UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

ÁREA DE CONCENTRAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

LUCAS PAULO JACINTO SAAVEDRA

PROGRAMAÇÃO PERINATAL PARA O DESENVOLVIMENTO DE OBESIDADE E DISFUNÇÃO METABÓLICA: O PAPEL DA GLICAÇÃO E DA ATIVAÇÃO DO PPARα

Maringá-PR, Brasil

Maio – 2023

LUCAS PAULO JACINTO SAAVEDRA

PROGRAMAÇÃO PERINATAL PARA O DESENVOLVIMENTO DE OBESIDADE E DISFUNÇÃO METABÓLICA: O PAPEL DA GLICAÇÃO E DA ATIVAÇÃO DO PPAR*α*

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

Orientador: Prof. Dr. Paulo Cezar de Freitas Mathias

Coorientador: Prof. Dr. Douglas Lopes Almeida

Maringá-PR, Brasil

Maio – 2023

Dados Internacionais de Catalogação na Publicação (CIP)
(Biblioteca Central - UEM, Maringá, PR, Brasil)

S112p

Saavedra, Lucas Paulo Jacinto
Programação perinatal para o desenvolvimento de obesidade e disfunção metabólica : o papel da glicação e da ativação do PPARα / Lucas Paulo Jacinto Saavedra. -- Maringá, 2023.
49 f. : il. color., tabs.

Orientador: Prof. Dr. Paulo Cezar de Freitas Mathias.
Coorientador: Prof. Dr. Douglas Lopes Almeida.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Biológicas, Departamento de Biotecnologia, Genética e Biologia Celular, Programa de Pós-Graduação em Ciências Biológicas (Biologia Celular), 2023.

1. Bioquímica. 2. PPAR alfa. 3. Obesidade pós-natal. 4. Hiperalimentação pós-natal. 5. Biologia molecular. I. Mathias, Paulo Cezar de Freitas, orient. II. Almeida, Douglas Lopes, coorient. III. Universidade Estadual de Maringá. Centro de Ciências Biológicas. Departamento de Biotecnologia, Genética e Biologia Celular. Programa de Pós-Graduação em Ciências Biológicas (Biologia Celular). IV. Título.

CDD 23.ed. 572.4

Síntique Raquel Eleuterio – CRB 9/1641

Universidade Estadual de Maringá
Centro de Ciências Biológicas
Programa de Pós-Graduação em Ciências Biológicas
Área de concentração: Biologia Celular e Molecular

ATA DA DEFESA DE TESE DE DOUTORADO DO PÓS-GRADUANDO LUCAS PAULO JACINTO SAAVEDRA. Aos nove dias do mês de maio de dois mil e vinte e três, terça-feira, realizou-se a sessão pública da defesa de tese intitulada: "Programação perinatal para o desenvolvimento de obesidade e disfunção metabólica: O papel da glicação e da ativação do PPARα", apresentada em inglês pelo pós-graduando **Lucas Paulo Jacinto Saavedra**, Bacharel em Nutrição pelo Centro Universitário de Maringá - UniCesumar , que concluiu os créditos exigidos para obtenção do grau de "Doutor em Ciências Biológicas". Os trabalhos foram instalados às 09:00 horas, pelo Prof. Dr. Paulo Cezar de Freitas Mathias (DBC/PBC-UEM), presidente da banca examinadora, constituída pelos seguintes membros: Profa. Dra. Susana Patrícia da Silva Pereira (Universidade do Porto - Portugal), Prof. Dr. José Carlos Jiménez Chillarón (Hospital Sant Joan de Déu Barcelona – Espanha), Prof. Dr. Daniel Badauê Passos Junior (Universidade Federal de Sergipe-UFS), Prof. Dr. Carlos Henrique Grossi Sponton (Universidade Estadual de Campinas-UNICAMP), Prof. Dr. Marcio Alberto Torsoni (Universidade Estadual de Campinas-UNICAMP) e Dr. Douglas Lopes de Almeida (pós-doutorando CNPq). A banca examinadora, tendo se decidido a aceitar a tese, passou à arguição pública do candidato. Encerrados os trabalhos de arguição às 12:30, os examinadores deram o parecer final, considerando a tese APROVADO. Proclamado o resultado pelo presidente da banca examinadora, foram encerrados os trabalhos e lavrada a presente ata que vai assinada pela presidente da banca examinadora e pelos demais membros. Maringá, aos nove dias do mês de maio de dois mil e vinte e três.

Prof. Dr. Paulo Cezar de Freitas Mathias

Profa. Dra. Susana Patrícia da Silva Pereira

Prof. Dr. José Carlos Jiménez Chillarón

Prof. Dr. Daniel Badauê Passos Junior

Prof. Dr. Carlos Henrique Grossi Sponton

Prof. Dr. Marcio Alberto Torsoni

Dr. Douglas Lopes de Almeida

BIOGRAFIA

Lucas Paulo Jacinto Saavedra, filho de Juan Carlos Saavedra Peña e Marinalva Jacinto Saavedra, nasceu em Paranavaí/PR em 30/09/1994. Formou-se bacharel em nutrição pelo Centro Universitário Maringá, no ano de 2016, com trabalho de conclusão de curso intitulado "Efeitos hepáticos em ratos Wistar adultos alimentados com uma dieta hiperlipídica durante a adolescência". Em 2018 obteve o título de mestre em ciências biológicas pelo Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Biologia Celular e Molecular da Universidade Estadual de Maringá (UEM), com a dissertação intitulada, "Exposição materna ao metilglioxal durante a lactação leva a disrupção da homeostase glicêmica da prole de ratos machos no início da vida" orientado pelo professor Dr. Paulo Mathias e Co-orientado pelo Dr. Douglas Almeida.

AGRADECIMENTOS

A todos os professores que contribuíram com a minha carreira acadêmica, em especial aos meus orientadores de doutorado, Dr. Paulo Cezar de Freitas Mathias, Dr. Douglas Lopes Almeida, e supervisor de estágio sanduíche, Dr. Paulo Matafome, por toda a paciência, dedicação e ensinamentos.

A professora, Dra. Maria Aparecida Fernandez, por ter gentilmente me recebido em seu laboratório.

A todos os colegas, amigos e técnicos do Laboratório Experimental de DOHaD (LExDOHaD), por toda amizade, incentivo e colaboração.

As agências de fomento brasileiras (CNPq, CAPES, JBS), pela concessão de bolsa de estudos e financiamento da pesquisa.

"O presente trabalho foi realizado com apoio da coordenação de aperfeiçoamento de pessoal de nível superior – Brasil (CAPES) – Código de financiamento 001".

"O presente trabalho foi realizado com apoio do CNPQ, Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brasil".

Agradeço a toda minha família, em especial aos meus pais, Juan Carlos Saavedra Peña e Marinalva Jacinto Saavedra, e avó, Arleida Marcos Jacinto, por todo o incentivo e apoio durante a minha trajetória acadêmica.

A Patricia Satie Maeda, por todo carinho e companheirismo.

A todos os amigos que me incentivaram e apoiaram.

APRESENTAÇÃO

Esta tese é composta de dois artigos científicos, uma revisão e um artigo experimental. Ambos os artigos exploram o conceito das origens desenvolvimentistas da saúde e da doença (DOHaD). No primeiro artigo, intitulado *"Early AGEing and metabolic diseases: is perinatal exposure to glycotoxins programming for adult-life metabolic syndrome?",* publicado na revista "*Nutrition Reviews – IF: 6.846",* explora os efeitos da exposição aos agentes avançados de glicação (AGE), durante as fases sensíveis do desenvolvimento. Como conclusão, evidências apontam para potenciais efeitos deletérios no organismo, com potencial prejuízo a saúde metabólica e cardiovascular sendo causado pela exposição aos AGEs durante os períodos críticos do desenvolvimento. O segundo artigo, experimental, intitulado "*Increased PPARα activation by an agonist during lactation protects against long-term hepatic steatosis and insulin resistance induced by post-natal overfeeding in male Wistar rats,* a ser submetido a revista *"Molecular Metabolism – IF: 8.5"*, neste estudo foi observado que em um modelo animal de obesidade durante a infância, uma maior ativação do PPAR*α* durante a lactação é capaz de atenuar o desenvolvimento de obesidade, esteatose hepática, insulino-resistência. Além disso, que é de nosso conhecimento pela primeira vez mostrou-se e a disfunção do eixo hepático-hipotalâmico simpático, FGF21-FGFR1 em um modelo de hiperalimentação pós-natal, a redução de ninhada, que foi revertida pela ativação do PPAR*α* na lactação.

“So, carry on

There's a meaning to life

Which someday we may find

And carry on, it's time to forget

The remains from the past, to carry on”

Angra – Carry On

RESUMO GERAL

Estudos experimentais e epidemiológicos apontam para o impacto que situações estressoras durante o desenvolvimento podem ter no desenvolvimento de saúde ou doença mais tarde na vida. A tese busca trazer novos conhecimentos sobre potenciais agentes etiológicos para esta programação, do organismo nomeadamente os agentes avançados de glicação (AGEs). Procura ainda em um artigo experimental propor mecanismos pelos quais a hiperalimentação pós-natal pode levar ao desenvolvimento de obesidade e disfunção metabólica mais tarde na vida, ressaltando a via do PPARα como um potencial alvo terapêutico para a prevenção do desenvolvimento deste fenótipo doente. No primeiro estudo, uma revisão narrativa, buscou-se reunir dados da literatura acerca dos potenciais efeitos deletérios dos AGEs no desenvolvimento de saúde da prole. As fontes de AGEs são diversas, indo desde alimentos preparados sob altas temperaturas, quanto o diabetes e tabagismo materno, até as fórmulas infantis. Conclui-se ao fim do estudo que a exposição as glicotoxinas durante fases sensíveis do desenvolvimento podem ser um importante fator para a programação metabólica para doenças, no entanto são necessários mais estudos para se elucidar os mecanismos que causam este fenótipo, bem como estudos clínicos afim de se verificar os efeitos á longo prazo na saúde da prole exposta precocemente aos AGEs. No segundo estudo experimental, em um modelo de hiperalimentação e obesidade pós-natal, onde a ninhada dos roedores é reduzida para três filhotes por mãe, observou-se o desenvolvimento de obesidade, resistência à insulina, dislipidemia e esteatose hepática, o que corrobora com estudos encontrados na literatura. Além disso observou-se, que é de nosso conhecimento, pela primeira vez a desregulação do eixo hepático-hipotalâmico simpático, FGF21-FGFR1 no modelo de redução de ninhada, como um dos mecanismos que contribuem para o desenvolvimento deste fenótipo. Ademais, o estudo aponta para um potencial alvo terapêutico, à medida que foi observado pela primeira vez, pelo que é de nosso conhecimento, que uma maior ativação do PPARα durante a lactação, induzida por um agonista, foi capaz de prevenir e atenuar o desenvolvimento de obesidade e disfunção autonômica e metabólica nos animais hiper alimentados durante a lactação.

GENERAL ABSTRACT

Experimental and epidemiological studies point to the impact that stressful situations during development can have on the development of health or disease later in life. This thesis looks to bring, in a review article, new knowledge about potential etiological agents for this programming of the organism, namely the advanced glycation end-products (AGEs). It also seeks in an experimental article to propose mechanisms by which postnatal overfeeding can lead to the development of obesity and metabolic dysfunction later in life, highlighting the PPARα pathway as a potential therapeutic target for preventing the development of this phenotype. In the first study, a narrative review, we sought to gather data from the literature about the potential deleterious effects of AGEs on the health development of the offspring. The sources of AGEs are diverse, ranging from food prepared at high temperatures, also diabetes, maternal smoking, and infant formulas. At the end of the study, it was concluded that exposure to glycotoxins during susceptible phases of development can be an important factor for metabolic programming for diseases, however further studies are needed to elucidate the mechanisms that cause this phenotype, as well as related clinical studies to verify the long-term health effects of the offspring of early exposure to AGEs. In the second experimental study, in a model of infant overfeeding, where the rodent litter is reduced to three pups per mother, we have observed a reduction in markers for lipid oxidation early in life, and during adulthood the development of obesity, insulin resistance, dyslipidemia and hepatic steatosis was observed, which corroborates with studies found in the literature. Furthermore, to our knowledge, for the first time the dysregulation of the sympathetic hepatic-hypothalamic axis, FGF21-FGFR1 in the small litter model, was observed as one of the potential mechanisms that contribute to the development of this diseased phenotype. Furthermore, the study points to a potential therapeutical target, as it was observed for the first time, to our knowledge, that a greater activation of PPARα du, a key regulator of lipid oxidation, during lactation, was able to prevent and attenuate the development of obesity and autonomic and metabolic dysfunction associated with post-natal overfeeding.

SUMÁRIO

CAPÍTULO 1 - Early AGEing and metabolic diseases: is perinatal exposure to glycotoxins programming for adult-life metabolic syndrome?

Lead Article

Early AGEing and metabolic diseases: is perinatal exposure to glycotoxins programming for adult-life metabolic syndrome?

Flávio A. Francisco*, Lucas P.J. Saavedra*, Marcos D.F. Junior, Cátia Barra, Paulo Matafome, Paulo C.F. Mathias, and Rodrigo M. Gomes

Perinatal early nutritional disorders are critical for the developmental origins of health and disease. Glycotoxins, or advanced glycation end-products, and their precursors such as the methylglyoxal, which are formed endogenously and commonly found in processed foods and infant formulas, may be associated with acute and long-term metabolic disorders. Besides general aspects of glycotoxins, such as their endogenous production, exogenous sources, and their role in the development of metabolic syndrome, we discuss in this review the sources of perinatal exposure to glycotoxins and their involvement in metabolic programming mechanisms. The role of perinatal glycotoxin exposure in the onset of insulin resistance, central nervous system development, cardiovascular diseases, and early aging also are discussed, as are possible interventions that may prevent or reduce such effects.

INTRODUCTION

The developmental origins of health and disease concept focuses on the potential associations between a suboptimal fetal and/or postnatal environment and several pathologies in the offspring, such as the metabolic syndrome. Several animal models have been developed to explore the pathophysiology and mechanisms of developmental programming of the metabolic syndrome. Features of cardiometabolic diseases have been found in the offspring of diabetic rodents, as well as in the offspring of rodents fed a high-fat diet or fructose-enriched diet.[1–5] High sugar intake is associated with harmful effects, such as cardiovascular diseases, obesity, insulin resistance, and diabetes. In this way, hyperglycemia is related to increased levels of advanced glycation end-products (AGEs), and these glycotoxins are closely associated with the development and progression of diabetes and its complications.[6–11] AGEs also are involved in the deterioration of metabolic homeostasis in obesity, namely the development of insulin resistance–associated pathologies such as cardio- and cerebrovascular diseases, nonalcoholic steatohepatitis, and central nervous system disorders, including dementia, in adult and pediatric patients.[12–26] Vascular aging due to AGEs exposure, or vascular AGEing, is related to oxidative stress due to increased generation of reactive species of oxygen and nitrogen,[27–30] endothelial dysfunction,[31–33] and changes in the extracellular matrix[32] and in inflammatory factors.[34] Infant formulas are used worldwide as a substitute for breast milk; previous studies have reported high AGE content in breast milk.[35–38] Thus, infants' exposure to these nutritional

Affiliation: *F.A. Francisco, L.P.J. Saavedra,* and *P.C.F. Mathias* are with the Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringa, Maringa, PR, Brazil. *M.D.F. Junior* and *R.M. Gomes* are with the Department of Physiological Sciences, Federal University of Goiás, Goiânia, GO, Brazil. *C. Barra* and *P. Matafome* are with the Institute of Physiology and Coimbra Institute of Clinical and Biomedical Research, Faculty of Medicine, and the Center for Innovative Biotechnology and Biomedicine, University of Coimbra; and the Clinical Academic Center of Coimbra, Coimbra, Portugal.

*These authors contributed equally.

Correspondence: *R.M. Gomes*, Department of Physiological Sciences, Biological Sciences Institute 2, room 101, Federal University of Goiás, Esperança Ave s/n, 74690-900 Goiânia, GO, Brazil. Email: Gomesrm@ufg.br.

Key words: advanced glycation end products (AGEs), glycotoxins, metabolic programming, metabolic syndrome, methylglyoxal.

doi: 10.1093/nutrit/nuaa074

contaminants early in life may contribute to the development of cardiometabolic disorders at adulthood.[37,39,40]

In this review, we provide an overview of the current knowledge about the contribution of perinatal glycotoxin exposure to metabolic programming and the development of metabolic syndrome–related pathologies. Evidence of increased glycotoxin exposure of the fetus or newborn, due to maternal or infant dietary AGE consumption, in addition to infant formula feeding, on developmental programming of metabolic syndrome are discussed, as are interventions to prevent the consequences of perinatal exposure to AGEs. Figure 1 provides an overview of main sources and potential mechanisms of the development of cardiometabolic disease development during adult life, due to the exposure to glycotoxins during perinatal life.

CLINICAL EVIDENCE OF PERINATAL PROGRAMMING FOR ADULT-LIFE METABOLIC SYNDROME

Pregnancy is a critical period for the health of both the fetus and the mother and is very sensitive to environmental disturbances. Several studies established a relationship between disturbances in the pregnancy and diseases in offspring throughout life.[41–50] The magnitude of such effects depends on the stage of gestation in which the fetus was exposed and the nature of the aggressive agent.[44] It is well established that tobacco, alcohol, distress, nutritional unbalances, and other metabolic disruptors affect the proper development of the fetus during intrauterine life.[46,48,50–52] One of the most common gestational disorders is gestational diabetes mellitus (GDM), which is associated with pregestational overweight and has been implicated in adverse perinatal outcomes such as increased weight gain during the gestational period and high sugar consumption.[45,53,54] Fetal development is very susceptible to diabetes, given that this condition can promote severe changes in tissues and organs, with cardiovascular and neural tube defects being the most frequent malformations.[43,46] Mothers with pregestational diabetes mellitus (PGDM) and a poorly controlled hyperglycemia during the first trimester have a 5% to 10% higher risk of having newborns with a major birth defect and a 15% to 20% higher risk of spontaneous abortion.[55] On the other hand, GDM is associated more with pregnancy complications, such as macrosomia, and pre- and perinatal mortality, than with congenital anomalies.[46] The offspring of mothers with PGDM have increased adiposity and overweight resulting from transplacental passage of maternal glucose and induction of fetal hyperinsulinemia.[46] Pregnant women with GDM have an increased risk of delivering large-for-gestational-age (LGA) newborns, who have an higher risk of being obese at childhood.[43,56]

Diet composition before and during pregnancy may influence the metabolic profile of both the mother and the newborn, and may affect the newborn's size at birth.[57,58] Nutritional changes may lead to impairment of fetal growth and intrauterine growth restriction, as well as fetal adiposity, insulin resistance, and pancreatic β-cell dysfunction.[59] In a case-control study, Amezcua-Prieto et al[58] suggest the increased consumption during pregnancy of industrial bakery products, pastries, and products containing refined sugar is associated with a higher risk of having a small-for-gestational-age (SGA) newborn. In contrast, higher consumption of whole-grain cereal and bread is related to a lower risk of delivering an SGA infant.[58] According to another cohort study, the daily consumption of artificially sweetened beverages during pregnancy induces a 2-fold higher risk of having a child with overweight at the child's first year.[60] Ornoy et al[46] showed that the offspring of mothers with GDM have a high frequency of overweight, as do babies who are breastfed by mothers with diabetes. Palatianou et al[61] found an increased association of the LGA condition with nondiabetic obesity compared with type 2 diabetes. On the other hand, LGA infants from mothers with diabetes (either GDM or PGDM) are above the 90th percentile in height and weight and have increased weight gain in the first 4 months of life.[46,62] A meta-analysis performed by Schellong et al[63] revealed a predisposition to adulthood overweight in LGA newborns but not in SGA newborns. However, both the LGA and SGA conditions have a similar risk for development of adulthood diabetes, with the risk that following a U-shaped and not a linear relationship.[64] Children who are SGA born to mothers with PGDM and associated nephropathy are more susceptible to prematurity, reduced growth at age 3 years and body weight and height below the 50th percentile when compared with children of mothers with PGDM without complications. As well, SGA individuals who gained a substantial amount of weight in early childhood exhibited higher risk of developing hypertension and diabetes and also higher coronary heart disease mortality in adulthood compared with their age-matched counterparts.[59]

Thus, maternal obesity and type 2 diabetes affect birth weight, and both the SGA and LGA conditions are associated with increased risk of metabolic impairment and related complications in the adult life. Moreover, the presence of diabetic complications in the

mother is apparently related to an increased risk to the newborn.

Metabolic effects of glycotoxins on metabolic syndrome

One of the main glycotoxins is methylglyoxal (MG), which may change cell behavior through modification of biomolecules, such as proteins and DNA, and consequent formation of AGEs.[18] Modification of arginine residues by MG leads to the formation of Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) and argpyrimidine from the imidazolones family, whereas lysine modification leads to the formation of methylglyoxal lysine dimer and (carboxyethyl)lysine.[65–69] Modification of amino acid residues by MG affects intracellular (i.e., transcription factors and cytoplasmic proteins, including the proteasome and stress-response pathways), circulating (ie, hemoglobin, albumin, or lipoproteins), and extracellular matrix proteins, changing cell behavior and activating inflammatory and death pathways.[18,70–77] Indeed, MG modifies proteasome subunits and protein quality-control pathways (namely, Hsc70, Hsp90, and Hsp27), causing endoplasmic reticulum stress and impaired degradation of misfolded proteins, in turn leading to a vicious circle of progressive accumulation of misfolded proteins and impaired activation of detoxification systems.[78–81] Besides directly modifying protein structure through modification of amino acid residues, MG also increases oxidative stress, namely, the formation of superoxide anion,[82–85] hydrogen peroxide, and peroxynitrite[82,83,86] in different types of cells, including endothelial cells,[87] rat kidney mesangial cells,[88] rat hepatocytes,[86,89] blood cells,[83,90] osteoblasts,[90] and in rat and mouse neurons.[91–94] MG also induces the depletion of antioxidant defenses, predisposing cells for oxidative damage.[82,88,95–99] Given that MG detoxification systems, namely the glyoxalase system, are glutathione dependent, such mechanisms lead to a self-perpetuating circle of reactive oxygen species and AGEs formation and mitochondrial dysfunction.[97]

Extracellular AGEs may change cell behavior through activation of membrane receptors, such as RAGE, which recognizes 2 major types of ligands: imidazolones (MG-derived) and Nε-(carboxymethyl)lysine (CML) adducts.[100] Upon activation, RAGE triggers intracellular signaling pathways such as NF-κB, involved in activation of inflammatory and proliferation or stress signals, as well as generation of oxidative stress.[75,101–106] Inhibition of RAGE or expression of soluble RAGE isoforms with the ability to scavenge AGEs prevented vascular disease in several animal models.[101,107,108] Thus, MG-induced changes in cell behavior involve several mechanisms, namely the modification of biomolecules, accumulation of misfolded proteins, activation of membrane receptors, generation of oxidative stress, changes in transcription factors, and activation of inflammatory or stress pathways.

MG has been implicated in the development of diabetes complications such as retinopathy, nephropathy, and peripheral neuropathy, given that its levels are increased in patients with diabetes patients, and insulin-independent cells like endothelial cells, podocytes, and neurons are more susceptible to hyperglycemia-driven MG formation.[18] Several studies have addressed the involvement of MG in the mechanisms governing the development of such pathologies, namely endothelial cell senescence and angiogenesis impairment,[70,77,109,110] podocyte effacement and death,[111,112] glomerular fibrosis,[76,101,105,113] apoptosis of retinal pericytes and retinal pigmented cells,[114–117] and changes in the nociception and pain stimuli (hyperalgesia).[118,119] Moreover, MG is involved in the pathophysiology of cardio- and cerebrovascular diseases. MG causes structural changes in the blood-brain barrier[120,121] and is involved in other neurodegenerative disorders, such as increased neurotoxicity,[122,123] β-amyloid protein neurotoxic effects,[124,125] and loss of dopaminergic neurons.[126–128] In the cardiovascular system, MG impairs calcium handling between sarcoplasmic reticulum and cytoplasm of cardiomyocytes[129] and also affects survival and apoptotic pathways during ischemia,[130,131] and angiogenic deficits.[132] Features of endothelial dysfunction, hypertension, and atherosclerosis have also been reported, such as oxidative stress and stiffness of the aorta, impaired elasticity, acetylcholine-dependent relaxation, nitric oxide bioavailability,[33,133–136] activation of the renin-angiotensin system,[137,138] increased glycoxidation of low-density lipoprotein particles[139,140] and increased risk of thrombosis and atherosclerosis through platelet hyperaggregation and RAGE activation.[141,142]

Besides being implicated in the development of diabetic complications or associated diseases, MG also contributes to the process of loss of metabolic homeostasis itself, namely in the development of β-cell dysfunction and insulin resistance. MG transiently activates insulin secretion due to β-cell depolarization,[143] but it hampers β-cell survival and long-term insulin synthesis and secretion.[144] In insulin signaling, MG causes a redox-independent inhibition of the insulin-receptor pathway and GLUT4 translocation in muscle cells and 3T3 adipocytes.[9,145,146] In vivo, MG caused insulin resistance in several animal models,[144,145,147] but only when supraphysiological doses were used.[18] Other studies did not show MG-induced insulin resistance, which was only observed in obese animal models.[25,148,149] Several studies have also shown AGE-induced overexpression of inflammatory mediators in the liver,[26,150,151] but again,

hepatic insulin resistance was only observed in obese animals.[26] Such results suggest that glycation may have an impact in obesity-associated insulin resistance, possibly through increased depletion of antioxidant and detoxifying mechanisms, but has a less dramatic effect in lean models. In humans, elevated MG and AGE levels have been reported in patients with diabetes and in metabolically unhealthy, obese patients, but no correlation was found between AGE levels and impaired glucose homeostasis.[152,153] Nevertheless, AGE-restricted diets improve insulin sensitivity in normal and overweight individuals, as well as patients with metabolic syndrome and type 2 diabetes.[154–157] Reports have shown the impact of oral AGE restriction in the improvement of insulin resistance, even in patients with metabolic syndrome, which may reduce the risk of progression from metabolically unhealthy and obese and having metabolic syndrome to type 2 diabetes.

In summary, MG and MG-derived AGEs are involved in several pathologies associated with metabolic syndrome and diabetes, but their progressive accumulation in biological systems may be also associated with impaired lipid handling and increased susceptibility to oxidative damage, which may contribute to the development of insulin resistance in adipose tissue and liver in obesity and predispose to the metabolically unhealthy, obese phenotype. Together with increased β-cell damage, such mechanisms are likely to contribute to the progressive deterioration of metabolic homeostasis and development of prediabetes and type 2 diabetes. Importantly, the impact of early glycotoxin exposure since the perinatal period is unknown, although recent evidence suggests such exposure may increase the risk of metabolic dysregulation and development of diabetes-like complications in adult life.

Sources of perinatal glycotoxins exposure

In utero exposure to AGEs during embryonic development. Similar to the other types of diabetes, GDM-related hyperglycemia increases serum levels of MG and AGEs, such as CML.[158,159] Increased serum AGE levels are associated with insulin resistance, oxidative stress, cardiovascular diseases, and diabetes comorbidities in normal individuals and pregnant women.[33,160–164] In addition to hyperglycemia, maternal AGEs may also derive from dietary absorption, given that industrialized foods are rich in AGEs[54] and given their possible transfer to the embryo through the placenta.[35] Accordingly, Konishi et al[165] reported the impairment of implantation and placental growth and function by the accumulation of AGEs through RAGE activation, oxidative stress, low human chorionic gonadotropin levels, and apoptosis in human first-trimester trophoblasts. Similarly, Hao et al[166] and Haucke et al[167] reported the adverse effects of GDM through raised AGEs levels during embryonic development, which promote RAGE activation, inflammation, and AGE accumulation in the embryo. This environmental stress may collaborate to cause embryo resorption, fetus malformation, or preterm birth.[168] On the other hand, knockout of soluble RAGE in pregnant diabetic rats prevents embryonic dysmorphogenesis,[169] and the administration of the soluble form of RAGE during pregnancy reduces NF-κB activity in rat fetal tissues.[170]

Elevated sugar-sweetened soft beverages and refined carbohydrates consumption during pregnancy are strongly correlated with high serum AGE levels, offspring congenital heart defects, SGA newborns, and increased risk of offspring overweight.[58,60,170,171] These data reinforce the role of AGE exposure on the diabetic embryopathy and its implications for proper fetus development, which are widely related to developmental origins of diseases at later stages of life. However, data regarding the mechanisms involved in AGEs passage through the placenta are not currently available, to our knowledge, and studies are necessary in this field.

Glycotoxin exposure during lactation period through breast milk and infant formula. The lactation period is essential to the proper development and maturation of different organs and systems of the newborn, because breast milk is to supply this nutritional demand. Given the abundance of evidence regarding the importance of breastfeeding in infant health, the World Health Organization recommends exclusive breastfeeding until 6 months of life and complementary until age 2 years.[172] Breastfeeding prevents diseases such as diabetes, multiple sclerosis, and celiac disease.[173] More than just a source of calories, breast milk is an important source of bioactive molecules such as antibodies, oligosaccharides, and hormones, which exert beneficial effects for the healthy development of newborns.[173,174] Insulin may be found in breast milk and plays an important role in the process of gut maturation, decreasing permeability to macromolecules.[175]

The milk composition depends on the maternal metabolic status and there is evidence that breast milk may also be a source of glycotoxins during lactation. Human studies have shown that the neonatal intake of breast milk from mothers with diabetes was related to overweight and glucose intolerance.[176] Mericq et al[35] found a correlation between blood AGE levels of lactating mothers and their infants, raising the question of whether maternal diet during lactation influences infant glycoxidative stress. Even in other diseases, such as

beriberi, when an accumulation of glucose metabolites such as MG occurs, there is an increased concentration of these substances in breast milk.[177] Infants whose mothers smoked during pregnancy and/or lactation have increased accumulation of AGEs in their skin, indicating that the transmission of glycotoxins from mother to child may also occur through breast milk.[37] Altered composition of breast milk from obese dams, caused by high-sugar consumption, programs rat offspring to develop obesity due to the impairment of melanocortin system.[178]

Other studies have demonstrated that the levels and effects of breast-milk AGEs may also originate in maternal diet. Cows fed a diet high in AGEs had increased glycated compounds in their milk, such as MG-H1.[179] On the other hand, a diet low in AGEs during pregnancy and the neonatal period prevented the development of type 1 diabetes in the offspring of NOD mice.[38] In this regard, it was previously demonstrated that oral administration of MG to lactating rats increased the content of the glycation intermediary fructosamine in their milk, which was related to the development of a diabetic phenotype in the offspring during adult life.[180] Such observations are in line with evidence that AGE levels in the blood could be derived from the diet and not just produced endogenously. In fact, a strong correlation between intake of AGEs and AGE levels in the plasma has been demonstrated.[181,182] Similarly, evidence from human studies have shown that dietary restriction of AGEs decreases their concentration in plasma and their renal excretion.[155,183–185] In animals fed a ^{14}C-labelled, AGE-rich diet, as in humans, 10% of dietary AGEs are absorbed.[183,186] Indeed, the glycation compound pirralyne, as well as major AGEs such as CML, (carboxyethyl)lysine, and MG-H1, are absorbed in the form of dipeptides via PEPT1 transporter in intestinal cells.[187,188]

Another source of glycotoxins during the perinatal period are infant formulas, which commonly contain high levels of AGEs, reaching almost a 35-fold higher concentration of CML than breast milk of healthy mothers.[39,189] AGEs are formed in heat-treated foods, as a product from Maillard reaction, or nonenzymatic browning. In fact, traditional methods of cooking that use high temperature (100°C–250°C), such as frying, baking, and grilling, contribute to a higher grade of AGE formation, because foods rich in reducing sugars and proteins are more prone to the formation of these compounds.[190–192] For instance, grilled beef has 5 times higher AGE levels (5963 kU/100 g) than boiled beef (1124 kU/100 g).[193] Also, infant formulas are rich in sugars and proteins, and their industrial production includes heat treatment. Hence, it was demonstrated that hydrolysate infant formulas, rich in whey, have higher concentrations of CML because whey proteins are subjected to great heat treatment during manufacturing of infant formula.[194]

A positive correlation between formula-derived AGEs, increased AGE circulating levels, and their urinary excretion was found in newborns, indicating its absorption.[189,195] In an animal model of intrauterine growth restriction, animals fed a high-AGE formula during suckling had CML accumulation in renal tubular cells that was associated with increased protein oxidation and expression of pro-inflammatory and apoptotic factors.[36] Similarly, intrauterine growth restricted piglets fed a high-AGE formula during suckling have increased liver oxidative stress at adulthood, due to impaired antioxidant activity.[196] Some authors suggest high consumption of glycation compounds through infant formulas during early life may predispose to the development of oxidative stress and diseases later in life, such as diabetes.[35,197] It was observed that increased maternal AGE levels were correlated with the infant AGE levels, which may precondition the young to high oxidative stress, inflammation, and insulin resistance.[35] A more recent investigation observed decreased insulin sensitivity in infants fed AGE-rich formula compared with those fed only breast milk, although the specific AGE contribution to decreased insulin sensitivity was not clear, because no differences were observed in infants fed a low-AGE formula.[198]

In this context, it was shown that glycation of dairy protein by MG or glyoxal may decrease the protein digestibility by proteases, mainly due to cross-linked AGEs.[199] On the other hand, noncross-linked AGEs, such as CML, (carboxyethyl)lysine, and MG-H1 are more prone to be absorbed by intestinal epithelial cells.[200] High-molecular-weight AGEs are harder to digest and absorb, so they are more able to advance in the intestinal tract and interact with the colonic microbiota.[200,201] In fact, dietary AGEs may influence the microbiota composition. In rats, dietary AGEs reduced the diversity of microbiota, decreasing short-chain fatty acid–producing bacteria and damaging the colonic epithelial barrier.[202] Human studies also report the interaction between dietary AGEs and changes in gut microbiota composition, highlighting the importance of this interaction to human health.[203,204] However, little is known about the mechanisms of AGE absorption in the neonatal gut. The newborn gut is not totally mature, and the epithelial gut barrier of newborns is still permeable to the passage of macromolecules, such as hormones, carbohydrates, and peptides.[175,205] Thus, the newborn gut may be more complacent to the passage of glycotoxins, making the

rat pup more susceptible to the absorption and accumulation of AGEs and their precursors. Newborn rats are more susceptible to the toxic effects of orally delivered MG, because the lethal dose is almost 4 times lower than that for an adult male rat (531 mg/kg vs 1990 mg/kg).[206] In short, exposure to increased AGE levels by infant formulas or via breast milk are detrimental to health and proper development of the infant. In general, the mechanisms of AGE absorption, digestion, and interaction with the microbiota are not well understood, and less is known about these mechanisms in infancy; thus, more studies are necessary to clarify them.

EFFECTS OF PERINATAL AGE EXPOSURE ON PROGRAMMING OF METABOLIC SYNDROME, CARDIOVASCULAR DISEASES AND EARLY AGING

Although several studies have reported high perinatal exposure to AGEs during embryonic development and lactation, little is known about their effects in metabolic programming and in increasing the risk of development of noncommunicable diseases in adulthood. Moreover, the consumption of AGEs through milk or infant formulas disturbs metabolic homeostasis in newborns and is associated with pancreatic dysfunction and cardiovascular and central nervous system diseases. Exposure of lactating rats to high dietary levels of sucrose or high-fructose corn syrup was observed to lead to increased free fatty acid levels, adiposity, and liver fat in the offspring at weaning.[207] Accordingly, Csongová et al[208] have shown increased predisposition for weight gain and insulin resistance in the progeny of females fed an AGE-rich diet during pregnancy, and Francisco et al[180] have shown a similar impact of increased maternal exposure to MG during lactation leading to an impaired lipid profile and adiposity in the offspring. The authors also reported decreased β-cell function in the offspring.[180] Accordingly, using type 1 diabetic NOD mice, 2 different studies have shown the impact of perinatal AGE exposure on β-cell function. Peppa et al[38] have shown that low-glycotoxin fetal and neonatal environments, through maternal AGE dietary restriction, decreased T-cell inflammatory activity in the pancreas, resulting in lower glycemia and increased survival. Accordingly, Borg et al[209] have shown deteriorated β-cell function in the progeny of NOD females exposed to increased dietary AGE levels during pregnancy and lactation.

The impact of perinatal AGEs exposure to other pathologies is less studied, although a few studies have implicated perinatal AGEing in the development of cardiovascular diseases and central nervous system disorders. Vascular diseases in adult life are associated with increased glycoxidative stress, and increased prenatal AGE exposure also resulted in early cardiac changes. Embryos of diabetic female rats accumulated higher levels of CML, which was associated with lower vascular endothelial growth factor levels.[210] As well, AGE levels were increased in the heart of newborns of streptozotocin-induced diabetic dams and were associated with increased oxidative stress and inflammatory markers.[168]

Recent reports have suggested impairment of the AGE-RAGE axis in preterm birth. Chiavaroli et al[211] have shown decreased levels of soluble RAGE and endogenous secretory RAGE in overweight prepubertal children who were LGA or SGA, and these were correlated with insulin resistance. In the central nervous system, increased hippocampal RAGE expression was observed in the offspring of streptozotocin-induced diabetic female rats, which was associated with increased excitability and behavioral changes.[212] Increased glycation during gestational diabetes has been implicated in impaired neural development, namely, in the decrease of cortical neural precursor cells.[213] Authors have shown that glyoxalase pathway disruption during embryonic development leads to premature neurogenesis, depletion of cortical neural precursor cells, and behavioral changes, which were found in the offspring of diabetic murine mothers.[213]

Thus, high AGE levels in mothers can predispose their progeny to impaired metabolic homeostasis, and recent data suggest defining cutoff values for maternal glycated albumin levels during pregnancy to prevent neonatal complications.[214,215]

INTERVENTIONS TO PREVENT PERINATAL AGE EXPOSURE AND METABOLIC PROGRAMMING

As previously described, exposure to glycotoxins during perinatal life may occur in utero, because AGEs can cross the placental barrier and impair fetal development, activating the RAGE axis and increasing oxidative stress, which may underlie the embryopathy related to GDM. Furthermore, the exposure during lactation may occur via breast milk, because maternal circulating AGE levels may influence AGE concentration in the milk. It is well established that uncontrolled glycemia in GDM increases MG and AGE circulating levels, to which the embryo is exposed. Thus, the first approach to prevent MG and AGE exposure should be a proper glycemic control. Metformin was suggested as an efficient and safe drug for GDM treatment.[216] Besides improving insulin sensitivity and decreasing hepatic gluconeogenesis, metformin may directly react with and scavenge MG, preventing the formation of MG-

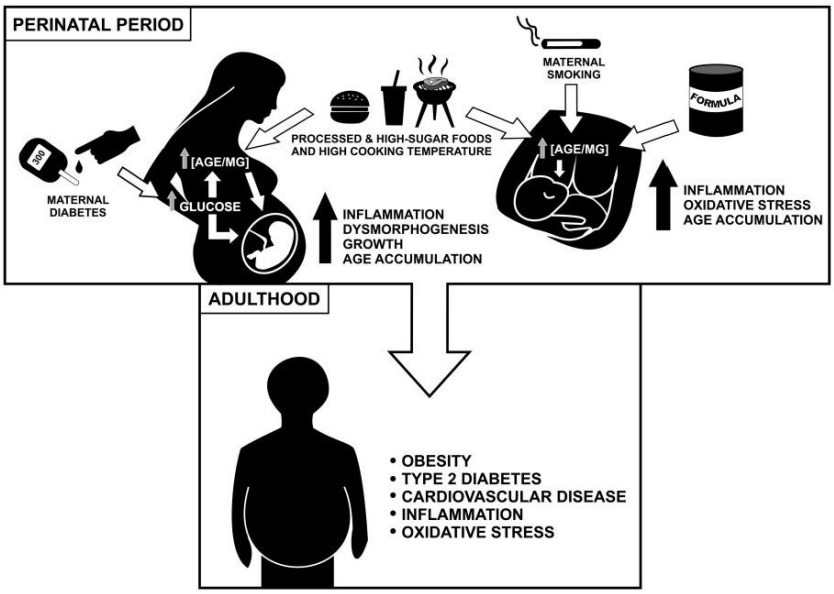


Figure 1 **Main sources and potential mechanisms by which exposure to glycotoxins during perinatal life (eg, gestation, lactation) may potentially program cardiometabolic disease development during adult life.** *Abbreviations:* AGE, advanced glycation end product; MG, methylglyoxal.

derived AGEs such as MG-H1.[217,218] During lactation, the same interventions may be administered to treat maternal diabetes, thus preventing the transmission of glycotoxins from mother to the infant through breast milk.

As previously described, the diet is one of the main sources of external glycotoxins. Because maternal AGEs may be transmitted to the infant via placenta or breast milk, the consumption of ultraprocessed or high-temperature cooked food should be discouraged or controlled, because they present high levels of AGEs. The intake of fresh foods should be encouraged, such as *in natura* vegetables and fruits as part of balanced diet. Attention should be taken in the cooking process, avoiding high temperature methods such as frying and grilling, opting for low-temperature methods such as boiling.

AGEs are largely found in infant formulas, contributing to increase the pool of AGEs in the infant. As recommended by the World Health Organization, breastfeeding must be exclusive during the first 6 months of life.[172] In this sense, infant formula must be implemented only when breast milk was not available, thus avoiding unnecessary use. As mentioned, the industrial process to obtain whey protein leads to a higher degree of AGE formation; therefore, the addition of whey protein should be avoided. The use of milk from different animals, such as goat, should be encouraged, because their amino acidic profile is more similar to the human milk, making the addition of whey protein unnecessary, thus reducing the amount of AGEs in the final product.[194]

Thus, some interventions may be taken to prevent AGE exposure during perinatal life, including proper glycemic control in mothers with diabetes and the adoption of a balanced diet low in ultraprocessed food. Quitting smoking may also be an important intervention, because smoking during lactation may increase AGE levels in breast milk.[37] Infant formulas should be prescribed with caution, and industry should be encouraged to develop infant formulas with low AGE levels.

CONCLUSION

More studies are needed to understand the mechanisms underlying the effects of perinatal, neonatal, and infancy exposure to glycotoxins to prevent the metabolic programming of diseases due to the embryo and infant exposure to AGEs. In clinical practice, the advice to

pregnant and lactating women about the importance of the diet and glycemic control is essential. To study the long-term effects of intrauterine and postnatal exposure to glycotoxins in humans, a long follow-up of the offspring and mother is required, given that studies about this issue are currently scarce.

Acknowledgments

Author contributions. F.A.F., P.M., and R.M.G. outlined and drafted the manuscript. All the authors contributed to the writing of the manuscript. P.M., P.C.F.M., and R.M.G. supervised the work. All the authors revised and approved the manuscript for publication.

Funding. Financial support was received from the following Brazilian funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento Pessoal de Nível Superior. None of the funding agencies were involved with the conception, design, performance, or approval of this study.

Declaration of interest. None.

REFERENCES

1. Albracht-Schulte K, Kalupahana NS, Ramalingam L, et al. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. *J Nutr Biochem*. 2018;58:1–16.
2. Desai M, Jellyman JK, Han G, et al. Maternal obesity and high-fat diet program offspring metabolic syndrome. *Am J Obstet Gynecol*. 2014;211:237.e1–237.e13.
3. Pan Y, Kong LD. High fructose diet-induced metabolic syndrome: pathophysiological mechanism and treatment by traditional Chinese medicine. *Pharmacol Res*. 2018;130:438–450.
4. Sarman I. Review shows that early foetal alcohol exposure may cause adverse effects even when the mother consumes low levels. *Acta Paediatr*. 2018;107:938–941.
5. Walters KA, Bertoldo MJ, Handelsman DJ. Evidence from animal models on the pathogenesis of PCOS. *Best Pract Res Clin Endocrinol Metab*. 2018;32:271–281.
6. McLellan AC, Thornalley PJ. Glyoxalase activity in human red blood cells fractioned by age. *Mech Ageing Dev*. 1989;48:63–71.
7. McLellan AC, Thornalley PJ, Benn J, et al. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci*. 1994;87:21–29.
8. Oliveira LM, Lages A, Gomes RA, et al. Insulin glycation by methylglyoxal results in native-like aggregation and inhibition of fibril formation. *BMC Biochem*. 2011;12:41.
9. Riboulet-Chavey A, Pierron A, Durand I, et al. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes*. 2006;55:1289–1299.
10. Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J*. 1990;269:1–11.
11. Thornalley PJ. Modification of the glyoxalase system in human red blood cells by glucose in vitro. *Biochem J*. 1988;254:751–755.
12. Baidoshvili A, Niessen HWM, Stooker W, et al. Nε-(carboxymethyl)lysine depositions in human aortic heart valves: similarities with atherosclerotic blood vessels. *Atherosclerosis*. 2004;174:287–292.
13. Cuccurullo C, Iezzi A, Fazia ML, et al. Suppression of RAGE as a basis of simvastatin-dependent plaque stabilization in type 2 diabetes. *Arterioscler Thromb Vasc Biol*. 2006;26:2716–2723.
14. Hanssen NMJ, Stehouwer CDA, Schalkwijk CG. Methylglyoxal and glyoxalase I in atherosclerosis. *Biochem Soc Trans*. 2014;42:443–449.
15. Hanssen NMJ, Wouters K, Huijberts MS, et al. Higher levels of advanced glycation endproducts in human carotid atherosclerotic plaques are associated with a rupture-prone phenotype. *Eur Heart J*. 2014;35:1137–1146.
16. Heier M, Margeirsdottir HD, Torjesen PA, et al. The advanced glycation end product methylglyoxal-derived hydroimidazolone-1 and early signs of atherosclerosis in childhood diabetes. *Diabetes Vasc Dis Res*. 2015;12:139–145.
17. Kume S, Takeya M, Mori T, et al. Immunohistochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. *Am J Pathol*. 1995;147:654–667.
18. Matafome P, Rodrigues T, Sena C, et al. Methylglyoxal in metabolic disorders: facts, myths, and promises. *Med Res Rev*. 2017;37:368–403.
19. Matafome P, Sena C, Seiça R. Methylglyoxal, obesity, and diabetes. *Endocrine*. 2013;43:472–484.
20. Nakamura Y, Horii Y, Nishino T, et al. Immunohistochemical localization of advanced glycosylation end products in coronary atheroma and cardiac tissue in diabetes mellitus. *Am J Pathol*. 1993;143:1649–1656.
21. Nakayama K, Nakayama M, Iwabuchi M, et al. Plasma α-oxoaldehyde levels in diabetic and nondiabetic chronic kidney disease patients. *Am J Nephrol*. 2008;28:871–878.
22. Odani H, Shinzato T, Matsumoto Y, et al. Increase in three α,β-dicarbonyl compound levels in human uremic plasma: specific in vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun*. 1999;256:89–93.
23. Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation-a mini-review. *Gerontology*. 2012;58:227–237.
24. Srikanth V, Westcott B, Forbes J, et al. Methylglyoxal, cognitive function and cerebral atrophy in older people. *J Gerontol A Biol Sci Med Sci*. 2013;68:68–73.
25. Rodrigues T, Matafome P, Sereno J, et al. Methylglyoxal-induced glycation changes adipose tissue vascular architecture, flow and expansion, leading to insulin resistance. *Sci Rep*. 2017;7:1698.
26. Neves C, Rodrigues T, Sereno J, et al. Dietary glycotoxins impair hepatic lipidemic profile in diet-induced obese rats causing hepatic oxidative stress and insulin resistance. *Oxid Med Cell Longev*. 2019;2019:1–14.
27. Lee HJ, Howell SK, Sanford RJ, et al. Methylglyoxal can modify GAPDH activity and structure. *Ann N Y Acad Sci*. 2005;1043:135–145.
28. Loske C, Neumann A, Cunningham AM, et al. Cytotoxicity of advanced glycation endproducts is mediated by oxidative stress. *J Neural Transm*. 1998;105:1005–1015.
29. Rosca MG, Mustata TG, Kinter MT, et al. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. *Am J Physiol Renal Physiol*. 2005;289:F420–F430.
30. Wu L, Juurlink B. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension*. 2002;39:809–814.
31. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813–820.
32. Funk SD, Yurdagul A, Orr AW. Hyperglycemia and endothelial dysfunction in atherosclerosis: lessons from type 1 diabetes. *Int J Vasc Med*. 2012;2012:1–19.
33. Sena CM, Matafome P, Crisóstomo J, et al. Methylglyoxal promotes oxidative stress and endothelial dysfunction. *Pharmacol Res*. 2012;65:497–506.
34. Su Y, Lei X, Wu L, et al. The role of endothelial cell adhesion molecules P-selectin, E-selectin and intercellular adhesion molecule-1 in leucocyte recruitment induced by exogenous methylglyoxal. *Immunology*. 2012;137:65–79.
35. Mericq V, Piccardo C, Cai W, et al. Maternally transmitted and food-derived glycotoxins: a factor preconditioning the young to diabetes? *Diabetes Care*. 2010;33:2232–2237.
36. Elmhiri G, Mahmood DFD, Niquet-Leridon C, et al. Formula-derived advanced glycation end products are involved in the development of long-term inflammation and oxidative stress in kidney of IUGR piglets. *Mol Nutr Food Res*. 2015;59:939–947.
37. Federico G, Gori M, Randazzo E, et al. Skin advanced glycation end-products evaluation in infants according to the type of feeding and mother's smoking habits. *SAGE Open Med*. 2016;4:205031211668212.
38. Peppa M, He C, Hattori M, et al. Fetal or neonatal low-glycotoxin environment prevents autoimmune diabetes in NOD mice. *Diabetes*. 2003;52:1441–1448.
39. Kutlu T. Dietary glycotoxins and infant formulas. *Turk Pediatri Ars*. 2016;51:179–185.
40. Pischetsrieder M, Henle T. Glycation products in infant formulas: chemical, analytical and physiological aspects. *Amino Acids*. 2012;42:1111–1118.
41. Angueira AR, Ludvik AE, Reddy TE, et al. New insights into gestational glucose metabolism: lessons learned from 21st century approaches. *Diabetes*. 2015;64:327–334.
42. Arnaout R, Nah G, Marcus G, et al. Pregnancy complications and premature cardiovascular events among 1.6 million California pregnancies. *Open Heart*. *2019*;6:1–10.
43. Farrar D, Simmonds M, Griffin S, et al. The identification and treatment of women with hyperglycaemia in pregnancy: an analysis of individual participant data, systematic reviews, meta-analyses and an economic evaluation. *Health Technol Assess*. 2016;20:1–382.
44. Holness N. High-risk pregnancy. *Nurs Clin North Am*. 2018;53:241–251.

45. Giannakou K, Evangelou E, Yiallouros P, et al. Risk factors for gestational diabetes: an umbrella review of meta-analyses of observational studies. *PLoS One*. 2019;14:E0215372.
46. Ornoy A, Reece EA, Pavlinkova G, et al. Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defect Res C Embryo Today*. 2015;105:53–72.
47. Papathakis PC, Singh LN, Manary MJ. How maternal malnutrition affects linear growth and development in the offspring. *Mol Cell Endocrinol*. 2016;435:40–47.
48. Sinzato YK, Bevilacqua EM, Volpato GT, et al. Maternal oxidative stress, placental morphometry, and fetal growth in diabetic rats exposed to cigarette smoke. *Reprod Sci*. 2019;26:1287–1293.
49. Zeng Z, Liu F, Li S. Metabolic adaptations in pregnancy: a Review. *Ann Nutr Metab*. 2017;70:59–65.
50. Silva CCV, Vehmeijer FOL, El Marroun H, et al. Maternal psychological distress during pregnancy and childhood cardio-metabolic risk factors. *Nutr Metab Cardiovasc Dis*. 2019;29:572–579.
51. Barker DJP, Osmond C, Golding J, et al. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *Br Med J*. 1989;298:564–567.
52. Eriksson UJ, Wentzel P. The status of diabetic embryopathy. *Ups J Med Sci*. 2016;121:96–112.
53. Li S, Yang H. Relationship between advanced glycation end products and gestational diabetes mellitus. *J Matern Neonatal Med*. 2019;32:2783–2789.
54. Takeuchi M, Takino JI, Furuno S, et al. Assessment of the concentrations of various advanced glycation end-products in beverages and foods that are commonly consumed in Japan. *PLoS One*. 2015;10:E0118652.
55. Reece EA. Diabetes-induced birth defects: what do we know? What can we do? *Curr Diab Rep*. 2012;12:24–32.
56. A. Lawlor D, Fraser A, Lindsay RS, et al. Association of existing diabetes, gestational diabetes and glycosuria in pregnancy with macrosomia and offspring body mass index, waist and fat mass in later childhood: findings from a prospective pregnancy cohort. *Diabetologia*. 2010;53:89–97.
57. Olmedo-Requena R, Gómez-Fernández J, Amezcua-Prieto C, et al. Pre-pregnancy adherence to the Mediterranean diet and gestational diabetes mellitus: a case-control study. *Nutrients*. 2019;11:1003– 1011.
58. Amezcua-Prieto C, Martínez-Galiano JM, Cano-Ibáñez N, et al. Types of carbohydrates intake during pregnancy and frequency of a small for gestational age newborn: a case-control study. *Nutrients*. 2019;11:523.
59. Vaiserman A, Lushchak O. Prenatal malnutrition-induced epigenetic dysregulation as a risk factor for type 2 diabetes. *Int J Genomics*. 2019;2019:1–11.
60. Mullie P, Clarys P. Consumption of artificially sweetened beverages during pregnancy is associated with a twofold higher risk of infant being overweight at 1 year. *Evid Based Nurs*. 2017;20:11–11.
61. Palatianou ME, Simos YV, Andronikou SK, et al. Long-term metabolic effects of high birth weight: a critical review of the literature. *Horm Metab Res*. 2014;46:911–920.
62. Plagemann A, Harder T, Rodekamp E, et al. Rapid neonatal weight gain increases risk of childhood overweight in offspring of diabetic mothers. *J Perinat Med*. 2012;40:557–563.
63. Schellong K, Schulz S, Harder T, et al. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. *PLoS One*.2012;7:E47776.
64. Harder T, Rodekamp E, Schellong K, et al. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. *Am J Epidemiol*. 2007;165:849–857.
65. Kalapos MP. Where does plasma methylglyoxal originate from? *Diabetes Res Clin Pract*. 2013;99:260–271.
66. Poulsen MW, Hedegaard RV, Andersen JM, et al. Advanced glycation end products in food and their effects on health. *Food Chem Toxicol*. 2013;60:10–37.
67. Allaman I, Bélanger M, Magistretti PJ. Methylglyoxal, the dark side of glycolysis. *Front Neurosci*. 9:23.
68. Falone S, D'Alessandro A, Mirabilio A, et al. Long term running biphasically improves methylglyoxal-related metabolism, redox homeostasis and neurotrophic support within adult mouse brain cortex. *PLoS One*. 2012;7:E31401.
69. Masania J, Malczewska-Malec M, Razny U, et al. Dicarbonyl stress in clinical obesity. *Glycoconj J*. 2016;33:581–589.
70. Yao D, Taguchi T, Matsumura T, et al. High glucose increases angiopoietin-2 transcription in microvascular endothelial cells through methylglyoxal modification of mSin3A. *J Biol Chem*. 2007;282:31038–31045.
71. Carlsson H, Törnqvist M. Strategy for identifying unknown hemoglobin adducts using adductome LC-MS/MS data: identification of adducts corresponding to acrylic acid, glyoxal, methylglyoxal, and 1-octen-3-one. *Food Chem Toxicol*. 2016;92:94–103.
72. Bose T, Bhattacherjee A, Banerjee S, et al. Methylglyoxal-induced modifications of hemoglobin: structural and functional characteristics. *Arch Biochem Biophys*. 2013;529:99–104.
73. Guerin-Dubourg A, Catan A, Bourdon E, et al. Structural modifications of human albumin in diabetes. *Diabetes Metab*. 2012;38:171–178.
74. Rabbani N, Godfrey L, Xue M, et al. Glycation of LDL by methylglyoxal increases arterial atherogenicity: a possible contributor to increased risk of cardiovascular disease in diabetes. *Diabetes*. 2011;60:1973–1980.
75. Goldin A, Beckman JA, Schmidt AM, et al. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation*. 2006;114:597–605.
76. Pedchenko VK, Chetyrkin SV, Chuang P, et al. Mechanism of perturbation of integrin-mediated cell-matrix interactions by reactive carbonyl compounds and its implication for pathogenesis of diabetic nephropathy. *Diabetes*. 2005;54:2952–2960.
77. Bento CF, Fernandes R, Matafome P, et al. Methylglyoxal-induced imbalance in the ratio of vascular endothelial growth factor to angiopoietin 2 secreted by retinal pigment epithelial cells leads to endothelial dysfunction. *Exp Physiol*. 2010;95:955–970.
78. Bento CF, Marques F, Fernandes R, et al. Methylglyoxal alters the function and stability of critical components of the protein quality control. *Cotterill S, ed. PLoS One*. 2010;5:E13007.
79. Padival AK, Crabb JW, Nagaraj RH. Methylglyoxal modifies heat shock protein 27 in glomerular mesangial cells. *FEBS Lett*. 2003;551:113–118.
80. Palsamy P, Bidasee KR, Ayaki M, et al. Methylglyoxal induces endoplasmic reticulum stress and DNA demethylation in the Keap1 promoter of human lens epithelial cells and age-related cataracts. *Free Radic Biol Med*. 2014;72:134–148.
81. Nam DH, Han JH, Lee TJ, et al. CHOP deficiency prevents methylglyoxal-induced myocyte apoptosis and cardiac dysfunction. *J Mol Cell Cardiol*. 2015;85:168–177.
82. Chang T, Wang R, Wu L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. *Free Radic Biol Med*. 2005;38:286–293.
83. Ward RA, McLeish KR. Methylglyoxal: a stimulus to neutrophil oxygen radical production in chronic renal failure? *Nephrol Dial Transplant*. 2004;19:1702–1707.
84. Škrha J, Gáll J, Buchal R, et al. Glucose and its metabolites have distinct effects on the calcium-induced mitochondrial permeability transition. *Folia Biol (Czech Republic)*. 2011;57:96–103.
85. Remor AP, de Matos FJ, Ghisoni K, et al. Differential effects of insulin on peripheral diabetes-related changes in mitochondrial bioenergetics: involvement of advanced glycosylated end products. *Biochim Biophys Acta Mol Basis Dis*. 2011;1812:1460–1471.
86. Kalapos MP, Littauer A, qde Groot H. Has reactive oxygen a role in methylglyoxal toxicity? A study on cultured rat hepatocytes. *Arch Toxicol*. 1993;67:369–372.
87. Akhand AA, Hossain K, Mitsui H, et al. Glyoxal and methylglyoxal trigger distinct signals for MAP family kinases and caspase activation in human endothelial cells. *Free Radic Biol Med*. 2001;31:20–30.
88. Uriuhara A, Miyata S, Liu BF, et al. Methylglyoxal induces prostaglandin E2 production in rat mesangial cells. *Kobe J Med Sci*. 2008;53:305–315.
89. Seo K, Ki SH, Shin SM. Methylglyoxal induces mitochondrial dysfunction and cell death in liver. *Toxicol Res*. 2014;30:193–198.
90. Suh KS, Choi EM, Rhee SY, et al. Methylglyoxal induces oxidative stress and mitochondrial dysfunction in osteoblastic MC3T3-E1 cells. *Free Radic Res*. 2014;48:206–217.
91. Di Loreto S, Caracciolo V, Colafarina S, et al. Methylglyoxal induces oxidative stress-dependent cell injury and up-regulation of interleukin-1β and nerve growth factor in cultured hippocampal neuronal cells. *Brain Res*. 2004;1006:157–167.
92. Dafre AL, Goldberg J, Wang T, et al. Methylglyoxal, the foe and friend of glyoxalase and Trx/TrxR systems in HT22 nerve cells. *Free Radic Biol Med*. 2015;89:8–19.
93. Kikuchi S, Shinpo K, Moriwaka F, et al. Neurotoxicity of methylglyoxal and 3-deoxyglucosone on cultured cortical neurons: synergism between glycation and oxidative stress, possibly involved in neurodegenerative diseases. *J Neurosci Res*. 1999;57:280–289.
94. Amicarelli F, Colafarina S, Cattani F, et al. Scavenging system efficiency is crucial for cell resistance to ROS-mediated methylglyoxal injury. *Free Radic Biol Med*. 2003;35:856–871.
95. Morgan PE, Sheahan PJ, Pattison DI, et al. Methylglyoxal-induced modification of arginine residues decreases the activity of NADPH-generating enzymes. *Free Radic Biol Med*. 2013;61:229–242.
96. Suravajjala S, Cohenford M, Frost LR, et al. Glycation of human erythrocyte glutathione peroxidase: effect on the physical and kinetic properties. *Clin Chim Acta*. 2013;421:170–176.
97. Pun PBL, Logan A, Darley-Usmar V, et al. A mitochondria-targeted mass spectrometry probe to detect glyoxals: implications for diabetes. *Free Radic Biol Med*. 2014;67:437–450.
98. Paget C, Lecomte M, Ruggiero D, et al. Modification of enzymatic antioxidants in retinal microvascular cells by glucose or advanced glycation end products. *Free Radic Biol Med*. 1998;25:121–129.
99. Di Loreto S, Zimmitti V, Sebastiani P, et al. Methylglyoxal causes strong weakening of detoxifying capacity and apoptotic cell death in rat hippocampal neurons. *Int J Biochem Cell Biol*. 2008;40:245–257.
100. Yan SF, Ramasamy R, Schmidt AM. Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. *J Mol Med*. 2009;87:235–247.

101. Negre-Salvayre A, Salvayre R, Augé N, et al. Hyperglycemia and glycation in diabetic complications. *Antioxidants Redox Signal*. 2009;11:3071–3109.
102. Yan SF, Ramasamy R, Naka Y, et al. Glycation, Inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. *Circ Res*. 2003;93:1159–1169.
103. Xue J, Rai V, Singer D, et al. Advanced glycation end product recognition by the receptor for AGEs. *Structure*. 2011;19:722–732.
104. Xue J, Ray R, Singer D, et al. The receptor for advanced glycation end products (RAGE) specifically recognizes methylglyoxal-derived AGEs. *Biochemistry*. 2014;53:3327–3335.
105. Liu BF, Miyata S, Hirota Y, et al. Methylglyoxal induces apoptosis through activation of p38 mitogen-activated protein kinase in rat mesangial cells. *Kidney Int*. 2003;63:947–957.
106. Du J, Cai S, Suzuki H, et al. Involvement of MEKK1/ERK/P21Waf1/Cip1 signal transduction pathway in inhibition of IGF-I-mediated cell growth response by methylglyoxal. *J Cell Biochem*. 2003;88:1235–1246.
107. Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes*. 2010;59:249–255.
108. Ueno H, Koyama H, Shoji T, et al. Receptor for advanced glycation end-products (RAGE) regulation of adiposity and adiponectin is associated with atherogenesis in apoE-deficient mouse. *Atherosclerosis*. 2010;211:431–436.
109. Thangarajah H, Yao D, Chang EI, et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. *Proc Natl Acad Sci USA*. 2009;106:13505–13510.
110. Berlanga J, Cibrian D, Guillén I, et al. Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. *Clin Sci*. 2005;109:83–95.
111. Diez-Sampedro A, Lenz O, Fornoni A. Podocytopathy in diabetes: a metabolic and endocrine disorder. *Am J Kidney Dis*. 2011;58:637–646.
112. Giacco F, Du X, D'Agati VD, et al. Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. *Diabetes*. 2014;63:291–299.
113. Mostafa AA, Randell EW, Vasdev SC, et al. Plasma protein advanced glycation end products, carboxymethyl cysteine, and carboxyethyl cysteine, are elevated and related to nephropathy in patients with diabetes. *Mol Cell Biochem*. 2007;302:35–42.
114. Kim J, Kim OS, Kim CS, et al. Accumulation of argpyrimidine, a methylglyoxal-derived advanced glycation end product, increases apoptosis of lens epithelial cells both in vitro and in vivo. *Exp Mol Med*. 2012;44:167–175.
115. Kim J, Kim OS, Kim CS, et al. Cytotoxic role of methylglyoxal in rat retinal pericytes: Involvement of a nuclear factor-kappaB and inducible nitric oxide synthase pathway. *Chem Biol Interact*. 2010;188:86–93.
116. Kim OS, Kim J, Kim CS, et al. KIOM-79 prevents methyglyoxal-induced retinal pericyte apoptosis in vitro and in vivo. *J Ethnopharmacol*. 2010;129:285–292.
117. Kim J, Son JW, Lee JA, et al. Methylglyoxal induces apoptosis mediated by reactive oxygen species in bovine retinal pericytes. *J Korean Med Sci*. 2004;19:95–100.
118. Bierhaus A, Fleming T, Stoyanov S, et al. Methylglyoxal modification of Na v 1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat Med*. 2012;18:926–933.
119. Skapare E, Konrade I, Liepinsh E, et al. Association of reduced glyoxalase 1 activity and painful peripheral diabetic neuropathy in type 1 and 2 diabetes mellitus patients. *J Diabetes Complications*. 2013;27:262–267.
120. Li W, Maloney RE, Aw TY. High glucose, glucose fluctuation and carbonyl stress enhance brain microvascular endothelial barrier dysfunction: implications for diabetic cerebral microvasculature. *Redox Biol*. 2015;5:80–90.
121. Li W, Maloney RE, Circu ML, et al. Acute carbonyl stress induces occludin glycation and brain microvascular endothelial barrier dysfunction: role for glutathione-dependent metabolism of methylglyoxal. *Free Radic Biol Med*. 2013;54:51–61.
122. Chun HJ, Lee Y, Kim AH, et al. Methylglyoxal causes cell death in neural progenitor cells and impairs adult hippocampal neurogenesis. *Neurotox Res*. 2016;29:419–431.
123. Heimfarth L, Loureiro SO, Pierozan P, et al. Methylglyoxal-induced cytotoxicity in neonatal rat brain: a role for oxidative stress and MAP kinases. *Metab Brain Dis*. 2013;28:429–438.
124. Li XH, Du LL, Cheng XS, et al. Glycation exacerbates the neuronal toxicity of β-amyloid. *Cell Death Dis*. 2013;4:e673.
125. Lovestone S, Smith U. Advanced glycation end products, dementia, and diabetes. *Proc Natl Acad Sci USA*. 2014;111:4743–4744.
126. Xie B, Lin F, Peng L, et al. Methylglyoxal increases dopamine level and leads to oxidative stress in SH-SY5Y cells. *Acta Biochim Biophys Sin (Shanghai)*. 2014;46:950–956.
127. Xie B, Lin F, Ullah K, et al. A newly discovered neurotoxin ADTIQ associated with hyperglycemia and Parkinson's disease. *Biochem Biophys Res Commun*. 2015;459:361–366.
128. Song DW, Xin N, Xie BJ, et al. Formation of a salsolinol-like compound, the neurotoxin, 1-acetyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, in a cellular model of hyperglycemia and a rat model of diabetes. *Int J Mol Med*. 2014;33:736–742.
129. Tian C, Alomar F, Moore CJ, et al. Reactive carbonyl species and their roles in sarcoplasmic reticulum Ca 2+ cycling defect in the diabetic heart. *Heart Fail Rev*. 2014;19:101–112.
130. Crisóstomo J, Matafome P, Santos-Silva D, et al. Methylglyoxal chronic administration promotes diabetes-like cardiac ischaemia disease in Wistar normal rats. *Nutr Metab Cardiovasc Dis*. 2013;23:1223–1230.
131. Almeida F, Santos-Silva D, Rodrigues T, et al. Pyridoxamine reverts methylglyoxal-induced impairment of survival pathways during heart ischemia. *Cardiovasc Ther*. 2013;31:e79–e85.
132. Molgat ASD, Tilokee EL, Rafatian G, et al. Hyperglycemia inhibits cardiac stem cell-mediated cardiac repair and angiogenic capacity. *Circulation*. 2014;130:S70–S76.
133. Su Y, Qadri SM, Wu L, et al. Methylglyoxal modulates endothelial nitric oxide synthase-associated functions in EA.hy926 endothelial cells. *Cardiovasc Diabetol*. 2013;12:134.
134. Su Y, Qadri SM, Hossain M, et al. Uncoupling of eNOS contributes to redox-sensitive leukocyte recruitment and microvascular leakage elicited by methylglyoxal. *Biochem Pharmacol*. 2013;86:1762–1774.
135. Mukohda M, Yamawaki H, Nomura H, et al. Methylglyoxal inhibits smooth muscle contraction in isolated blood vessels. *J Pharmacol Sci*. 2009;109:305–310.
136. Semba RD, Najjar SS, Sun K, Lakatta EG, et al. Serum carboxymethyl-lysine, an advanced glycation end product, is associated with increased aortic pulse wave velocity in adults. *Am J Hypertens*. 2009;22:74–79.
137. Dhar I, Dhar A, Wu L, et al. Methylglyoxal, a reactive glucose metabolite, increases renin angiotensin aldosterone and blood pressure in male Sprague-Dawley rats. *Am J Hypertens*. 2014;27:308–316.
138. Dhar I, Dhar A, Wu L, et al. Increased methylglyoxal formation with upregulation of renin angiotensin system in fructose fed Sprague Dawley rats. *PLoS One*. 2013;8:E74212.
139. Rabbani N, Chittari MV, Bodmer CW, et al. Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin. *Diabetes*. 2010;59:1038–1045.
140. Beisswenger PJ, Howell SK, Touchette AD, et al. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes*. 1999;48:198–202.
141. Tikellis C, Pickering RJ, Tsorotes D, et al. Dicarbonyl stress in the absence of hyperglycemia increases endothelial inflammation and atherogenesis similar to that observed in diabetes. *Diabetes*. 2014;63:3915–3925.
142. Hadas K, Randriamboavonjy V, Elgheznawy A, et al. Methylglyoxal induces platelet hyperaggregation and reduces thrombus stability by activating PKC and inhibiting PI3K/Akt pathway. *PLoS One*. 2013;8:E74401.
143. Yang Y, Konduru AS, Cui N, et al. Acute exposure of methylglyoxal leads to activation of KATP channels expressed in HEK293 cells. *Acta Pharmacol Sin*. 2014;35:58–64.
144. Dhar A, Dhar I, Jiang B, et al. Chronic methylglyoxal infusion by minipump causes pancreatic β-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes*. 2011;60:899–908.
145. Jia X, Wu L. Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats. *Mol Cell Biochem*. 2007;306:133–139.
146. Engelbrecht B, Stratmann B, Hess C, et al. Impact of GLO1 knock down on GLUT4 trafficking and glucose uptake in L6 myoblasts. *PLoS One*. 2013;8:E65195.
147. Dhar A, Desai KM, Wu L. Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in Sprague-Dawley rats. *Br J Pharmacol*. 2010;159:166–175.
148. Hofmann SM, Dong HJ, Li Z, et al. Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes*. 2002;51:2082–2089.
149. Rodrigues T, Matafome P, Santos-Silva D, et al. Reduction of methylglyoxal-induced glycation by pyridoxamine improves adipose tissue microvascular lesions. *J Diabetes Res*. 2013;2013:1–9.
150. Wei Y, Wang D, Moran G, et al. Fructose-induced stress signaling in the liver involves methylglyoxal. *Nutr Metab*.10:32.
151. Gaens KHJ, Niessen PMG, Rensen SS, et al. Endogenous formation of N$^{\varepsilon}$-(carboxymethyl)lysine is increased in fatty livers and induces inflammatory markers in an in vitro model of hepatic steatosis. *J Hepatol*. 2012;56:647–655.
152. Uribarri J, Cai W, Woodward M, et al. Elevated serum advanced glycation end products in obese indicate risk for the metabolic syndrome: a link between healthy and unhealthy obesity? *J Clin Endocrinol Metab*. 2015;100:1957–1966.
153. Kong X, zhe MM, Huang K, et al. Increased plasma levels of the methylglyoxal in patients with newly diagnosed type 2 diabetes. *J Diabetes*. 2014;6:535–540.
154. Uribarri J, Cai W, Ramdas M, et al. Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. *Diabetes Care*. 2011;34:1610–1616.
155. De Courten B, De Courten MPJ, Soldatos G, et al. Diet low in advanced glycation end products increases insulin sensitivity in healthy overweight individuals: a double-blind, randomized, crossover trial. *Am J Clin Nutr*. 2016;103:1426–1433.
156. Macías-Cervantes MH, Rodríguez-Soto JMD, Uribarri J, et al. Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition*. 2015;31:446–451.
157. Vlassara H, Cai W, Tripp E, et al. Oral AGE restriction ameliorates insulin resistance in obese individuals with the metabolic syndrome: a randomised controlled trial. *Diabetologia*. 2016;59:2181–2192.

158. Jones ML, Buhimschi IA, Zhao G, et al. Acute glucose load, inflammation, oxidative stress, nonenzymatic glycation, and screening for gestational diabetes. *Reprod Sci*. 2019;193371911983177.
159. Bartakova V, Kollarova R, Kuricova K, et al. Serum carboxymethyl-lysine, a dominant advanced glycation end product, is increased in women with gestational diabetes mellitus. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2016;160:70–75.
160. Kellow NJ, Coughlan MT. Effect of diet-derived advanced glycation end products on inflammation. *Nutr Rev*. 2015;73:737–759.
161. Villegas-Rodríguez ME, Uribarri J, Solorio-Meza SE, et al. The AGE-RAGE axis and its relationship to markers of cardiovascular disease in newly diagnosed diabetic patients. *PLoS One*. 2016;11:E0159175.
162. Šebeková K, Brouder Šebeková K. Glycated proteins in nutrition: friend or foe? *Exp Gerontol*. 2019;117:76–90.
163. Guosheng L, Hongmei S, Chuan N, et al. The relationship of serum AGE levels in diabetic mothers with adverse fetal outcome. *J Perinatol*. 2009;29:483–488.
164. Lappas M. Activation of inflammasomes in adipose tissue of women with gestational diabetes. *Mol Cell Endocrinol*. 2014;382:74–83.
165. Konishi H, Nakatsuka M, Chekir C, et al. Advanced glycation end products induce secretion of chemokines and apoptosis in human first trimester trophoblasts. *Hum Reprod*. 2004;19:2156–2162.
166. Hao L, Noguchi S, Kamada Y, et al. Adverse effects of advanced glycation end products on embryonal development. *Acta Med Okayama*. 2008;62:93–99.
167. Haucke E, Santos AN, Simm A, et al. Accumulation of advanced glycation end products in the rabbit blastocyst under maternal diabetes. *Reproduction*. 2014;148:169–178.
168. Kawaharada R, Masuda H, Chen Z, et al. Intrauterine hyperglycemia-induced inflammatory signalling via the receptor for advanced glycation end products in the cardiac muscle of the infants of diabetic mother rats. *Eur J Nutr*. 2018;57:2701–2712.
169. Ejdesjö A, Brings S, Fleming T, et al. Receptor for advanced glycation end products (RAGE) knockout reduces fetal dysmorphogenesis in murine diabetic pregnancy. *Reprod Toxicol*. 2016;62:62–70.
170. Tang X, Qin Q, Xie X, et al. Protective effect of sRAGE on fetal development in pregnant rats with gestational diabetes mellitus. *Cell Biochem Biophys*. 2015;71:549–556.
171. Dale MTG, Magnus P, Leirgul E, et al. Intake of sucrose-sweetened soft beverages during pregnancy and risk of congenital heart defects (CHD) in offspring: a Norwegian pregnancy cohort study. *Eur J Epidemiol*. 2019;34:383–396.
172. World Health Organization. Global Strategy for Infant and Young Child Feeding. Geneva: World Health Organization; 2003.
173. Vieira Borba V, Sharif K, Shoenfeld Y. Breastfeeding and autoimmunity: programing health from the beginning. *Am J Reprod Immunol*. 2018;79:E12778.
174. Donovan SM, Odle J. Growth factors in milk as mediators of infant development. *Annu Rev Nutr*. 1994;14:147–167.
175. Shehadeh N, Shamir R, Berant M, et al. Insulin in human milk and the prevention of type 1 diabetes. *Pediatr Diabetes*. 2001;2:175–177.
176. Plagemann A, Harder T, Schellong K, et al. Early postnatal life as a critical time window for determination of long-term metabolic health. *Best Pract Res Clin Endocrinol Metab*. 2012;26:641–653.
177. Knowles JA. Breast milk: a source of more than nutrition for the neonate. *Clin Toxicol*. 1974;7:69–82.
178. Gomes RM, Bueno FG, Schamber CR, et al. Maternal diet-induced obesity during suckling period programs offspring obese phenotype and hypothalamic leptin/insulin resistance. *J Nutr Biochem*. 2018;61:24–32.
179. Schwarzenbolz U, Hofmann T, Sparmann N, et al. Free Maillard reaction products in milk reflect nutritional intake of glycated proteins and can be used to distinguish "organic" and "conventionally" produced milk. *J Agric Food Chem*. 2016;64:5071–5078.
180. Francisco FA, Barella LF, Silveira S da S, et al. Methylglyoxal treatment in lactating mothers leads to type 2 diabetes phenotype in male rat offspring at adulthood. *Eur J Nutr*. 2018;57:477–486.
181. Uribarri J, Cai W, Sandu O, et al. Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. *Ann N Y Acad Sci*. 2005;1043:461–466.
182. Uribarri J, Cai W, Peppa M, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. *J Gerontol A Biol Sci Med Sci*. 2007;62:427–433.
183. Koschinsky T, He CJ, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci USA*. 1997;94:6474–6479.
184. Birlouez-Aragon I, Saavedra G, Tessier FJ, et al. A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *Am J Clin Nutr*. 2010;91:1220–1226.
185. Mark AB, Poulsen MW, Andersen S, et al. Consumption of a diet low in advanced glycation end products for 4 weeks improves insulin sensitivity in overweight women. *Diabetes Care*. 2014;37:88–95.
186. He C, Sabol J, Mitsuhashi T, et al. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. *Diabetes*. 1999;48:1308–1315.
187. Hellwig M, Geissler S, Peto A, et al. Transport of free and peptide-bound pyrraline at intestinal and renal epithelial cells. *J Agric Food Chem*. 2009;57:6474–6480.
188. Hellwig M, Geissler S, Matthes R, et al. Transport of free and peptide-bound glycated amino acids: synthesis, transepithelial flux at Caco-2 cell monolayers, and interaction with apical membrane transport proteins. *ChemBioChem*. 2011;12:1270–1279.
189. Dittrich R, Hoffmann I, Stahl P, et al. Concentrations of Nε-carboxymethyllysine in human breast milk, infant formulas, and urine of infants. *J Agric Food Chem*. 2006;54:6924–6928.
190. Han L, Li L, Li B, et al. Review of the characteristics of food-derived and endogenous Nε-carboxymethyllysine. *J Food Prot*. 2013;76:912–918.
191. O'Brien J, Morrissey PA, Ames JM. Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Crit Rev Food Sci Nutr*. 1989;28:211–248.
192. Zamora R, Hidalgo FJ. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit Rev Food Sci Nutr*. 2005;45:49–59.
193. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc*. 2010;110:911–916.e12.
194. Prosser CG, Carpenter EA, Hodgkinson AJ. N ε -carboxymethyllysine in nutritional milk formulas for infants. *Food Chem*. 2019;274:886–890.
195. Šebeková K, Saavedra G, Zumpe C, et al. Plasma concentration and urinary excretion of Nε- (carboxymethyl)lysine in breast milk- and formula-fed infants. *Ann N Y Acad Sci*. 2008;1126:177–180.
196. Firmin S, Elmhiri G, Crepin D, et al. Formula derived Maillard reaction products in post-weaning intrauterine growth-restricted piglets induce developmental programming of hepatic oxidative stress independently of microRNA-21 and microRNA-155. *J Dev Orig Health Dis*. 2018;9:566–572.
197. Elliott RB. Diabetes - a man made disease. *Med Hypotheses*. 2006;67:388–391.
198. Klenovics KS, Boor P, Somoza V, et al. Advanced glycation end products in infant formulas do not contribute to insulin resistance associated with their consumption. *PLoS One*. 2013;8:E53056.
199. Renzone G, Arena S, Scaloni A. Proteomic characterization of intermediate and advanced glycation end-products in commercial milk samples. *J Proteomics*. 2015;117:12–23.
200. Zhao D, Le TT, Larsen LB, et al. Effect of glycation derived from α-dicarbonyl compounds on the in vitro digestibility of β-casein and β-lactoglobulin: A model study with glyoxal, methylglyoxal and butanedione. *Food Res Int*. 2017;102:313–322.
201. Snelson M, Coughlan MT. Dietary advanced glycation end products: digestion, metabolism and modulation of gut microbial ecology. *Nutrients*. 2019;11:215.
202. Qu W, Yuan X, Zhao J, et al. Dietary advanced glycation end products modify gut microbial composition and partially increase colon permeability in rats. *Mol Nutr Food Res*. 2017;61:1700118.
203. Seiquer I, Rubio LA, Peinado MJ, et al. Maillard reaction products modulate gut microbiota composition in adolescents. *Mol Nutr Food Res*. 2014;58:1552–1560.
204. Yacoub R, Nugent M, Cai W, et al. Advanced glycation end products dietary restriction effects on bacterial gut microbiota in peritoneal dialysis patients; a randomized open label controlled trial. *PLoS One*. 2017;12:E0184789.
205. Harada E, Syuto B. Precocious cessation of intestinal macromolecular transmission and sucrase development induced by insulin in adrenalectomized suckling rat. *Comp Biochem Physiol – Part A Physiol*. 1991;99:327–331.
206. Peters MA, Hudson PM, Jurgelske W. The acute toxicity of methylglyoxal in rats: the influence of age, sex, and pregnancy. *Ecotoxicol Environ Saf*. 1978;2:369–374.
207. Toop CR, Muhlhausler BS, O'Dea K, et al. Impact of perinatal exposure to sucrose or high fructose corn syrup (HFCS-55) on adiposity and hepatic lipid composition in rat offspring. *J Physiol*. 2017;595:4379–4398.
208. Csongová M, Gurecká R, Koborová I, et al. The effects of a maternal advanced glycation end product-rich diet on somatic features, reflex ontogeny and metabolic parameters of offspring mice. *Food Funct*. 2018;9:3432–3446.
209. Borg DJ, Yap FYT, Keshvari S, et al. Perinatal exposure to high dietary advanced glycation end products in transgenic NOD8.3 mice leads to pancreatic beta cell dysfunction. *Islets*. 2018;10:10–24.
210. Roest PAM, Molin DGM, Schalkwijk CG, et al. Specific local cardiovascular changes of n ε-(carboxymethyl) lysine, vascular endothelial growth factor, and smad2 in the developing embryos coincide with maternal diabetes-induced congenital heart defects. *Diabetes*. 2009;58:1222–1228.
211. Chiavaroli V, D'Adamo E, Giannini C, et al. Serum levels of receptors for advanced glycation end products in normal-weight and obese children born small and large for gestational age. *Diabetes Care*. 2012;35:1361–1363.
212. Chandna AR, Kuhlmann N, Bryce CA, et al. Chronic maternal hyperglycemia induced during mid-pregnancy in rats increases RAGE expression, augments hippocampal excitability, and alters behavior of the offspring. *Neuroscience*. 2015;303:241–260.

213. Yang G, Cancino GI, Zahr SK, et al. A Glo1-methylglyoxal pathway that is perturbed in maternal diabetes regulates embryonic and adult neural stem cell pools in murine offspring. *Cell Rep*. 2016;17:1022–1036.
214. Shimizu I, Hiramatsu Y, Omori Y, et al. Comparison of HbA1c and glycated albumin as a control marker for newborn complications in diabetic women in a multicentre study in Japan (Japan Glycated Albumin Study Group: Study 2). *Ann Clin Biochem*. 2018;55:639–646.
215. Sugawara D, Sato H, Ichihashi K, et al. Glycated albumin level during late pregnancy as a predictive factor for neonatal outcomes of women with diabetes. *J Matern Neonatal Med*. 2018;31:2007–2012.
216. Feng Y, Yang H. Metformin–a potentially effective drug for gestational diabetes mellitus: a systematic review and meta-analysis. *J Matern Neonatal Med*. 2017;30:1874–1881.
217. Kinsky OR, Hargraves TL, Anumol T, et al. Metformin scavenges methylglyoxal to form a novel imidazolinone metabolite in humans. *Chem Res Toxicol*. 2016;29:227–234.
218. Foretz M, Guigas B, Bertrand L, et al. Metformin: from mechanisms of action to therapies. *Cell Metab*. 2014;20:953–966.

CAPÍTULO 2 - Increased PPARα activitation by an agonist during lactation protects against long-term hepatic steatosis and insulin resistance induced by post-natal overfeeding in male Wistar rats.

INCREASED PPARα ACTIVATION BY AN AGONIST DURING LACTATION PROTECTS AGAINST LONG-TERM HEPATIC STEATOSIS AND INSULIN RESISTANCE INDUCED BY POST-NATAL OVERFEEDING IN MALE WISTAR RATS

Lucas Paulo Jacinto Saavedra[1#]; Scarlett Rodrigues Raposo[1]; Ana Letícia Manso Assakawa[1]; Naiara Cristina Lucredi[5]; Maria Natália Chimirri Peres[1]; Silvano Piovan[2]; Gessica Dutra Gonçalves[1]; Veridiana Mota Moreira[1]; Letícia Ferreira Barbosa[1]; Diana Sousa[4]; Flávia Caroline Farias dos Santos[3]; Jones Bernardes Graceli[3]; Paulo Matafome[4]; Jurandir Fernando Comar[5]; Rodrigo Mello Gomes[6]; Douglas Lopes Almeida[1*]; Paulo Cezar de Freitas Mathias[1*].

[1]Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringá, Maringá, PR, Brazil.

[2]Department of Biochemistry, State Univerisity of West Paraná, Cascavel, PR, Brazil.

[3]Department of morphology, Federal University of Espirito Santo, Vitória, ES, Brazil.

[4]Institute of Physiology and Institute of Clinical and Biomedical Research, Faculty of Medicine and Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal Coimbra Health School, ESTeSC, Instituto Politécnico de Coimbra, Coimbra, Portugal

Clinical Academic Center of Coimbra, Coimbra, Portugal

[5]Department of Biochemistry, State University of Maringá, Maringá, PR, Brazil.

[6]Department of Physiological Sciences, Federal University of Goiás, Goiânia, GO, Brazil.

*Both authors contributed equally.

[#]**Corresponding Author:** Lucas Paulo Jacinto Saavedra, Department of Biotechnology, Cell Biology 10 and Genetics, State University of Maringá, 5790 Av Colombo, Sala 19, Maringá, PR, 87020-900, 11 Brazil. Phone/fax: +55 44 3011-4892; e-mail: saavedralpj@gmail.com. ORCiD - 0000-0003-0825-426X.

Declarations of interest: none.

Highlights:

- Post-natal overfeeding decreased offspring hypothalamic expression of FGFR1 and sympathetic activity of BAT innervation;
- Post-natal overfeeding promotes liver microsteatosis, oxidative stress, insulin resistance and increased liver FGF21 expression;
- Increased PPARα activity during breastfeeding protects against the development of obesity, metabolic and autonomic dysfunction induced by post-natal overfeeding during adulthood

Abstract: Introduction/Hypothesis: Obesity and their associated comorbidities are of great concern worldwide. Evidences points out to the importance of lactation in later disease development. In this sense, the model of post-natal overfeeding is linked to the development of obesity, insulin resistance and steatosis. PPARα activation promote expression of key enzymes involved in lipid oxidation, and their agonists, show beneficial effects in animal models of metabolic dysfunction and steatosis. Therefore, we have hypothesized that post-natal activation of PPARα by an agonist, would prevent the development of obesity and metabolic dysfunction induced in animal model of post-natal overfeeding. **Methods:** a well characterized model of post-natal overfeeding was used, litter reduction, in which Wistar rats were treated with an PPARα agonist, fenofibrate, from post-natal day (PN) 1 until weaning (PN21), in order to verify if increased PPARα activation would prevent disease development during adulthood (PN120). **Results:** Post-natal overfeeding leads to increased visceral adipose tissue mass and oxidative stress, insulin resistance, hepatic microsteatosis and increased FGF21 expression, followed by decreased brown adipose tissue (BAT) sympathetic activity and decreased FGFR1 hypothalamic expression. Animals whose received the agonist during lactation were protected against the development of these alterations at adulthood. **Conclusion:** Offspring increased PPARa activation during lactation is able to protect against the development of visceral obesity, steatosis, insulin resistance and hyposympathetic activity of BAT nerve, in an animal model of post-natal overfeeding.

Keywords: FGF21; small litter; neonatal overfeeding; childhood obesity.

INTRODUCTION

Obesity is a condition of major health concern. In 2016, 650 millions of adults and 340 million of children and adolescents were obese worldwide [1]. Often accompanied with metabolic dysfunction, obesity may increase the risk for the onset of type 2 diabetes and cardiovascular disease, the leading causes of death worldwide [2; 3]. Epidemiological and experimental evidences point out to the role of intercurrences during development in increasing or reducing the susceptibility of an organism for obesity and their comorbidities [4; 5]. Lactation indeed is a well-established "window" for metabolic programming for disease later in life [6; 7]. Epidemiological data have shown the protective effect of six months exclusive breastfeeding in promoting protection against the development of disease [8]. Different stimulus during lactation may confer protective effects in the metabolic dysfunction induced during adulthood, such as maternal low-protein diet and exercise [9; 10]. On the other hand, several experimental studies have shown that stresses in this critical phase of development, such as maternal poor diet, increased glycation and smoking, may have long-term effects on the metabolism offspring, promoting the development of obesity, cardiovascular and metabolic dysfunction [11-13].

An animal model for obesity during infancy, named litter reduction, in which the offspring is reduced to 2 to 4 pups per mother causing post-natal overfeeding, have shown to cause early in life metabolic dysfunction and obesity which persists and aggravate later in life [14]. In fact, post-natal overfeeding is reported to cause liver steatosis, insulin resistance, cardiovascular dysfunction, dyslipidemia and dysregulation of glycemic homeostasis [14; 15]. Preliminary studies from our group, have shown that litter reduction leads to consistent reduction in the expression of markers for lipid oxidation (Supplementary Information, Figure 1). The Peroxisome Proliferator Activated by Receptor – alpha (PPARα) has a major role in lipid oxidation since it is a transcriptional factor for the expression of key genes involved in lipid oxidation and ketogenesis [16]. During gestation, fetus is nourished through cord blood where glucose is the main energy source, but after birth, the breastmilk, rich in lipids, is the primary source of nutrition to the neonate, thus characterizing a nutrition switch from carbohydrate to lipid metabolism [17]. Breastmilk derived FFA's activates PPARα into the neonate liver, promoting DNA demethylation and increasing transcription of genes involved in β-oxidation [18]. It was shown that maternal increased PPARα activation by the end of gestation ad beginning of

lactation, may prevent the development of obesity and metabolic dysfunction induced by post-natal high-fat diet (HFD) in the offspring [19]. In adult animals, increased activation of PPARα, prevented obesity and ameliorated metabolic dysfunction induced by HFD [20]. These field of studies may contribute for understanding the mechanisms behind long-term programming for obesity and metabolic dysfunction during lactation and pave the way for the design of new interventions during this period focused in modulation of the PPARα activity.

Considering the metabolic dysfunction and dysregulation of lipid oxidation induced by early post-natal overfeeding, and the effects of PPARα activation in lipid oxidation, we have hypothesized that increase post-natal PPARα activation, by an agonist, would prevent the development of metabolic dysfunction and obesity induced by post-natal overfeeding.

METHODS

Experimental design and treatment

Wistar rats were obtained from the Maringá State University Central Animals Facility and housed in the Animal Facility of the Experimental Laboratory of DOHaD (LExDOHaD), kept under controlled condition of temperature (22±2ºC), and photoperiod (12 hours dark-light cycle). Animals had ad libitum access to standard chow and water throughout the experimental period. Birth was considered postnatal-day zero (PN0). At PN 1 all litters were standardized for nine pups per mother, maintaining the proportion of five-to-six males and four-to-three females in the NL group. Fenofibrate (Infinity Pharma, COD: 1454, LT: 20F15-B030-063706) was dissolved in 10% DMSO (Sigma, D2650) and 15% kolliphor HS 15 (Sigma, 42 966). At PN1 litters were assigned to Vehicle (V - 15% Koliphor and 10%DMSO in distilled water) or Treated (F – 12.5mg/kg in vehicle) by intraperitoneal injections from PN1 until weaning, PN21 [21]. At PN3 the number of pups was adjusted to three males per dam for small litters (SL). Forming the experimental groups with the following number of litters: NL-V (n=10), NL-F (n=10), SL-V (n=10) and SL-F (n=11). Considering the sexual dimorphism, since females do not develop marked insulin resistance and non-hepatic steatosis (NAFLD) when reared in SL [22], only males were used in this study for assessments during lactation and after weaning. During lactation biometric parameters were measured using a pachymeter, whereas

glucose and β-hydroxybutyrate levels were assessed using a glucometer (FreeStyle Optimum®, Abbot). After weaning PN21, animals were housed at three per cage until PN119, when experimental procedures and euthanasia for tissue collection were performed. Food intake and bodyweight (BW) gain were measured weekly during this period. All the animal handling, housing and experimental procedures followed the guidelines of the Brazilian National Council for Control of Animal Experimentation (CONCEA) and were approved by the Ethic Commission in The Use of Animals (CEUA) from State University of Maringá, Brazil (Protocol Number 8934110422).

Indirect calorimetry and sympathetic nerve activity

From each group, a separated set of PN119 were used for indirect calorimetry and nerve register. O_2 consumption and CO_2 production were assessed for 24 hours (Oxylet system; Pan Lab/Harvard Instruments, Barcelona, Spain). 24 hours energy expenditure (EE) and respiratory quotient (RQ) were then calculated using the O_2 and CO_2 data by Metabolism® Software (Pan Lab/Harvard Instruments) [23]. During the procedures rats had *ad libtum* access to food and water.

For sympathetic nerve register, animals were anesthetized using Sodium Thiopental (45 mg⁄kg of body weight) after overnight fasting (± 12hous). A Sympathetic Nerve (SNS) branch innervating brown adipose tissue (BAT) was surgically exposed and placed over a pair of platinum recording electrodes. Recordings were made with a Bio-Amplificator (Insight®, Ribeirão Preto, Brazil) in the 1–80 kHz range and amplified 10000-fold, as previously described [24]. The nerve activity was recorded for 10 min, and the average numbers of spikes/5 s were used to calculate the nerve firing rate from five to seven sections of 15s recordings for each rat.

Insulin sensitivity, tissue collection and biochemical analyzes

From each group, a separated set of PN119 were submitted to intra-peritoneal insulin tolerance test (ipITT). Animals were fasted for 6 hours and glycemia was measured (0’ time), then animals received an intraperitoneal injection of insulin (1U/kg – NovoRapid®,), and glycemia was measured at 15’, 30’ and 60 minutes using a glucometer (FreeStyle Optimum®, Abbot) as previously described [25]. The plasma glucose disappearance rate was calculated (K_{itt}).

After 48 hours of recovery, following an overnight fasting, animals were euthanized by decapitation for tissue and blood sample collection. Blood was centrifuged (10000 RPM, 5 min), plasma was collected and stored at -20°C. Plasmatic concentrations of glucose, total cholesterol, and triglycerides were quantified by spectrophotometry in a microplate reader (Kasuaki®), using commercial kits by following manufacturer's instructions (Gold Analise®, Belo Horizonte/MG, Brazil). Samples from retroperitoneal, liver, BAT and hypothalamus were quickly frozen in liquid nitrogen and then stored in -80°C for total lipid and molecular biology analyzes, or fixed in 10% formalin for histological procedures.

RNA isolation and real-time quantitative RT-qPCR

Liver and hypothalamus samples previously stored at -80°C had their RNA isolated using TRIzol™ (Invitrogen®) following manufacturer's instructions. Total RNA concentration was measured spectrophotometrically at 260 nm (NanoDrop ND 1000 NanoDrop Technologies, Wilmington, DE). Total RNA was used for reverse transcription followed by real-time quantitative PCR (RT-qPCR). For cDNA synthesis 2 µg of RNA was reverse transcribed by the GoScript Reverse Transcription System (Promega, Madison, USA), and the quantitation of the tissue expression of selected genes (Supplementary information, Table 2) was done by quantitative PCR in a Corbett Rotor-Gene 6000® (Qiagen) with GoTaq® qPCR Master Mix (Promega). β-actin was used as housekeeping gene. The $2^{-\Delta CT}$ method was used for the relative quantification analysis and data were expressed in arbitrary units (AU) [26].

Western blotting

Tissue samples of BAT were homogenized in a RIPA lysis buffer (25 mM Tris/HCl pH 7.6, 150mM NaCl, EDTA 5mM, 1% Triton x100, sodium deoxycolate 1%, SDS 1%, 1Mm PMSF, sodium orthovanadate 1mM, pyrophasphate 2mM, sodium fluoride 10mM, EGTA 1mM and Aprotinin 2µg/mL) and centrifuged two times 12.000×g for 15 min. The pellet was discarded and the supernatant was used. Extracted sample total protein was determined according using Pierce™ BCA Protein Assay Kit (Thermofischer). Samples containing 20 µg protein were combined with Laemmli sample buffer (50 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, 0.025% bromophenol blue, 5%

β-mercaptoethanol), heated at 98°C for 5 minutes for protein denaturation, applied on 10% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), and transferred to PVDF membranes for one-hour 100V. These membranes were blocked in a Tris-buffered saline (TBS-T - 10 mM Tris base, 150 mM NaCl and 0.25% (v/v) of Tween 20) containing 5% (w/v) non-fat dried milk for 1h at room temperature. Afterwards, membranes were incubated overnight at 4°C with primary antibodies (anti-UCP1 - U6382, Sigma Aldrich; anti-β-actin, Santa Cruz Biotechnology cat. sc-47778), all of them were diluted 1:1000. Afterwards washed in TBS-T and incubated with a whole molecule peroxidase-conjugated secondary antibody (Anti-Goat, Sigma Aldrich, A5420, diluted 1:5000) for two hours and then washed in TBS-T. Band detection was performed by chemiluminescence (Amersham™ ECL Prime Western Blotting Detection Reagent, GE Healthcare, Chicago, IL, USA) and the bands were visualized using the ImageQuant LAS 500® (GE Healthcare Life Sciences, Chicago, IL, USA). Band intensities were analyzed using ImageJ software (National Institute of Health, Maryland, USA). β-actin was used as a housekeeping protein.

Liver lipid Extraction

Total lipid content in the liver was determined using the gravimetric method adapted from Folch et al. (1957) [27]. Briefly, liver samples (150mg) were homogenized in a chloroform-methanol mixture (2:1). The result referring to the content of total lipids was expressed in percentage (g/100g of wet liver weight). Liver triglycerides were determined by spectrophotometry in the total lipid extracted diluted in isopropyl alcohol, using commercial kit as previously described in the biochemical analyzes section of this paper.

Histology

Liver, BAT and VAT (Visceral Adipose Tissue - retroperitoneal) tissues were fixed in formalin (40% formaldehyde in PBS, pH 6.8) and embedded in paraffin to non-serial sections of 5 μm. Three non-serial sections per animal were placed in glass slides and stained in hematoxylin/eosin. The morphological study was performed utilizing digital images, acquired at random (TIFF format, 36-bit color, 1360x1024 pixels) with an CMOS 12.0 MP camera (Bioptika) and a microscopy Axioscop (Zeiss). For WAT adipocyte diameter, the area of all adipocytes in 10 different pictures of the sections were measured by circulating adipocytes with free hand tool using ImageJ software (National

Institute of Health, Maryland, USA), and calculating the total area of the adipocyte after calibration with a ruler. For liver, 10 photos of 3 different sections per animal was used, where microsteatosis quantification was measured by point counting in a system consisting of 36 test points (Pt), were the density of micro steatosis was (Vv) was estimated as the ratio of points hitting microsteatotic vesicles (Pp), Vv [steatosis, liver] = Pp[steatosis]/Pt, as previously described [28; 29]. For BAT percentual lipid area 10 photos of 3 different sections per animal used. Images were converted to black and white using and image analyzes software (Image J), and a threshold (Shanbhag) was applied in order to highlight the white vacuoles from lipids, and then the white are was measured, using the tool "particles analyze", and divided from the total area of the picture.

Oxidative stress

Liver and VAT tissue were collected to evaluate oxidative stress using thiobarbituric acid reactive substance assay (TBARS) and reduced glutathione (GSH) assays (#set4). Thus, Liver and white adipose tissue liver tissues were prepared for a GSH and TBARS reactive species quantification assay, as previously described [30; 31].

Statistical analyzes

Data analyze was performed using GraphPad-Prism® Software version 9.00 for Windows (GraphPad Software Inc., La Jolla California USA, www.graphpad.com). Results were presented as mean ± standard error of the mean (SEM). The data were analyzed through, two-way ANOVA and post-test of Tukey, considering the factors Litter (L); Treatment (T) and the Interaction (I) between them. For the correlation analyze, a "simple linear regression" was used. Values of $p<0.05$ were considered as the significance level.

RESULTS

Post-natal PPARα agonist mitigate the development of overweight, hypertriglyceridemia and insulin resistance caused by early overfeeding

Table 1 shows the effects of litter reduction (L) and post-natal PPARα agonist treatment (T) on biometric and biochemical parameters. Litter reduction increased

bodyweight gain (p_L<0.05) and final bodyweight (p_L<0.001) in SL-V, and post-natal PPARα agonist was able to prevent both, bodyweight gain (p_T<0.05) and final bodyweight (p_T<0.05), particularly in SL-F animals since an interaction between factor was observed (p_I>0.05). Food intake during the period (AUC PN28-119, p_L<0.01) and final food intake (p_L<0.01) were increased in SL rats. Regarding to biochemical parameters, it was observed a significant interaction (p_I>0.05) on triglyceride levels, demonstrating that post-natal PPARα agonist was able to attenuate these parameters on SL-F animals. The analysis of K_{itt} shows that the treatment was also able to attenuate insulin resistance in SL-F animals (p_I>0.01).

Post-natal PPARα agonist reduce visceral obesity and oxidative damage on visceral adipose tissue

Litter reduction increased perigonadal (Figure 1b, p_L<0.001), mesenteric (Figure 1c, p_L<0.001) and retroperitoneal (Figure 1d, p_L<0.01) fat mass, which was prevented by PPARα agonist treatment in SL-F animals (Figure 1b, p_I>0.001; Figure 1c, p_I>0.001; Figure 1d, p_I>0.01, respectively). In VAT oxidative stress a significant interaction was observed (Figure 1h, p_I>0.01), demonstrating that PPARα agonist was able to attenuate oxidative damage tissue of SL-F animals. Early overfeeding leads to adipose tissue remodeling towards a trend to increase in median VAT adipocyte diameter (Figure 1e, p_L=0.06), and increased frequency of larger adipocytes (Figure 1f).

Post-natal PPARα agonist prevent liver triglycerides accumulation and microsteatosis

Post-natal overfeeding increased liver mass **(**Figure 1i, p_L<0.05) and showed a trend towards increasing FAS expression **(**Figure 1j, p_L=0.07). Post-natal PPARα agonist was able to reduce the percentual of total lipids (data not shown) and triglycerides (Figure 1k, p_I>0.01), in the liver of SL-F rodents. Regarding to oxidative stress, it was observed a trend towards increased oxidative damage by litter reduction (Figure 1m, p_L=0.1). Post-natal overfeeding increased FGF21 expression (Figure 1n, p_L<0.05) which was reduced in SL-F animals (Figure 1o, p_I>0.05) and positive correlated with oxidative damage in the liver (Figure 1n, r=0.4721, p=0.01). Morphological analyzes revealed increased microsteatosis by post-natal overfeeding (Figure 1l, p_L=0.05), which was abolished by early exposure to PPARα agonist **(**Figure 1l, p_I>0.01). The evaluation of inflammatory

markers showed that treatment was able to reduce the expression of the inflammatory transcription factor NFKβ (Figure 1q, $p_T>0.01$).

Post-natal PPARα agonist prevent BAT low sympathetic nervous system activity and hypothalamic expression of FGFR1

Litter reduction increased BAT mass **(**Figure 3a, $p_L<0.05$) and reduced 24 hours total EE (Figure 3d, $p_L<0.05$). A significant interaction was observed between factors indicating an effect of agonist in preventing alterations in lipid droplet area in the BAT (Figure 3x, $p_I>0.05$). Sympathetic nerve activity showed a significant interaction (Figure 3f, $p_I>0.01$), indicating that post-natal PPARα agonist was able to increase sympathetic activity in SL-F animals accompanied by upregulated FGFR1 expression in the hypothalamus (Figure 3g, $p_I>0.05$).

DISCUSSION

Litter reduction is a well-recognized animal model for childhood obesity and metabolic dysfunction [15; 22; 32]. As expected, in the present study early post-natal overfeeding lead to obesity at weaning, and at adulthood increased visceral obesity and insulin resistance, liver microsteatosis, accompanied by upregulation of FGF21 expression and reduced BAT sympathetic nervous activity. The concomitant PPARα agonist administration during lactation was able to prevent the development of obesity, normalized sympathetic nervous activity, insulin resistance and liver steatosis, caused by litter reduction.

Previous studies have shown that the development of metabolic dysfunction in SL would be related to increased accumulation of diacylglycerol (DAG) within the liver [22]. DAG have been proposed as mechanism linking liver steatosis to oxidative stress and insulin resistance [33; 34]. In the liver DAG is formed by a reaction catalyzed by the enzyme MOGAT1, which uses monoacylglycerol as a precursor, catalyzes the binding of an acyl group to the intermediary, forming DAG as product[33]. It was shown that high DAG levels activate PKC, a phosphokinase, once activated PKC inhibit the phosphorylation of IRS-1, an important factor in the insulin intracellular signaling cascade, therefore promoting insulin resistance in the cell [33; 34]. Also, it was shown that PKC is able to activate the enzyme NADPH oxidase, thus leading to reactive oxygen species formation, oxidative stress and, consequently, local inflammation [33; 34]. Despite not showing marked inflammation, post-natal overfeeding has been reported to

leads to oxidative stress within the liver, which corroborates to our findings towards an increased trend for oxidative damage in this tissue [29; 32]. Interestingly, previous studies have shown that SL have increased expression of MOGAT1 and DAG during lactation, which persists until adulthood, mechanistic linking it to the development of hepatic steatosis and insulin resistance later in life, corroborating our findings [22].

Litter reduction leads to alterations in breastmilk composition, markedly increased fat content which is accompanied by increased total milk intake to pups, therefore increasing fat intake by pups during breastfeeding [35]. This increased flux of FFA from milk would increase hepatic triacylglycerol and DAG in the liver early in life[22]. The intracellular metabolism and oxidation, particularly in the liver is dependent of the transcription factor PPARα which is activated by lipids and which increases the expression of key enzymes in fatty acids oxidation such as CPT1a, ACOX1 and ACADM [16]. We have observed that post-natal fenofibrate administration prevented several disturbances relate to lipid metabolism such as visceral obesity, dyslipidemia, liver steatosis and insulins resistance. Fenofibrate is a well-known PPARα agonist, which effects in promoting lipid oxidation is stablished [16; 36]. Previous studies with animal model of glycogen storage disease type Ia, which leads to severe hepatic steatosis during lactation, have shown that fenofibrate treatment during five days was able to significantly reduce hepatic steatosis by increasing mitochondrial fatty acid oxidation since it was observed decreased levels of plasmatic acyl-carnitine and increased expression of genes related to lipid oxidation in the liver [21]. Therefore, despite not being addressed in our study, its plausible to hypothesize that fenofibrate may have prevented hepatic steatosis in the neonatal liver in a similar mechanism, which in turn would prevent lipid accumulation and insulin resistance at adulthood.

We have observed that SL-V animals had increased liver FGF21 expression, which was prevent by PPARα agonist. Clinical and pre-clinical studies have shown that hepatic steatosis is accompanied by upregulated expression of FGF21 with elevated FGF21 bloodstream levels. FGF21 is an hepatokyne secreted mainly by the liver in stressful conditions, having a particular influence to the serum levels of the hormone, but also by several tissues, such as muscle, white adipose tissue, BAT and pancreas [37]. We have observed that post-natal overfeeding leads to steatosis and a trend towards oxidative damage, which in turn may be triggering FGF21 expression. Increased expression of FGF21 have protective effects in metabolism, by promoting increased insulin sensitivity,

decreasing inflammation, increasing browning in VAT and BAT thermogenesis and fatty acid oxidation [38; 39].

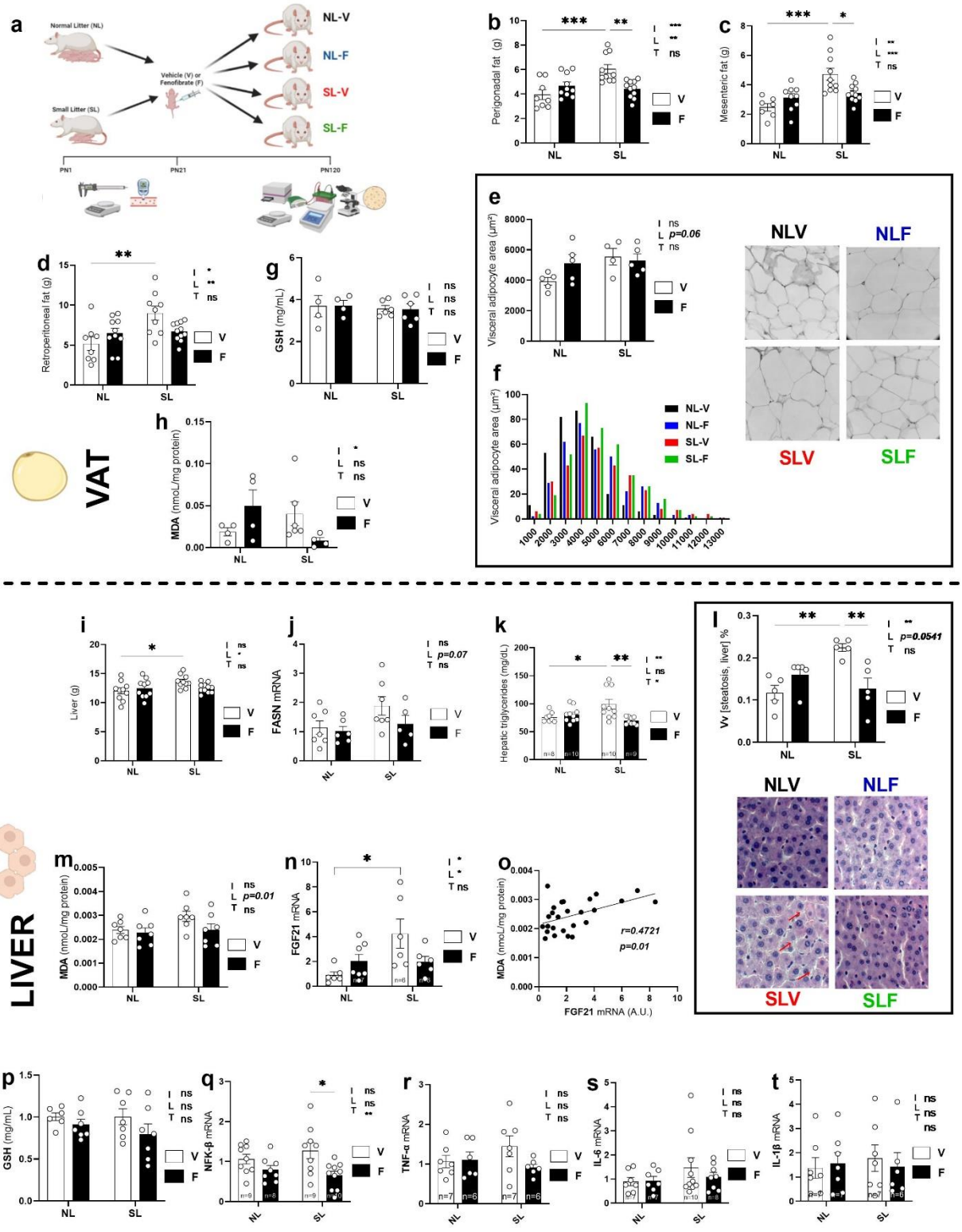
Similarly to our findings, despite having increased FGF21 expression, different models of obesity and hepatic steatosis are not protected against the development of obesity and insulin resistance [40]. FGF21 is a polypeptide that binds to a membrane receptor, FGFR1, and its co-receptor β-klotho. therefore, it has been proposed that obesity and hepatic steatosis reassembles a FGF21 resistant state, where the hormone is not able to promoted their effects within the cells [41; 42]. Previous studies have shown that diet induced obesity leads to an FGF21 resistant state, by decreased expression of FGFR1 in peripheral tissues such as liver and VAT, however, in that model a causal relationship between FGF21 and obesity was not established since mice knockout for *Fgf21* did not develop increased obesity when fed a high-fat diet [41]. In post-natal overfed animals, increased metabolic and oxidative stress within the liver may be triggering increased FGF21 expression, which is prevented by PPARα agonist, however, since it is observed a worse metabolic function, it is possible to conjecture an FGF21 resistant state.

Post-natal overfeeding leads to decreased sympathetic nervous activity which was accompanied by decreased FGFR1 expression in the hypothalamus. Beyond their peripheral effects, FGF21 was shown to have central effects in the hypothalamus, by promoting increased sympathetic nervous system activity and thermogenesis in BAT [43; 44]. Regarding epigenetic programming, some studies have found a link between FGF21 expression and protection against the development of obesity and metabolic dysfunction later in life. Metabolic programming for disease during the critical phases of development have been proposed as an important factor for disease development later in life [4; 5]. In fact, several studies relate these effects by alterations in gene expression modulated by epigenetic marks, such as DNA methylation, histone modification and mi-RNAs [45; 46]. Maternal administration of an PPARα agonist perinatally, was able to confer protection against diet-induced obesity and metabolic dysfunction later in life, which was attributed for increased FGF21 expression, being observed a reduced degree of repressive methylation on *Fgf21* promoter gene, which persisted until adulthood [19]. In our study, an increased PPARα activity induced by directly administration of an agonist to the pup was able to prevent the long-term metabolic dysfunction induced by post-natal overfeeding, despite also preventing increased FGF21 expression, showing that the protective effect may be related to a different mechanism. An animal study has found that

delayed weaning was able to confer this protection by increasing FGF21 levels in the offspring when fed HFD [47]. Also, it was found that the hypothalamic signaling via FGF21-*d2r*-expressing GABAergic neurons in the hypothalamus, was necessary to the observed effect [47]. We have observed that SL animals shows decreased sympathetic nervous activity which was accompanied by decreased FGFR1 expression in the hypothalamus, therefore, despite the increased FGF21 expression in the liver, which in turn would be associated with increased sympathetic activity, animals show decreased activity, which could be cause by a central resistance to FGF21 effects.

Our study has several limitations since we were not able to measure the effect of treatment on liver DAG levels or PKC activation, and p-ERK, or the peripherical expression of FGFR1 in tissues such as BAT, VAT and liver. Or to test the central FGF21 resistance through and intracerebroventricular injection of an analogue. Despite these limitations, as of our knowledge, for the first time it was shown the relationship of the FGF21-FGFR1- sympathetic axis in an animal model of early post-natal obesity. Also, we have shown that post-natal increased PPARα activity, induced by an agonist, may prevent the insulin resistance and hepatic steatosis long-term programmed by post-natal overfeeding. Finally, we conclude that post-natal overfeeding dysregulated FGF21-FGFR1-sympathetic system, leads to obesity, insulin resistance and hepatic steatosis, that is prevented by increased PPARα activation during lactation. Therefore, there's an open field of studies for interventions focusing in increasing PPARα activity during lactation, such as exercise and natural compounds, in order to prevent or attenuate the degree of obesity and metabolic dysfunction later in life.

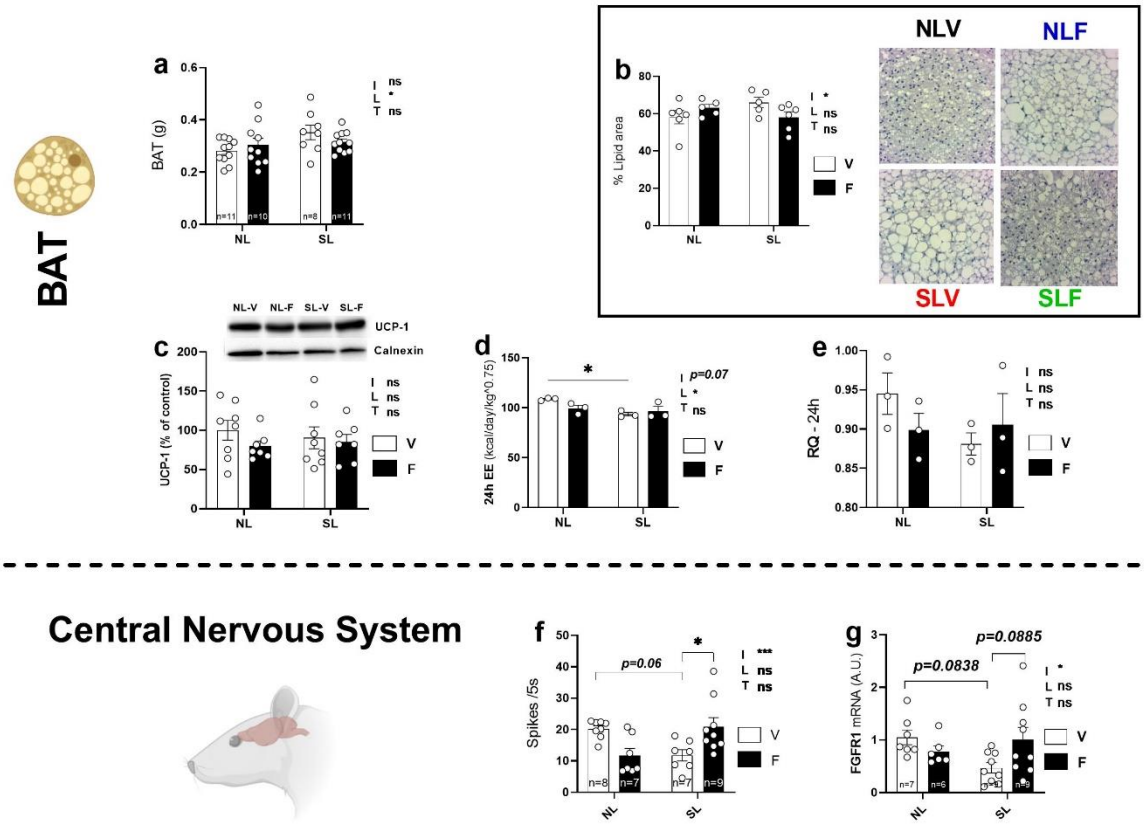
449 **Figures**



450

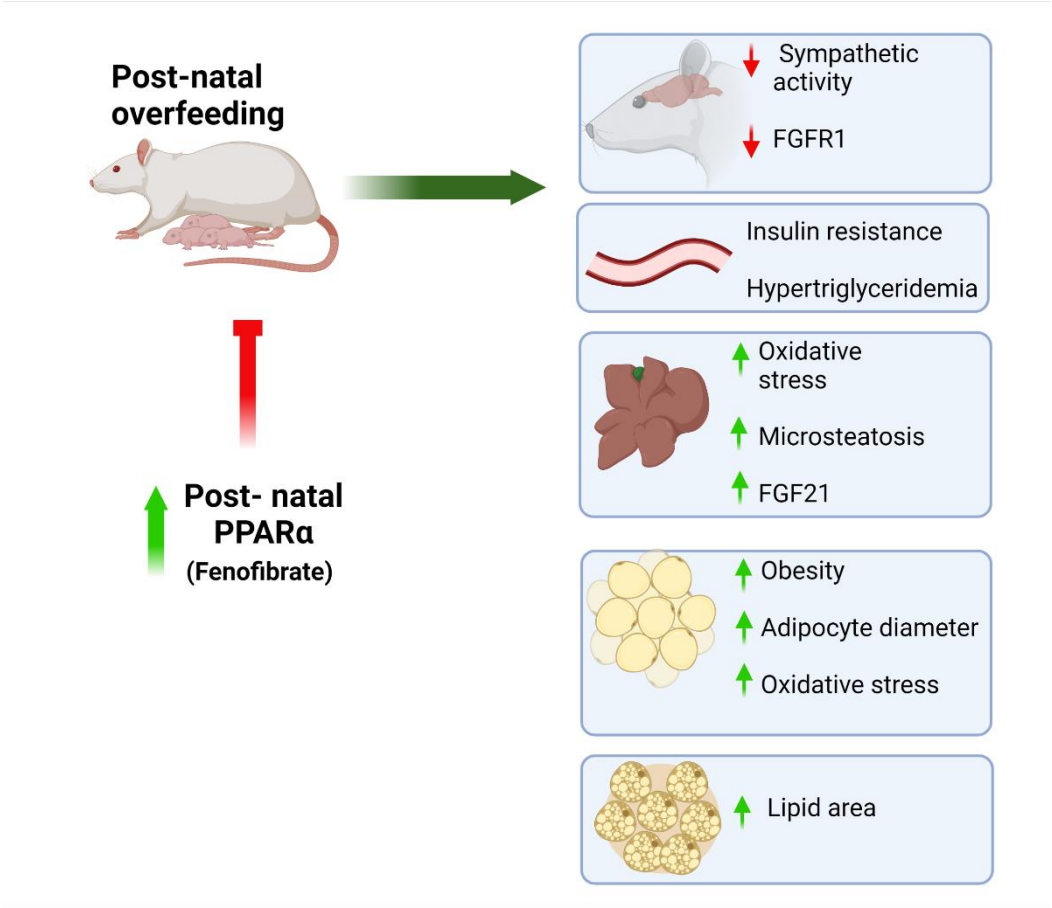
451 **Figure 1: (a)** Schematic representation of the experimental protocol, were normal (NL)
452 and reduced litters (SL) were submitted from PN1 to PN21 to intraperitoneal
453 administrations with either, a Vehicle (V) or PPARα agonist (F - fenofibrate) and then

their tissue collection and experimental procedures at adulthood (±PN120). **(b)** Perigonadal fat mass (NL-V n=8; SL-V n=10; NL-F n=10; SL-F n=11). **(c)** Mesenteric fat mass (NL-V n=7; SL-V n=10; NL-F n=9; SL-F n=11). **(d)** Retroperitoneal fat mass (NL-V n=8; SL-V n=9; NL-F n=10; SL-F n=11). **(e)** Visceral retroperitoneal adipocyte area (NL-V n=5; SL-V n=4; NL-F n=5; SL-F n=5). **(f)** Distribution of adipocytes stratified by adipocyte diameter of visceral retroperitoneal fat area (NL-V n=5; SL-V n=4; NL-F n=5; SL-F n=5). **(g)** Glutathione (GSH) levels in the visceral retroperitoneal tissue (NL-V n=4; SL-V n=6; NL-F n=4; SL-F n=6). **(h)** Malondialdehyde (MDA) levels in the visceral retroperitoneal tissue (NL-V n=4; SL-V n=6; NL-F n=4; SL-F n=4). **(i)** Liver mass (NL-V n=10; SL-V n=10; NL-F n=10; SL-F n=11). **(j)** mRNA expression levels of Fatty acid synthase (FAS) (NL-V n=7; SL-V n=7; NL-F n=6; SL-F n=5). **(k)** Hepatic triacylglycerol content (NL-V n=8; SL-V n=10; NL-F n=10; SL-F n=9). **(l)** Microsteatosis level (Vv) in the liver (NL-V n=5; SL-V n=5; NL-F n=5; SL-F n=5). **(m)** MDA levels in the liver (NL-V n=7; SL-V n=7; NL-F n=7; SL-F n=7). **(n)** mRNA expression of FGF21 (NL-V n=6; SL-V n=6; NL-F n=7; SL-F n=6). **(o)** Correlation through simple linear regression of FGF21 and MDA. **(p)** GSH levels in the liver (NL-V n=6; SL-V n=7; NL-F n=7; SL-F n=7). mRNA expression of NFK-β (NL-V n=9; SL-V n=9; NL-F n=8; SL-F n=10) **(q)**, TNF-α (NL-V n=7; SL-V n=7; NL-F n=6; SL-F n=6) **(r)**, IL-6 (NL-V n=7; SL-V n=8; NL-F n=7; SL-F n=8) **(s)** and IL-1β (NL-V n=7; SL-V n=7; NL-F n=7; SL-F n=5) **(t)**. Data are presented as mean ± SEM. To compare the experimental groups two-way ANOVA and post-test of Tukey were used.



481

482 **Figure 2:** (a) BAT mass (NL-V n=11; SL-V n=8; NL-F n=10; SL-F n=11). (b) Percentual
483 of lipid area in BAT (NL-V n=6; SL-V n=5; NL-F n=5; SL-F n=6). (c) Western blotting
484 of UCP-1 in BAT (NL-V n=8; SL-V n=8; NL-F n=7; SL-F n=8). (d) 24 hours animals'
485 EE (NL-V n=3; SL-V n=3; NL-F n=3; SL-F n=3). (e) Respiratory quotient during 24
486 hours in animals (NL-V n=3; SL-V n=3; NL-F n=3; SL-F n=3). (f) Sympathetic nerve
487 activity of BAT (NL-V n=8; SL-V n=7; NL-F n=7; SL-F n=9). (g) mRNA expression of
488 FGFR1 in the hypothalamus (NL-V n=7; SL-V n=9; NL-F n=6; SL-F n=9). Data are
489 presented as mean ± SEM. To compare the experimental groups two-way ANOVA and
490 post-test of Tukey were used.



Graphical Abstract. Post-natal overfeeding decreased BAT nerve sympathetic activity and hypothalamic expression of FGFR1, despite increased hepatic FGF21 levels, thus characterizing a central resistance to FGF21. Also, it was observed that litter reduction promoted insulin resistance, liver oxidarive stress and microsteatosis, with increased visceral adiposity, adipocyte diameter and oxidative stress in white visceral adipose tissue. Increased lipid area was observed in brown adipose tissue. Also, it was observed that increase PPARα activation during lactation was able to prevent or attenuate obesity and autonomic and metabolic dysfunction induced by post-natal overfeeding during adulthood. Created with BioRender.com.

Acknowledgements: We would like acknowledge all the collaborators from the Experimental Laboratory of DOHaD (LexDOHaD). Also, we would like to acknowledge Dr. Maria Aparecida Fernandez for the use of her laboratory facilities. We would like to thank the Brazilian Funding Agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES). And to JBS, “Fazer o bem faz bem”.

REFERENCES

[1] WHO, 2021. Obesity and overweight: Fact Sheet.

[2] WHO, 2021. Diabetes: Fact Sheet.

[3] WHO, 2021. Cardiovascular diseases (CVDs): Fact Sheet.

[4] Almeida, D.L., Pavanello, A., Saavedra, L.P., Pereira, T.S., de Castro-Prado, M.A.A., de Freitas Mathias, P.C., 2019. Environmental monitoring and the developmental origins of health and disease. J Dev Orig Health Dis 10(6):608-615.

[5] Hsu, C.N., Hou, C.Y., Hsu, W.H., Tain, Y.L., 2021. Early-Life Origins of Metabolic Syndrome: Mechanisms and Preventive Aspects. Int J Mol Sci 22(21).

[6] Pico, C., Reis, F., Egas, C., Mathias, P., Matafome, P., 2021. Lactation as a programming window for metabolic syndrome. Eur J Clin Invest 51(5):e13482.

[7] Lisboa, P.C., Miranda, R.A., Souza, L.L., Moura, E.G., 2021. Can breastfeeding affect the rest of our life? Neuropharmacology 200:108821.

[8] Victora, C.G., Bahl, R., Barros, A.J., Franca, G.V., Horton, S., Krasevec, J., et al., 2016. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 387(10017):475-490.

[9] Martins, I.P., de Oliveira, J.C., Pavanello, A., Matiusso, C.C.I., Previate, C., Tofolo, L.P., et al., 2018. Protein-restriction diet during the suckling phase programs rat metabolism against obesity and insulin resistance exacerbation induced by a high-fat diet in adulthood. J Nutr Biochem 57:153-161.

[10] Song, L., Cui, J., Wang, N., Wang, R., Yan, J., Sun, B., 2020. Maternal exercise during gestation and lactation decreases high-fat diet preference by altering central reward system gene expression in adult female offspring from high-fat fed dams. Behav Brain Res 390:112660.

[11] Francisco, F.A., Saavedra, L.P.J., Junior, M.D.F., Barra, C., Matafome, P., Mathias, P.C.F., et al., 2021. Early AGEing and metabolic diseases: is perinatal exposure to glycotoxins programming for adult-life metabolic syndrome? Nutr Rev 79(1):13-24.

[12] Miranda, R.A., Gaspar de Moura, E., Lisboa, P.C., 2020. Tobacco smoking during breastfeeding increases the risk of developing metabolic syndrome in adulthood: Lessons from experimental models. Food Chem Toxicol 144:111623.

[13] Grilo, L.F., Tocantins, C., Diniz, M.S., Gomes, R.M., Oliveira, P.J., Matafome, P., et al., 2021. Metabolic Disease Programming: From Mitochondria to Epigenetics, Glucocorticoid Signalling and Beyond. Eur J Clin Invest 51(10):e13625.

[14] Souza, L.L., Moura, E.G., Lisboa, P.C., 2022. Litter Size Reduction as a Model of Overfeeding during Lactation and Its Consequences for the Development of Metabolic Diseases in the Offspring. Nutrients 14(10).

[15] Parra-Vargas, M., Ramon-Krauel, M., Lerin, C., Jimenez-Chillaron, J.C., 2020. Size Does Matter: Litter Size Strongly Determines Adult Metabolism in Rodents. Cell Metab 32(3):334-340.

[16] Rakhshandehroo, M., Knoch, B., Muller, M., Kersten, S., 2010. Peroxisome proliferator-activated receptor alpha target genes. PPAR Res 2010.

[17] Hashimoto, K., Ogawa, Y., 2018. Epigenetic Switching and Neonatal Nutritional Environment. Adv Exp Med Biol 1012:19-25.

[18] Ehara, T., Kamei, Y., Yuan, X., Takahashi, M., Kanai, S., Tamura, E., et al., 2015. Ligand-activated PPARalpha-dependent DNA demethylation regulates the fatty acid beta-oxidation genes in the postnatal liver. Diabetes 64(3):775-784.

[19] Yuan, X., Tsujimoto, K., Hashimoto, K., Kawahori, K., Hanzawa, N., Hamaguchi, M., et al., 2018. Epigenetic modulation of Fgf21 in the perinatal mouse liver ameliorates diet-induced obesity in adulthood. Nat Commun 9(1):636.

[20] Abdelmoneim, D., El-Adl, M., El-Sayed, G., El-Sherbini, E.S., 2021. Protective effect of fenofibrate against high-fat-high-fructose diet induced non-obese NAFLD in rats. Fundam Clin Pharmacol 35(2):379-388.

[21] Yavarow, Z.A., Kang, H.R., Waskowicz, L.R., Bay, B.H., Young, S.P., Yen, P.M., et al., 2020. Fenofibrate rapidly decreases hepatic lipid and glycogen storage in neonatal mice with glycogen storage disease type Ia. Hum Mol Genet 29(2):286-294.

[22] Ramon-Krauel, M., Pentinat, T., Bloks, V.W., Cebria, J., Ribo, S., Perez-Wienese, R., et al., 2018. Epigenetic programming at the Mogat1 locus may link neonatal overnutrition with long-term hepatic steatosis and insulin resistance. FASEB J:fj201700717RR.

[23] Branco, R.C.S., Camargo, R.L., Batista, T.M., Vettorazzi, J.F., Lubaczeuski, C., Bomfim, L.H.M., et al., 2019. Protein malnutrition mitigates the effects of a high-fat diet on glucose homeostasis in mice. J Cell Physiol 234(5):6313-6323.

[24] Almeida, D.L., Moreira, V.M., Cardoso, L.E., Junior, M.D.F., Pavanelo, A., Ribeiro, T.A., et al., 2022. Lean in one way, in obesity another: effects of moderate exercise in brown adipose tissue of early overfed male Wistar rats. Int J Obes (Lond) 46(1):137-143.

[25] Martins, I.P., Vargas, R., Saavedra, L.P.J., Rickli, S., Matiusso, C.C.I., Pavanello, A., et al., 2022. Protein-caloric restriction induced HPA axis activation and altered the milk composition imprint metabolism of weaned rat offspring. Nutrition 108:111945.

[26] Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402-408.

[27] Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226(1):497-509.

[28] Catta-Preta, M., Mendonca, L.S., Fraulob-Aquino, J., Aguila, M.B., Mandarim-de-Lacerda, C.A., 2011. A critical analysis of three quantitative methods of assessment of hepatic steatosis in liver biopsies. Virchows Arch 459(5):477-485.

[29] Conceicao, E.P., Moura, E.G., Soares, P.N., Ai, X.X., Figueiredo, M.S., Oliveira, E., et al., 2016. High calcium diet improves the liver oxidative stress and microsteatosis in adult obese rats that were overfed during lactation. Food Chem Toxicol 92:245-255.

[30] Suvitha Syam, A.B., Najihah Mohd Hashim, Mostafa Ghaderian, Yahya Hasan Hobani, Anwar Makeen, Siddig Ibrahim Abdelwahab, Syam Mohan, 2016. β-Mangostin suppresses LA-7 cells proliferation in vitro and in vivo: Involvement of antioxidant enzyme modulation; suppression of matrix metalloproteinase and α6β4 integrin signalling pathways.

Journal of Functional Foods 22:504-517.

[31] Podratz, P.L., Merlo, E., de Araujo, J.F.P., Ayub, J.G.M., Pereira, A.F.Z., Freitas-Lima, L.C., et al., 2020. Disruption of fertility, placenta, pregnancy outcome, and multigenerational inheritance of hepatic steatosis by organotin exposure from contaminated seafood in rats. Sci Total Environ 723:138000.

[32] Conceicao, E.P., Franco, J.G., Oliveira, E., Resende, A.C., Amaral, T.A., Peixoto-Silva, N., et al., 2013. Oxidative stress programming in a rat model of postnatal early overnutrition--role of insulin resistance. J Nutr Biochem 24(1):81-87.

[33] Erion, D.M., Shulman, G.I., 2010. Diacylglycerol-mediated insulin resistance. Nat Med 16(4):400-402.

[34] Ter Horst, K.W., Gilijamse, P.W., Versteeg, R.I., Ackermans, M.T., Nederveen, A.J., la Fleur, S.E., et al., 2017. Hepatic Diacylglycerol-Associated Protein Kinase Cepsilon Translocation Links Hepatic Steatosis to Hepatic Insulin Resistance in Humans. Cell Rep 19(10):1997-2004.

[35] João Lucas P Xavier 1, D.X.S., Catherine C Pontes 1, Paulo Roberto Ribeiro 1, Maiara M Cordeiro 1, Jessica A Marcondes 1, Felipe O Mendonça 1, Makcine T da Silva 1, Fabio B de Oliveira 1, Gilson C N Franco 1, Sabrina Grassiolli 2, 2019. Litter Size Reduction Induces Metabolic and Histological Adjustments in Dams throughout Lactation with Early Effects on Offspring. Anais da Academia Brasileira de Ciências 91.

[36] Rachid, T.L., Penna-de-Carvalho, A., Bringhenti, I., Aguila, M.B., Mandarim-de-Lacerda, C.A., Souza-Mello, V., 2015. PPAR-alpha agonist elicits metabolically active brown adipocytes and weight loss in diet-induced obese mice. Cell Biochem Funct 33(4):249-256.

[37] Lewis, J.E., Ebling, F.J.P., Samms, R.J., Tsintzas, K., 2019. Going Back to the Biology of FGF21: New Insights. Trends Endocrinol Metab 30(8):491-504.

[38] Kim, K.H., Lee, M.S., 2015. FGF21 as a mediator of adaptive responses to stress and metabolic benefits of anti-diabetic drugs. J Endocrinol 226(1):R1-16.

[39] Geng, L., Lam, K.S.L., Xu, A., 2020. The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic. Nat Rev Endocrinol 16(11):654-667.

[40] Tucker, B., Li, H., Long, X., Rye, K.A., Ong, K.L., 2019. Fibroblast growth factor 21 in non-alcoholic fatty liver disease. Metabolism 101:153994.

[41] Fisher, F.M., Chui, P.C., Antonellis, P.J., Bina, H.A., Kharitonenkov, A., Flier, J.S., et al., 2010. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes 59(11):2781-2789.

[42] Liu, Q., Wang, S., Wei, M., Huang, X., Cheng, Y., Shao, Y., et al., 2019. Improved FGF21 Sensitivity and Restored FGF21 Signaling Pathway in High-Fat Diet/Streptozotocin-Induced Diabetic Rats After Duodenal-Jejunal Bypass and Sleeve Gastrectomy. Front Endocrinol (Lausanne) 10:566.

[43] Douris, N., Stevanovic, D.M., Fisher, F.M., Cisu, T.I., Chee, M.J., Nguyen, N.L., et al., 2015. Central Fibroblast Growth Factor 21 Browns White Fat via Sympathetic Action in Male Mice. Endocrinology 156(7):2470-2481.

[44] Owen, B.M., Ding, X., Morgan, D.A., Coate, K.C., Bookout, A.L., Rahmouni, K., et al., 2014. FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. Cell Metab 20(4):670-677.

[45] Keith M Godfrey, P.M.C., Karen A Lillycrop, 2016. Development, Epigenetics and Metabolic Programming. Nestle Nutr Inst Workshop Ser 85:71-80.

[46] Hanson, P.G.a.M., 2008. Developmental and epigenetic pathways to obesity:

an evolutionary-developmental perspective. International Journal of Obesity 32.

[47] Pena-Leon, V., Folgueira, C., Barja-Fernandez, S., Perez-Lois, R., Da Silva Lima, N., Martin, M., et al., 2022. Prolonged breastfeeding protects from obesity by hypothalamic action of hepatic FGF21. Nat Metab 4(7):901-917.

661

662

663

664

665

666

667

668

669

670

671

672

673

674

675

676

677

678

679

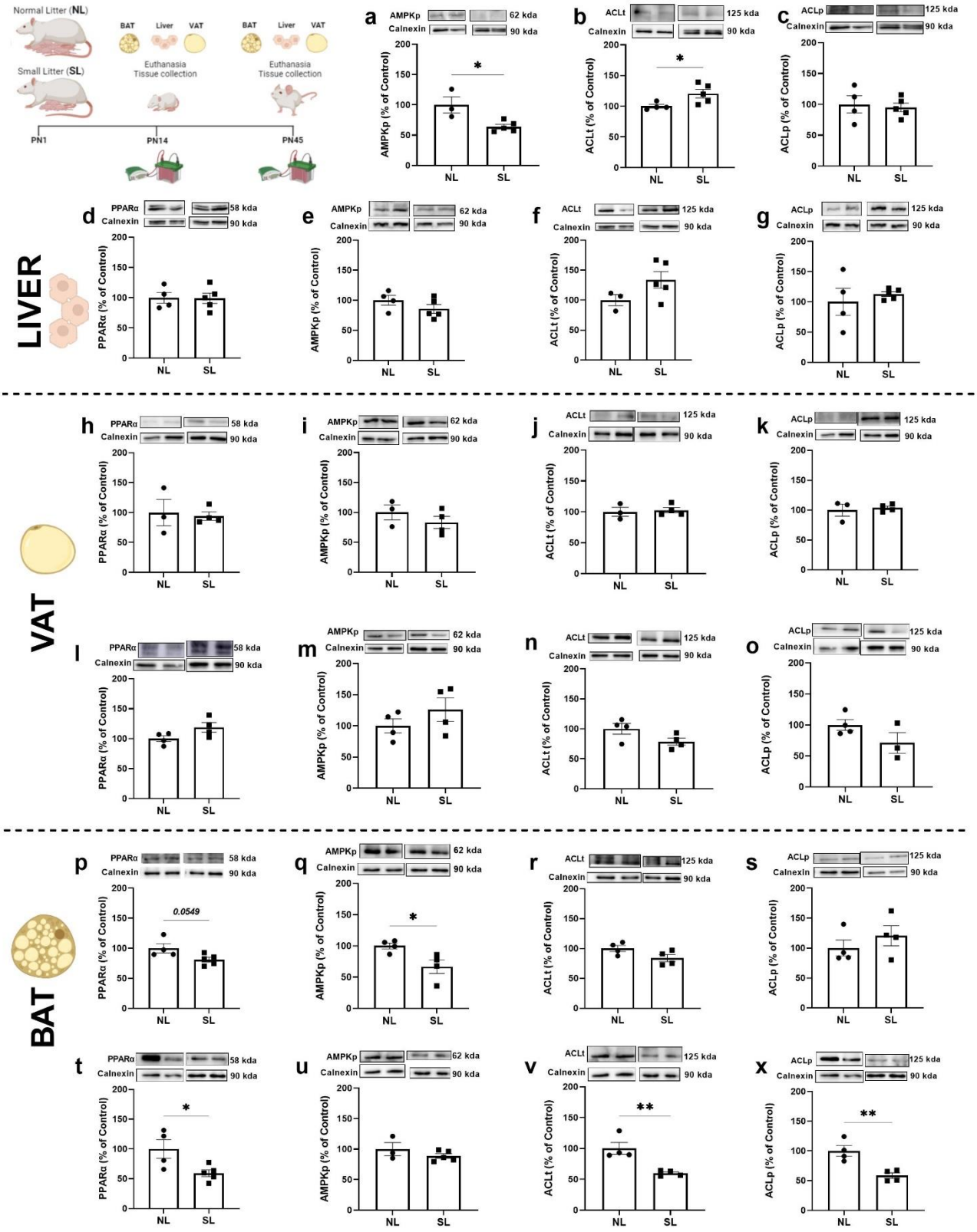
680

Tables

Parameters	Data (mean ± SEM)						
	NL-V	NL-F	SL-V	SL-F	Factors		
					L	T	I
Final bodyweight – PN 119 (g)	416.8 ± 5.7	417.5 ± 8.2	460.9 ± 6.8	432.4 ± 6.2	***	*	*
Bodyweight gain - PN 22-119 (AUC; g)	19978.6 ± 317.2	20122.9 ± 52.4	21779.1 ± 440.5	20079.2 ± 272.8	*	*	*
Final food intake – PN 119 (g)	26.6 ± 1.3	26.7 ± 1.5	31.3 ± 1	28.6 ± 0.5	**	ns	ns
Food intake (AUC; g)	1942.2 ± 84.6	1960.3 ± 107.2	2269.4 ± 52.8	2139.3 ± 56	**	ns	ns
Total Cholesterol (mg/dL)	83.4 ± 4.9	79.6 ± 3.7	84.6 ± 6.3	87.1 ± 5.1	ns	ns	ns
Triglycerides (mg/dL)	102.8 ± 14.4	109.7 ± 13.7	131.5 ± 12.5	88.9 ± 5.5	ns	ns	*
Glucose (mg/dL)	81.4 ± 5.3	80.3 ± 3.1	87.4 ± 3.1	82 ± 2.8	ns	ns	ns
K_{itt} (%/min)	1.1 ± 0.18	0.9 ± 0.08	0.8 ± 0.081	1.1 ± 0.05	ns	ns	**

Table 1. Biometric and biochemical parameters of. Data are presented as mean ± SEM. To compare the experimental groups two-way ANOVA and post-test of Tukey were used.

693 **Supplemental Material**



694

695 **Supplemental information, Figure 1.**

	Data (mean ± SEM)						
Parameters	**NL-V**	**NL-F**	**SL-V**	**SL-F**	**Factors**		
					L	**T**	**I**
Bodyweight gain during lactation – PN1-21 (AUC; g)	358.48 ± 8,4	330.61 ± 8,1	467.87 ± 9,7	411.10 ± 9,9	****	****	ns
Body weight on weaning - PN21 (g)	45,68 ± 1,1	42,25 ± 1,1	57,56 ± 1,0	50,95 ± 1,2	****	****	ns
Abdominal diameter – PN21 (mm)	34.08 ± 0.8	34.80 ± 0.8	38.54 ± 0.5	37.50 ± 1.1	**	ns	ns
Naso-anal length – PN21 (mm)	111.15 ± 1.6	104.76 ± 1.8	115.47 ± 1.6	112.07 ± 1.4	**	**	ns
β-hydroxybutyrate (mmol/L) – PN7	0.90 ± 0.05	0.82 ± 0.06	1.35 ± 0.08	1.42 ± 0.11	****	ns	ns
β-hydroxybutyrate (mmol/L) – PN14	1.23 ± 0.08	0.94 ± 0.09	1.25 ± 0.03	1.32 ± 0.11	*	ns	*
β-hydroxybutyrate (mmol/L) – PN21	0.87 ± 0.6	0.87 ± 0.06	0.78 ± 0.05	0.67 ± 0.06	*	ns	ns
Glucose (mg/dL) – PN7	124.40 ± 4.1	135.20 ± 3.3	135.75 ± 3.2	131.25 ± 3.8	ns	ns	ns
Glucose (mg/dL) – PN14	136.42 ± 5.4	134.20 ± 2.4	142.00 ± 2.1	134.00 ± 2.9	ns	ns	ns
Glucose (mg/dL) – PN21	129.00 ± 2.2	127.28 ± 4.4	131.25 ± 5.3	128.00 ± 4.2	ns	ns	ns

696

697 **Supplemental information, Table 1.**

698

699

700

Gene	5’ → 3’	3’ → 5’	NM_CODE
Fgf21	CCTTGAAGCCAGGGGTCATT	GGATCAAAGTGAGGCGATCC	NM_130752.1
Tnfα	ATGGGCTCCCTCTCATCAGT	GCTTGGTGGTTTGCTACGAC	NM_012675.3
Il-6	CATTCTGTCTCGAGCCCACC	GCTGGAAGTCTCTTGCGGAG	NM_012589.2
IL-1β	GCTTCCTTGTGCAAGTGTCT	TCTGGACAGCCCAAGTCAAG	NM_031512.2
Fgfr1	AGCTGCCAAGACGGTGAAAT	AGTGGCGTAACGAACCTTGT	NM_024146.1
Fasn	ACCTGTGGAATTCCCGGTTC	ACTCGGAACTGGCGTCAATG	NM_017332.2

701

702 **Supplemental information, Table 2.**