis: from sugar to fat to insulin resistance. Trends in Endocrinology & Metabolism. 2011;22(2):60-5.
86. Wende AR, Abel ED. Lipotoxicity in the Heart. Biochimica et biophysica acta. 2010;1801(3):311-9.
87. Goldberg IJ, Trent CM, Schulze PC. Lipid Metabolism and Toxicity in the Heart. Cell metabolism. 2012;15(6):805-12.
88. Akrouh A, Halcomb SE, Nichols CG, Sala-Rabanal M. Molecular Biology of K(ATP) Channels and Implications for Health and Disease. IUBMB life. 2009;61(10):971-8.
89. Yang H, Li X. The role of fatty acid metabolism and lipotoxicity in pancreatic β-cell injury: Identification of potential therapeutic targets. Acta Pharmaceutica Sinica B. 2012;2(4):396-402.
90. Cusi K. Role of Insulin Resistance and Lipotoxicity in Non-Alcoholic Steatohepatitis. Clinics in liver disease. 2009;13(4):545-63.
91. Martins AR, Nachbar RT, Gorjao R, Vinolo MA, Festuccia WT, Lambertucci RH, et al. Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. Lipids in health and disease. 2012;11:30-.
92. Drosatos K, Bharadwaj KG, Lymperopoulos A, Ikeda S, Khan R, Hu Y, et al. Cardiomyocyte lipids impair β-adrenergic receptor function via PKC activation. American Journal of Physiology - Endocrinology and Metabolism. 2011;300(3):E489-E99.
93. Park M, Sweeney G. Direct effects of adipokines on the heart: focus on adiponectin. Heart Failure Reviews. 2013;18(5):631-44.
94. Fang X, Palanivel R, Cresser J, Schram K, Ganguly R, Thong FSL, et al. An APPL1-AMPK signaling axis mediates beneficial metabolic effects of adiponectin in the heart. American Journal of Physiology - Endocrinology and Metabolism. 2010;299(5):E721-E9.
95. Park M, Youn B, Zheng X-l, Wu D, Xu A, Sweeney G. Globular Adiponectin, Acting via AdipoR1/APPL1, Protects H9c2 Cells from Hypoxia/Reoxygenation-Induced Apoptosis. PloS one. 2011;6(4):e19143.

96. Pirola L, Ferraz JC. Role of pro- and anti-inflammatory phenomena in the physiopathology of type 2 diabetes and obesity. World journal of biological chemistry. 2017;8(2):120-8. Epub 2017/06/08.
97. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K-i, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of clinical investigation. 2006;116(6):1494-505.
98. Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. The Journal of nutrition. 2009;139(1):1-4. Epub 2008/12/06.
99. Pimentel GD, Lira FS, Rosa JC, Oliveira JL, Losinskas-Hachul AC, Souza GI, et al. Intake of trans fatty acids during gestation and lactation leads to hypothalamic inflammation via TLR4/NFkappaBp65 signaling in adult offspring. The Journal of nutritional biochemistry. 2012;23(3):265-71. Epub 2011/05/06.
100. Grayson BE, Levasseur PR, Williams SM, Smith MS, Marks DL, Grove KL. Changes in melanocortin expression and inflammatory pathways in fetal offspring of nonhuman primates fed a high-fat diet. Endocrinology. 2010;151(4):1622-32. Epub 2010/02/24.
101. Thaler J, Yi C-X, Schur EA, Guyenet SJ, Hwang B, Dietrich M, et al. Obesity is associated with hypothalamic injury in rodents and humans (The Journal of Clinical Investigation (2012) 122, 1, (153-162) DOI: 10.1172/JCI59660)2012.
102. Timper K, Bruning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. Disease models & mechanisms. 2017;10(6):679-89. Epub 2017/06/09.