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FLÁVIO ANDRADE FRANCISCO

THE EARLY GLYCOTOXIN EXPOSURE AND METABOLIC PROGRAMMING: INFLAMMATION, OXIDATIVE STRESS AND METABOLIC DYSFUNCTION AT ADULT LIFE

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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
Co-orientador: Prof. Dr. Rodrigo Mello Gomes

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BANCA EXAMINADORA

Prof. Dr. Paulo Cezar de Freitas Mathias
Orientador

Prof. Dr. Rodrigo Mello Gomes
Co-orientador

Prof. Dr. Paulo Nuno C. Matafome

Prof. Dr. Alex Rafacho

Prof. Dr. Paul Taylor

BIOGRAPHY

Flávio Andrade Francisco was born in Osasco - SP in 1981 and soon afterwards he went to Ivaiporã - PR, where his family already lived. Second son of Israel Francisco and Lenir Luiza Andrade Francisco, he started his studies at the age of 6 at Santa Olga School, where he also studied his two brothers, Filipe and Fúlvio.

In 1992, he moved with his whole family to Maringá - PR where, in 1999, he entered the State University of Maringá, in the Biological Sciences course. He graduated in 2004 and in 2005 he moved to Curitiba - PR in order to study medicine. In 2008 he dedicated himself to music and theater traveling throughout Brazil in concerts and presentations and after that, in 2009 he moved to Cascavel - PR, where he worked for 4 years with Microbiology and in 2012, invited by UniCesumar (Maringá - PR) to work with artificial insemination of cattle, he returned to Maringá, already married to Érica Mota Vieira Andrade. In 2014, he started his Master's studies in Biological Sciences, developing his work at the Cellular Biology Laboratory of the Secretion (UEM), supervised by Professor Dr. Paulo Cezar de Freitas Mathias, who had already guided him during the entire scientific initiation, during graduation and by Professor Dr. Rodrigo Mello Gomes, with whom he developed a new scientific partnership.

Right after the master's degree, in March 2016, he started his PhD studies in Biological Sciences in the same laboratory and with the same supervisors as in the previous internship. In October of that same year his article was developed in the master's degree published by the European Journal of Nutrition.

In July 2018, was born in Maringá - PR, his first son, Kadu Vieira Francisco and a year later was approved in a public examination for the position of Biologist - Technical Responsible - in the laboratory of CISNAP - Intermunicipal Health Consortium of Nova Alta Paulista in Dracena - SP, where he has lived with his family since December 2019.

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I thank my wonderful wife, Erica, who helps me and is my companion in good times and bad ... in health and disease ... in wealth and poverty ... who defends me as a lioness and whom I admire and love with all my heart;

I thank my little Kadu, who even without having the slightest notion of the importance of this moment in Dad's life, makes my life lighter (even in the middle of bad nights), happier (even with a work routine much more tiring inside and outside the home), more hopeful and with more meaning (in the face of all the achievements and frustrations of life) and more motivating (in the face of the constant challenges of professional life and participatory partnership);

I thank my parents, Israel and Lenir, who, being strong and responsible, knew how to educate and instruct me with good moral principles, not preventing me from questioning, investigating, inquiring and having an independent spirit since childhood, sparing no effort for my education, they were with me in all the important moments of my life and that are still present, despite the distance that separates us;

I thank my brothers Filipe and Fúlvio who were so important pieces for me to have a happy childhood, even with the stumbles that life imposed on us, who were strong arms and legs in childhood and adolescence games and who are friendly shoulders in hard adult life;

I thank my competent and dedicated teachers, from the first scribbles to the graphics ... from the first letters to the thesis ... from teacher Lubina, from the first school year, to the

professors Dr. Paulo Cezar de Freitas Mathias and Dr. Rodrigo Mello Gomes who always guided me with extreme competence throughout my career;

I thank my extraordinary colleagues in the laboratory, who helped me in the development of all this work and who helped me - each one in his own way - to be a better and more mature researcher;

I thank the State University of Maringá and the Postgraduate Program in Biological Sciences for all their support in this long time of learning and development in Undergraduate, Master and Doctorate;

I thank for the opportunity to have my work evaluated, receiving important contributions from such a competent examination committee with Professor Dr. Paulo Nuno Centeio Matafome, from the University of Coimbra, Professor Dr. Alex Rafacho, from the Federal University of Santa Catarina, Professor Dr Paul Taylor, from the Royal College of London, Professor Dr. Douglas de Almeida, from the State University of Londrina and Professor Dra. Késia Palma-Rigo, from the Adventits Institute of Paraná;

I thank to the research promotion agencies in Brazil - CAPES and CNPq - that, even in times of crisis, cuts and contingencies, financed the entire development of this work and remained faithful in the payments of my scholarship.

DEDICATION

To my wife, Erica;

To my son, Kaduzinho;

To my parents, Israel and Lenir;

To my brothers, sisters-in-law and nephews;

knowing that they will understand very little of what is written here,

but that, empathetically and because they are not bothered by the failures,

they will be even more proud than I am for completing this work.

“Knowledge makes us responsible.”

Che Guevara

PRESENTATION

This thesis is entitled "**The early glycotoxin exposure and metabolic programming: Inflammation, Oxidative stress and Metabolic dysfunction at adult life**" and consists of 2 scientific articles.

First a review article entitled "**Early AGEing and metabolic diseases: is perinatal exposure to glycotoxins programming to adult-life metabolic syndrome?**" And then a full scientific article entitled "**Neonatal methylglyoxal exposure leads wistar rats offspring to inflammation, oxidative stress and metabolic dysfunctions at adulthood**"and aimed to study the physiological effects caused by glycotoxins, in a thorough survey of the existing literature, followed by laboratory experiments where the effect of administering a glycotoxin precursor, methylglioxal, was evaluated in puppies during the first 2 third of lactation, on glycemic and lipid metabolism, pancreatic, hepatic and renal function, in addition to oxidative stress and inflammatory profile of offspring in adulthood.

In accordance with the rules of the Graduate Program in Biological Sciences, the articles presented here were written according to the rules of the following journals, respectively:

Journal: Nutrition Reviews

Impact factor: 5,779

Qualis CAPES (Biological Sciences I): A1.

Journal: Journal of Nutritional Biochemistry

Impact factor: 4,490

Qualis CAPES (Biological Sciences I): A1.

RESUMO GERAL

O conceito DOHaD (Developmental Origins of Health and Disease) preconiza que distúrbios de ordem ambientais e/ou nutricionais precoces (pré- ou pós-natais) são críticos para a manutenção da saúde ou para o surgimento de doenças não comunicáveis nos indivíduos na fase adulta.

As glicotoxinas, ou produtos finais de glicação avançada (AGEs – Advanced Glycation End-products) e seus precursores, como o metilglioxal (MG), são formados endogenamente e também podem ser comumente encontrados em alimentos processados em altas temperaturas e estocados como as e fórmulas infantis, entre diversos outros, e podem estar associados a distúrbios nutricionais precoces. Além de aspectos gerais das glicotoxinas, como a produção endógena, os mecanismos fisiológicos de detoxificação, como o ciclo das glioxalases, fontes exógenas e seu papel no desenvolvimento da síndrome metabólica, o artigo de revisão apresentado tem como objetivo discutir as fontes de exposição perinatal às glicotoxinas e seu envolvimento nos mecanismos da programação metabólica. Discuti-se também o papel da exposição perinatal às glicotoxinas no desenvolvimento da resistência à insulina, desenvolvimento do sistema nervoso central, doenças cardiovasculares e envelhecimento precoce. Finalmente, o primeiro artigo apresentado discute possíveis intervenções que podem prevenir ou reduzir os efeitos da exposição pós-natal a glicotoxinas.

O segundo artigo teve como objetivo investigar os efeitos de exposição precoce ao MG na programação da prole para disfunção metabólica na vida adulta. Ao nascimento (PN1), os animais foram divididos em dois grupos: grupo controle (CO), tratados com salina 0,9% e metilglioxal (MG), tratado com MG (20mg/kg de peso corporal i.p.) durante as duas primeiras semanas do período de lactação. Durante o período experimental, a ingestão de alimentar e o peso dos animais foram verificados diariamente e ambos os grupos (CO e MG) foram avaliados na idade adulta (PN90). O grupo MG apresentou diminuição do peso

corporal, tecido adiposo e massa hepática e renal. Contrariamente, esse mesmo grupo apresentou aumento da ingesta alimentar, além de aumento nos níveis de frutosamina, insulina e no índice HOMA-IR, evidenciando resistência à insulina. Além disso, os animais MG apresentaram dislipidemia, estresse oxidativo e inflamação.

Em conclusão, a exposição precoce pós-natal ao MG induz estresse oxidativo e estado inflamatório que leva à síndrome metabólica na prole de ratos adultos e aumento do risco de doença cardiovascular. Tais resultados sustentam a hipótese de que a lactação é um importante período para a programação de saúde ou doença.

Palavras-chave: Programação metabólica; Glicotoxinas; Produtos finais de glicação avançada (AGEs); Metilglioxal; Síndrome metabólica.

GENERAL ABSTRACT

The DOHaD (Developmental Origins of Health and Disease) concept advocates that early environmental and/or nutritional disorders (pre- or post-natal) are critical for maintaining health or for the emergence of non-communicable diseases in individuals in adulthood .

Glycotoxins, or advanced glycation end-products (AGEs) and their precursors, such as methylglyoxal (MG), are formed endogenously and can also be commonly found in foods processed at high temperatures and stored as the infant formulas among others, and may be associated with early nutritional disorders. In addition to general aspects of glycotoxins, such as endogenous production, physiological detoxification mechanisms, such as the glyoxalase cycle, exogenous sources and their role in the development of the metabolic syndrome, the review article presented aims to discuss the sources of perinatal exposure to glycotoxins and their involvement in the mechanisms of metabolic programming. The role of perinatal exposure to glucotoxins in the development of insulin resistance, development of the central nervous system, cardiovascular diseases and premature aging is also discussed. Finally, the first article presented discusses possible interventions that can prevent or reduce the effects of postnatal exposure to glycotoxins.

The second article aimed to investigate the effects of early exposure to MG on the offspring's programming for metabolic dysfunction in adulthood. At birth (PN1), the animals were divided into two groups: control group (CO), treated with saline 0.9% and methylglyoxal (MG), treated with MG (20mg/kg body weight ip) during the first two weeks of the lactation period. During the experimental period, food intake and body weight were checked daily and both groups (CO and MG) and the animals were assessed in adulthood (PN90). The MG group showed a decrease in body weight, adipose tissue and hepatic and renal mass. In contrast, this same group showed an increase in food intake, in addition to an

increase in fructosamine, insulin levels and the HOMA-IR index, showing insulin resistance. In addition, MG animals showed dyslipidemia, oxidative stress and inflammation.

In conclusion, early postnatal exposure to MG induces oxidative stress and an inflammatory state that leads to metabolic syndrome in the offspring of adult rats and increased risk of cardiovascular disease. These results support the hypothesis that lactation is an important period for health or disease programming.

Key words: Metabolic programming; Glycotoxins, Advanced Glycation end Products (AGEs); Methylglyoxal; Metabolic syndrome.

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ATTACHMENT 01:

ARTICLE 01:

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

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

Flávio Andrade Francisco[1*]; Lucas Paulo Jacinto Saavedra[1*]; Marcos Divino Ferreira Junior[2]; Cátia Barra[3]; Paulo Cezar de Freitas Mathias[1] and Rodrigo Mello Gomes[2]

[1]Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringá, Maringá, PR, Brazil
[2]Department of Physiological Sciences, Federal University of Goiás, Goiânia, GO, Brazil
[3]Institute of Physiology and Coimbra Institute of Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra

Short title: Perinatal glycotoxins exposure and metabolic programming

ABSTRACT

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

Keywords: Metabolic programming; Glycotoxins, Advanced Glycation end Products (AGEs); Methylglyoxal; Metabolic syndrome.

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1. INTRODUCTION

Developmental origins of health and disease (DOHaD) concept alert to the potential associations between a suboptimal foetal and/or postnatal environment with 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 metabolic syndrome. Features of the cardiometabolic diseases have been found in the offspring of diabetic rodents or feeding a high-fat diet or a 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 with Advanced Glycation End-Products (AGEs), and these glycotoxins are closely related to the installation, progression and development of diabetes and its complications [6–11]. AGEs have also been implicated in the deterioration of metabolic homeostasis in obesity, namely the development of insulin resistance-associated pathologies such as cardio- and cerebrovascular diseases, non-alcoholic steatohepatitis and central nervous system disorders including dementia, in both adult and pediatric patients [12,13,22–26,14–21]. Vascular AGEing is associated to oxidative stress, with generation of reactive oxygen and nitrogen species [27–30], endothelial dysfunction [31–33], changes in the extracellular matrix [32] and in inflammatory factors [34]. Infant formulas used worldwide as a substitute for breast milk have been demonstrated high AGEs levels, thus exposing infants to these nutritional contaminants during the postnatal period early in life may contribute to the development of cardiometabolic disorders at adulthood [35–37].

This review provides an overview of the current knowledge about the contribution of perinatal glycotoxins exposure to metabolic programming and development of metabolic syndrome-related pathologies. Here, we emphasize the evidences of increased glycotoxins exposure of the foetus in gestational diabetes, the impact of maternal dietary AGE consumption during embryonic development, lactation and infant formula feeding on

developmental programming of metabolic syndrome and interventions to prevent the consequences of their perinatal exposure.

2. CLINICAL EVIDENCES OF PERINATAL PROGRAMMING FOR ADULT-LIFE METABOLIC SYNDROME

Pregnancy is a critical period for the health of both the foetus and the mother and is a sensitive period to environmental disturbances. Several studies established a relationship between disturbances in the pregnancy and offspring diseases throughout life [38–47]. The magnitude of the effects depends on the stage of gestation in which the foetus was exposed and the nature of the aggressive agent [41]. It is well established that the tobacco, alcohol, distress, nutritional unbalances and other metabolic disorders impact the proper development of the foetus in the intrauterine life [43,45,47–49]. One of the most common gestational disorders is the gestational diabetes mellitus (GDM), which is associated with pre-gestational overweight and has been implicated in adverse perinatal outcomes such as increased weight-gain during the gestational period and high sugar consumption [42,50,51]. Foetal development is very susceptible to diabetes and this condition can promote severe changes in tissues and organs, being cardiovascular and neural tube defects the most frequent malformations [40,43]. Mothers with pregestational diabetes mellitus (PGDM) and a poorly controlled hyperglycemia during the first trimester have 5-10% possibility to have newborns with a major birth defects and 15-20% of spontaneous abortion [52]. On the other hand, GDM is more related with pregnancy complications, such as macrosomia, pre and perinatal mortality, than congenital anomalies [43]. The offspring from mothers with PGDM presents increased adiposity and overweigh resulting from transplacental passage of maternal glucose and induction of foetal hyperinsulinemia [43]. Pregnant women with GDM were shown to have an increased risk to deliver large gestational for age (LGA) newborns, which have higher risk be obese in childhood [40,53].

Some authors showed that diet composition prior and during pregnancy may have impact on the metabolic profile of both mother and newborn, and in children size at birth [54,55]. Nutritional changes may lead to impairment of foetal growth and intrauterine growth restriction (IUGR), as well as foetal adiposity, insulin resistance and pancreatic beta-cell dysfunction [56]. A case-control study from Amezcua-Prieto *et al.* [55] suggests that the increased consumption of industrial bakery products, pastries and refined sugar products during pregnancy is associated to having a small gestational for age (SGA) newborn. In contrast, higher consumption of wholegrain cereal and bread is related to a lower risk to deliver a SGA infant [55]. Another cohort study suggests that the daily consumption of artificially sweetened beverage during pregnancy has a twofold higher risk to have a child with overweight at first year [57].Ornoy *et al.* [43] showed that the offspring of GDM mothers have a high frequency of overweight, as well as the babies who are breastfeeding from diabetic mothers. In another study conducted by Palatianou *et al.* [58] have found increased association of LGA with nondiabetic obesity than with type 2 diabetes. On the other hand, LGA infants from diabetic mothers (GDM or PGDM) present a height and weight above the 90th percentile as well as increased weight gain in the first four months of life [43,59]. A meta-analysis performed by Schellong *et al.* [60] revealed a predisposition to adulthood overweight in LGA infants but not in SGA newborns. However, both LGA and SGA have been shown to have a similar risk to adulthood diabetes development, following a U-shaped and not a linear relationship [61]. Children SGA born from mothers with PGDM and associated nephropathy are more susceptible to prematurity, reduced growth at 3 years old and body weight and height below to 50th percentile when compared to children of PGDM mothers without complications. As well, SGA individuals who significantly gained 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 [56].

Thus, obesity and type 2 diabetes of the mother affects birth weight and both SGA and LGA 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.

3. 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 AGE (reviewed by [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 methylglyoxal lysine dimer (MOLD) and (carboxyethyl)lysine (CEL) formation [62,63] reviewed by [64–66]. Modification of aminoacid residues by MG affects circulating (hemoglobin, albumin or lipoproteins), extracellular matrix and intracellular proteins (cytoplasmic proteins and transcription factors), changing cell behavior and activating inflammatory and death pathways [67–74] reviewed by [18]. Moreover, MG was shown to modify proteasome subunits and protein quality control pathways (Hsc70, Hsp90 and Hsp27) causing endoplasmic reticulum stress and impaired degradation of misfolded proteins [75–78]. Besides directly modifying protein structure through modification of aminoacid residues, MG was also shown to increase oxidative stress, namely the formation of superoxide anion [79–82], hydrogen peroxide and peroxynitrite [79,80,83] in different types of cells, including endothelial cells [84], rat kidney mesangial cells [85], rat hepatocytes [83,86], blood cells [80,87], osteoblasts [87] and in rat and mouse neurons [88–91]. MG was shown to induce the depletion of antioxidant defenses, predisposing cells for oxidative damage [79,85,92–96]. Given that MG detoxification systems are GSH-dependent, such mechanisms lead to a self-perpetuating cycle of ROS/AGE formation and mitochondrial dysfunction [94].

Extracellular AGEs may change cell behavior through activation of membrane receptors, such as RAGE, which is known to recognize two major types of ligands, imidazolones (MG-derived) and Nε-(carboxymethyl)lysine (CML) adducts (reviewed by [97]). Upon activation, RAGE triggers intracellular signaling pathways such as NF-kB, involved in activation of inflammatory and proliferation/stress signals, as well as generation of oxidative stress [72,98–103]. Inhibition of RAGE was shown to prevent vascular disease in several animal models [98,104,105]. Thus, MG-induced changes in cell behavior have been shown to involve several mechanisms, namely through the modification of biomolecules, accumulation of misfolded proteins, activation of membrane receptors, generation of oxidative stress, changes in transcription factors and activation of inflammatory/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 diabetic patients and insulin-independent cells like endothelial cells, podocytes and neurons are more susceptible to hyperglycemia-driven MG formation. Several studies have addressed the involvement of MG in the mechanisms governing the development of such pathologies, namely endothelial cell senescence and angiogenesis impairment [67,74,106,107], podocyte effacement and death [108,109], glomerular fibrosis [73,98,102,110], apoptosis of retinal pericytes and retinal pigmented cells [111–114], and changes in the nociception and pain stimuli (hyperalgesia) [115,116]. Moreover, MG has been involved in the pathophysiology of cardio- and cerebrovascular diseases. MG was observed to cause structural changes in the blood-brain barrier [117,118], but to be also involved in other neurodegenerative disorders such as increased neurotoxicity [119,120], beta-amyloid protein neurotoxic effects [121,122], and loss of dopaminergic neurons [123–125]. In the cardiovascular system, it was shown that MG impairs the calcium handling between sarcoplasmic reticulum and cytoplasm of cardiomyocytes [126], and also impacts survival and apoptotic pathways during ischemia [127,128], besides angiogenic deficits

[129]. Features of endothelial dysfunction, hypertension and atherosclerosis have also been reported, such as oxidative stress and stiffness of the aorta, impairing elasticity, acetylcholine-dependent relaxation and NO bioavailability [33,130–133], activation of the renin-angiotensin system [134,135], increased glycoxidation of LDL particles [136,137] and increased risk of thrombosis and atherosclerosis through platelet hyperaggregation and RAGE activation [138,139].

Moreover, MG has been also implicated in the process of loss of metabolic homeostasis itself, namely in the development of beta-cell dysfunction and insulin resistance. MG was shown to transiently activate insulin secretion [140], but hamper beta-cell survival and long-term insulin synthesis and secretion [141]. In insulin signaling, MG was observed to cause a redox-independent inhibition of insulin receptor pathway and GLUT4 translocation in muscle cells and 3T3 adipocytes [9,142,143]. *In vivo*, MG was observed to cause insulin resistance in several animal models [141,142,144] but only when supraphysiological doses were used (Reviewed by [18]). Other studies failed to show MG-induced insulin resistance and this was only observed in obese animal models [25,145,146]. Several studies have also shown AGE-induced overexpression of inflammatory mediators in the liver [26,147,148], 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 diabetic patients and in metabolically unhealthy obese (MUO) patients, but no correlation was found between AGE levels and impaired glucose homeostasis [149,150]. Nevertheless, AGE-restricted diets have been shown to improve insulin sensitivity in normal, overweighed and diabetic patients, showing the link between increased glycoxidative stress and impaired metabolic homeostasis [151–153].

In summary, MG and MG-derived AGE are involved in several metabolic syndrome and diabetes-related pathologies, 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 MUO phenotype. Together with increased beta-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 completely unknown, although recent evidences have suggested that such exposure may increase the risk of metabolic dysregulation and development of diabetes-like complications in the adult life.

4. SOURCES OF PERINATAL GLYCOTOXINS EXPOSURE

4.1. In utero exposure to AGE during the embryonic development

GDM-related hyperglycemia was shown to increase serum MG and AGEs, such as CML. Raised serum AGEs are associated with insulin resistance, oxidative stress, cardiovascular diseases and diabetes comorbidities in normal individuals and pregnants [33,154–158]. Besides hyperglycemia, maternal AGEs may also derive from dietary absorption given that industrialized foods are very rich in AGEs [51] and such AGEs are transferred to the embryo through the placenta [159]. Accordingly, Konishi et al. [160] reported the impairment of implantation and placentation, and placenta function by the accumulation of AGEs, through RAGE activation, oxidative stress, low hCG levels and apoptosis in human first trimester trophoblasts. Similarly, Hao et al. [161] and Haucke et al. [162] reported the adverse effects of GDM through raised AGE levels during embryonal development, which promotes RAGE activation, inflammation, and AGE accumulation in the embryo. This environmental stress may collaborate to embryo resorption, foetus malformation or preterm birth. On the other hand, knockout of RAGE in pregnant diabetic rats prevents embryonic dysmorphogenesis, mitigating the effects of AGEs in the foetus [163]. Elevated sugar-sweetened soft beverages and

refined carbohydrates consumption during pregnancy are strongly correlated with offspring congenital heart defects, SGA newborns and increased risk of offspring overweight [55,57,164]. Such data reinforce the role of AGEs exposure on the diabetic embryopathy and its implications to proper foetus development, which are widely related to developmental origins of diseases at later stages of life.

4.2. Exposure during lactation and digestion and intestinal absortion of glycotoxins in newborns through infant formulas

The period of lactation is of extreme importance to the neonate development and maturation of different organs and systems, being the breastmilk enough to completely supply the neonate necessities. Given the abundance of evidences regarding the importance of breastfeeding in infant health, the World Health Organization recommends exclusive breast feeding until 6 months of life and complementary until 2 years old [165]. Breastfeeding was shown to prevent the incidence of diseases such as diabetes, multiple sclerosis and celiac disease [166].

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 in the infant's health and development [166,167]. Insulin may be found in milk and plays an important role in the process of gut maturation , decreasing permeability to macromolecules [168].

Human studies have found that the neonatal intake of breastmilk from diabetic mothers was related to overweight and glucose intolerance [169]. There are evidences that breastmilk may also be a source of glycotoxins in the neonate period. Mericq *et al.* [159] have shown a correlation between blood AGE levels of lactating mothers and their infants, raising the question whether maternal diet during lactation influences infant glycoxidative stress [159]. Even in other diseases, such as beriberi, when occurs an accumulation of glucose

intermediaries, such as MG, it was observed an increased concentration of these substances in breastmilk [170]. Also, it was observed that infants whose mothers smoked during pregnancy and lactation have increased accumulation of AGEs in their skin, indicating that the transmission of glycotoxins from the mother to the child may also occur through breastmilk [35].

An animal study has found that cows with a high AGEs diet presents increased glycated compounds in their milk, such as MG-H1 [171]. A AGE diet during pregnancy and neonatal period prevented the development of type 1 diabetes in the offspring of NOD mice [172]. In this regard, our group has previously demonstrated that oral administration of MG to breastfeeding rats increased the content of the glycation intermediary fructosamine in their milk, which was associated to the development of a diabetic phenotype in offspring during adult life [173].

Another source of glycotoxins during the perinatal period are infant formulas, given that many have high AGEs levels , reaching almost 35-fold higher concentration of CML than breastmilk of healthy mothers [36,174]. AGEs are formed in heat-treated foods, as a product from Maillard reaction, or non-enzymatic browning. In fact, traditional methods of cooking which use high temperature (100 to 250 °C), such as frying, baking and grilling, contribute to a higher degree of AGE formation, being the foods rich in reducing sugars and proteins more prone to the formation of this compounds [175–177]. For instance, grilled beef presents five times higher AGEs levels (5,963 kU/100 g) than boiled one (1,124 kU/100 g) [178]. Infant formulas are rich in both sugars and proteins, and their industrial production includes heat exposition. In fact, it was demonstrated that hydrolysate infant formulas, rich in whey, presents higher concentration of CML since whey proteins are subjected to great heat treatment during its manufacturing [179].

A positive correlation between formula AGEs and increased circulating levels and urinary excretion of AGEs was found in newborns, indicating it absorption [174,180]. In an animal model of IUGR, animals that were fed high AGEs formula during suckling, presented accumulation of CML in renal tubular cells, associated with increased protein oxidation and expression of pro-inflammatory and apoptotic factors [181]. IUGR piglets present increased oxidative stress and the early consumption of high AGEs formula during suckling programs these animals to the development of liver oxidative stress in adult life by impairment of antioxidant defenses [182]. Some authors discuss that the high consumption of glycation compounds by infant formulas during early life may predispose to the development of oxidative stress and diseases later in life, such as diabetes [159,183]. It was observed that increased maternal AGEs levels were correlated to the infant AGEs levels, which may precondition the young to high oxidative stress, inflammation and insulin resistance [159]. A more recent investigation observed decreased insulin sensitivity in AGEs-rich formula-fed infants than those fed only breastmilk, although the specific AGEs contribution to decreased insulin sensitivity was not clear since no differences were observed to infants fed a low-AGEs formula [184].

The impact of neonates' exposition to glycotoxins is still controversial. The influence of maternal diabetes in milk composition during breastfeeding is not well understood, such as the role of MG and AGEs in the neonatal health and programming to diseases during adult life.

In the last years, the role of dietary AGEs in the development of metabolic diseases has been deeply discussed, but an important question remains to be elucidated: Are dietary AGEs digested and absorbed? In fact, a strong correlation between AGEs intake and its levels in the plasma has been demonstrated [185,186]. Similarly, evidences from human studies shown that dietary restriction of AGEs decreases plasma concentration and their renal excretion

[152,187–189]. It was shown in animals fed a ^{14}C labeled AGEs-rich diet, as well as in humans, that 10% of dietary AGEs are absorbed [187,190]. Indeed, the glycation compound Pirralyne, as well as major AGEs such as CML, CEL and MG-H1, were shown to be absorbed in the form of dipeptides via PEPT1 transporter in intestinal cells [191,192].

Regarding the AGEs digestion, it was shown that glycation of dairy protein by MG or glyoxal, may decrease their digestibility by proteases, mainly due to cross-linked AGEs. On the other hand, non-cross linked AGEs, such as CML, CEL and MG-H1 are more prone to be absorbed by intestinal epithelial cells [193]. High molecular-weight AGEs are harder to digest and absorb, turning them more able to advance in the intestinal tract and interact with the colonic microbiome [193,194]. In fact, it has been demonstrated that dietary AGEs may influence the microbiome composition. In rats, dietary AGEs reduced the diversity of microbiota, decreasing short-chain fatty acids-producing bacteria and damaging colonic epithelial barrier [195]. Human studies also report the interaction between dietary AGEs and changes in gut microbiome composition, highlighting the importance of this interaction to human health [196,197]. However, few articles have addressed the mechanisms of AGEs absorption in adults and much less is known about this process in the neonatal gut. In fact, the newborn gut is not totally mature, being shown that the epithelial gut barrier of newborns is still permeable to the passage of macromolecules, such as hormones, carbohydrates and peptides [168,198]. Thus, the newborn gut may be more complacent to the passage of glycotoxins, turning the pup more susceptible to the absorption and accumulation of AGEs and their precursors. In fact, it was shown that newborn rats are more susceptible to the toxic effects of oral delivered MG, since the lethal dose is almost 4 times lower than in an adult male rat (531 mg/kg vs 1990 mg/kg) [199]. As previously discussed, exposition to increased AGE levels by infant formulas or via breastmilk may be of great importance to health and development of the neonate. In general, the mechanisms of AGEs absorption, digestion and interaction with the microbiome are not

well understood and much less is known about the neonatal period, showing that more studies are necessary to clarify these mechanisms.

5. 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 really known about their effects in metabolic programming and in increasing the risk of developing certain diseases in the adult life. Moreover, the consumption of AGE through milk or infant formulas was shown to disturb metabolic homeostasis in newborns, being associated to pancreatic dysfunction, cardiovascular and central nervous system diseases. Exposure of rat lactating females to high dietary levels of sucrose or high-fructose corn syrup was observed to lead to increased FFA levels, adiposity and liver fat in the offspring at weaning [200]. Accordingly, Csongová *et al.* [201] have recently shown increased predisposition for weight gain and insulin resistance in the progeny of females fed a AGEs-rich diet during pregnancy, and Francisco *et al.* [173] have shown a similar impact of increased maternal exposure to MG during lactation, conducing to impaired lipid profile and adiposity in the offspring.

In the study of Francisco *et al.* [173] authors have also shown decreased beta-cell function in the offspring. Accordingly, using type 1 diabetic NOD mice, two different studies have shown the impact of perinatal AGE exposure of beta-cell function. Peppa *et al.* [172] have shown that low-glycotoxin foetal 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.* [202] have shown deteriorated beta cell function in the progeny of NOD females exposed to increased dietary AGE levels during pregnancy and lactation.

The impact of perinatal AGE 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 the adult life are known to be associated to increased glycoxidative stress and increased prenatal AGE exposure was also shown to result in early cardiac changes. Embryos of diabetic female rats were observed to accumulate higher levels of CML, which was associated to lower VEGF levels [203]. As well, AGE levels were increased in the heart of newborns of STZ-induced diabetic dams, being associated with increased oxidative stress and inflammatory markers [204].

Recent reports have suggested impairment of AGE-RAGE axis in preterm birth. Chiavaroli *et al.* [205] have shown decreased levels of soluble RAGE and endogenous secretory RAGE in overweigh prepubertal children who were LGA or SGA, being correlated with insulin resistance. In the central nervous system, increased hippocampal RAGE expression was observed in the offspring of STZ-induced diabetic female rats, which was associated with increased excitability and behavioral changes [206]. Increased glycation during gestational diabetes was also recently implicated in impaired neural development, namely in the decrease of cortical neural precursor cells [207]. Authors have shown that glyoxalase pathway disruption during the embryonic development leads to premature neurogenesis, depletion of cortical neural precursor cells and behavioural changes, which was also found in the offspring of diabetic mothers [207].

Thus, high AGE levels in mothers can predispose the progeny to impaired metabolic homeostasis and recent data suggest the definition of cut-off values for mother glycated albumin levels during pregnancy in order to prevent neonatal complications [208,209].

6. INTERVENTIONS TO PREVENT PERINATAL AGE EXPOSURE AND METABOLIC PROGRAMMING

As previously described, the exposition to glycotoxins during the perinatal life may occur in utero, since AGEs are able to cross the placental barrier and impair foetal development, activating the RAGE axis and increasing oxidative stress, which may underlie the embryopathy related to GDM. Furthermore, the exposition during lactation may occur via breastmilk since maternal AGEs levels may influence its concentration in the milk. Also, infant formulas and complementary foods constitute an important source of AGEs in neonatal life. Regarding gestational diabetes, it is well established that uncontrolled diabetes increases MG and AGEs circulating levels, exposing de embryo to it. Thus, the first approach to prevent MG and AGEs exposure, should be a proper glycemic control. Metformin was suggested as a efficient and safe drug for GDM management [210]. Besides improving insulin resistance and decreasing hepatic gluconeogenesis, metformin may directly react with and scavenge MG, preventing the formation of MG derived AGEs such as MG-H1 [211,212]. During lactation, the same interventions may be taken in order to treat maternal diabetes, thus preventing the transmission of glycotoxins from mother to the infant through breastmilk.

As previously described, one of the main sources of external glycotoxins is the diet. Since maternal AGEs may be transmitted to the infant via placenta or breastmilk, the consumption of ultra-processed AGEs-rich foods should be discouraged since they present high levels of AGEs. The intake of foods *in natura* should be encouraged, such as fresh vegetables, fruits and meats as part of balanced diet. Attention should be taken in the cooking process, avoiding high temperature methods such as frying and grilling, favouring 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 WHO, breastfeeding must be exclusive during the first 6

months of life [165]. In this sense, infant formula must be implemented only when breastmilk was not available, thus avoiding unnecessary uses. As previously described, the industrial process to obtain whey protein lead to a higher degree of AGEs formation, therefore the addition of whey protein should be avoided. The use of milk from different animals, such as goat should be encouraged, since their amino acidic profile is more similar do the human milk, removing the need for the addition of whey protein, thus reducing the amount of AGEs in the final product [179].

Thus, some interventions may be taken to prevent AGEs exposition during perinatal life, including proper glycemic control in diabetic mothers and the adoption of a balanced diet low in ultra-processed foods. Quit smoking may also be an important intervention, since smoking during lactation may increase AGEs levels in breastmilk [35]. The consumption of infant formulas, rich in AGEs may be taken with caution and the industry should be encouraged to develop infant formulas with low AGEs levels.

7. FUTURE CHALLENGES AND CONCLUSION

More studies are needed to understand the mechanisms underlying the effects of perinatal exposure to glycotoxins, in order to prevent the comorbidities in adult life related to the embryo and infant exposure to adverse conditions such as diabetes and diet rich in AGEs. In the clinical practice, the advice of pregnant and lactating women about the importance of the diet and glycemic control is essential. In order 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 the studies about this issue are currently scarce.

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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. doi:10.1016/j.jnutbio.2018.02.012
2. Desai M, Jellyman JK, Han G, Beall M, Lane RH, Ross MG. Maternal obesity and high-fat diet program offspring metabolic syndrome. *Am J Obstet Gynecol*. 2014;211(3):237.e1-237.e13. doi:10.1016/j.ajog.2014.03.025
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. doi:10.1016/j.phrs.2018.02.020
4. Sarman I. Review shows that early foetal alcohol exposure may cause adverse effects even when the mother consumes low levels. *Acta Paediatr Int J Paediatr*. 2018;107(6):938-941. doi:10.1111/apa.14221
5. Walters KA, Bertoldo MJ, Handelsman DJ. Evidence from animal models on the pathogenesis of PCOS. *Best Pract Res Clin Endocrinol Metab*. 2018;32(3):271-281. doi:10.1016/j.beem.2018.03.008
6. McLellan AC, Thornalley PJ. Glyoxalase activity in human red blood cells fractioned by age. *Mech Ageing Dev*. 1989;48(1):63-71. http://www.ncbi.nlm.nih.gov/pubmed/2725076.
7. McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci (Lond)*. 1994;87(1):21-29. http://www.ncbi.nlm.nih.gov/pubmed/8062515.
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(1):41. doi:10.1186/1471-2091-12-41
9. Riboulet-Chavey A, Pierron A, Durand I, Murdaca J, Giudicelli J, Van Obberghen E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes*. 2006;55(5):1289-1299. doi:10.2337/db05-0857
10. Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J*. 1990;269(1):1-11. doi:10.1042/bj2690001
11. Thornalley PJ. Modification of the glyoxalase system in human red blood cells by

glucose in vitro . *Biochem J*. 1988;254(3):751-755. doi:10.1042/bj2540751

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(2):287-292. doi:10.1016/j.atherosclerosis.2004.02.012
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(12):2716-2723. doi:10.1161/01.ATV.0000249630.02085.12
14. Hanssen NMJ, Stehouwer CDA, Schalkwijk CG. Methylglyoxal and glyoxalase I in atherosclerosis. *Biochem Soc Trans*. 2014;42(2):443-449. doi:10.1042/bst20140001
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(17):1137-1146. doi:10.1093/eurheartj/eht402
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(2):139-145. doi:10.1177/1479164114560910
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(3):654-667. http://www.ncbi.nlm.nih.gov/pubmed/7545874.
18. Matafome P, Rodrigues T, Sena C, Seiça R. Methylglyoxal in Metabolic Disorders: Facts, Myths, and Promises. *Med Res Rev*. 2017;37(2):368-403. doi:10.1002/med.21410
19. Matafome P, Sena C, Seiça R. Methylglyoxal, obesity, and diabetes. *Endocrine*. 2013;43(3):472-484. doi:10.1007/s12020-012-9795-8
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(6):1649-1656. http://www.ncbi.nlm.nih.gov/pubmed/8256853.
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(6):871-878. doi:10.1159/000139653
22. Odani H, Shinzato T, Matsumoto Y, Usami J, Maeda K. 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(1):89-93. doi:10.1006/bbrc.1999.0221

23. Sell DR, Monnier VM. Molecular basis of arterial stiffening: Role of glycation-a mini-review. *Gerontology*. 2012;58(3):227-237. doi:10.1159/000334668
24. Srikanth V, Westcott B, Forbes J, et al. Methylglyoxal, cognitive function and cerebral atrophy in older people. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2013;68(1):68-73. doi:10.1093/gerona/gls100
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(1):1698. doi:10.1038/s41598-017-01730-3
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;2019:6362910. doi:10.1155/2019/6362910
27. Lee HJ, Howell SK, Sanford RJ, Beisswenger PJ. Methylglyoxal can modify GAPDH activity and structure. *Ann N Y Acad Sci*. 2005;1043(1):135-145. doi:10.1196/annals.1333.017
28. Loske C, Neumann A, Cunningham AM, et al. Cytotoxicity of advanced glycation endproducts is mediated by oxidative stress. *J Neural Transm*. 1998;105(8-9):1005-1015. doi:10.1007/s007020050108
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 Physiol*. 2005;289(2):F420-F430. doi:10.1152/ajprenal.00415.2004
30. Wu L, Juurlink BHJ. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension*. 2002;39(3):809-814. doi:10.1161/hy0302.105207
31. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414(6865):813-820. doi:10.1038/414813a
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. doi:10.1155/2012/569654
33. Sena CM, Matafome P, Crisóstomo J, et al. Methylglyoxal promotes oxidative stress and endothelial dysfunction. *Pharmacol Res*. 2012;65(5):497-506. doi:10.1016/j.phrs.2012.03.004
34. Su Y, Lei X, Wu L, Liu L. 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(1):65-79. doi:10.1111/j.1365-2567.2012.03608.x

35. Federico G, Gori M, Randazzo E, Vierucci F. 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. doi:10.1177/2050312116682126
36. Kutlu T. Dietary glycotoxins and infant formulas. *Türk Pediatr Arşivi*. 2016;51(4):179-185. doi:10.5152/turkpediatriars.2016.2543
37. Pischetsrieder M, Henle T. Glycation products in infant formulas: Chemical, analytical and physiological aspects. *Amino Acids*. 2012;42(4):1111-1118. doi:10.1007/s00726-010-0775-0
38. Angueira AR, Ludvik AE, Reddy TE, Wicksteed B, Lowe WL, Layden BT. New insights into gestational glucose metabolism: Lessons learned from 21st century approaches. *Diabetes*. 2015;64(2):327-334. doi:10.2337/db14-0877
39. Arnaout R, Nah G, Marcus G, et al. Pregnancy complications and premature cardiovascular events among 1.6 million California pregnancies. *Open Hear*. 2019;6(1):1-10. doi:10.1136/openhrt-2018-000927
40. 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 (Rockv)*. 2016;20(86):1-382. doi:10.3310/hta20860
41. Holness N. High-Risk Pregnancy. *Nurs Clin North Am*. 2018;53(2):241-251. doi:10.1016/j.cnur.2018.01.010
42. 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(4):1-19. doi:10.1371/journal.pone.0215372
43. Ornoy A, Reece EA, Pavlinkova G, Kappen C, Miller RK. Effect of maternal diabetes on the embryo, fetus, and children: Congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defects Res Part C - Embryo Today Rev*. 2015;105(1):53-72. doi:10.1002/bdrc.21090
44. Papathakis PC, Singh LN, Manary MJ. How maternal malnutrition affects linear growth and development in the offspring. *Mol Cell Endocrinol*. 2016;435:40-47. doi:10.1016/j.mce.2016.01.024
45. Sinzato YK, Bevilacqua EM, Volpato GT, Hernandez-Pando RE, Rudge MVC,

Damasceno DC. Maternal Oxidative Stress, Placental Morphometry, and Fetal Growth in Diabetic Rats Exposed to Cigarette Smoke. *Reprod Sci*. 2018. doi:10.1177/1933719118815589

46. Zeng Z, Liu F, Li S. Metabolic Adaptations in Pregnancy: A Review. *Ann Nutr Metab*. 2017;70(1):59-65. doi:10.1159/000459633
47. Silva CCV, Vehmeijer FOL, El Marroun H, Felix JF, Jaddoe VWV, Santos S. Maternal psychological distress during pregnancy and childhood cardio-metabolic risk factors. *Nutr Metab Cardiovasc Dis*. 2019;(xxxx). doi:10.1016/j.numecd.2019.02.008
48. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ*. 1989;298(6673):564-567. doi:10.1136/bmj.298.6673.564
49. Eriksson UJ, Wentzel P. The status of diabetic embryopathy. *Ups J Med Sci*. 2016;121(2):96-112. doi:10.3109/03009734.2016.1165317
50. Li S, Yang H. Relationship between advanced glycation end products and gestational diabetes mellitus. *J Matern Neonatal Med*. 2018;0(0):1-7. doi:10.1080/14767058.2018.1449201
51. 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(3):5-15. doi:10.1371/journal.pone.0118652
52. Reece EA. Diabetes-induced birth defects: What do we know? What can we do? *Curr Diab Rep*. 2012;12(1):24-32. doi:10.1007/s11892-011-0251-6
53. 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(1):89-97. doi:10.1007/s00125-009-1560-z
54. Olmedo-Requena R, Gómez-Fernández J, Amezcua-Prieto C, Mozas-Moreno J, Khan KS, Jiménez-Moleón JJ. Pre-pregnancy adherence to the mediterranean diet and gestational diabetes mellitus: A case-control study. *Nutrients*. 2019;11(5):1-11. doi:10.3390/nu11051003
55. Amezcua-Prieto C, Martínez-Galiano JM, Cano-Ibáñez N, Olmedo-Requena R, Bueno-Cavanillas A, Delgado-Rodríguez M. Types of carbohydrates intake during pregnancy and frequency of a small for gestational age newborn: A case-control study. *Nutrients*. 2019;11(3):523. doi:10.3390/nu11030523
56. Vaiserman A, Lushchak O. Prenatal Malnutrition-Induced Epigenetic Dysregulation as

a Risk Factor for Type 2 Diabetes. *Int J Genomics*. 2019;2019:1-11. doi:10.1155/2019/3821409

57. 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(1):11. doi:10.1136/eb-2016-102558
58. Palatianou ME, Simos Y V., Andronikou SK, Kiortsis DN. Long-term metabolic effects of high birth weight: A critical review of the literature. *Horm Metab Res*. 2014;46(13):911-920. doi:10.1055/s-0034-1395561
59. Plagemann A, Harder T, Rodekamp E, Kohlhoff R. Rapid neonatal weight gain increases risk of childhood overweight in offspring of diabetic mothers. *J Perinat Med*. 2012;40(5):557-563. doi:10.1515/jpm-2011-0270
60. Schellong K, Schulz S, Harder T, Plagemann A. 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(10). doi:10.1371/journal.pone.0047776
61. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: A meta-analysis. *Am J Epidemiol*. 2007;165(8):849-857. doi:10.1093/aje/kwk071
62. 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(2). doi:10.1371/journal.pone.0031401
63. Masania J, Malczewska-Malec M, Razny U, et al. Dicarbonyl stress in clinical obesity. *Glycoconj J*. 2016;33(4):581-589. doi:10.1007/s10719-016-9692-0
64. Kalapos MP. Where does plasma methylglyoxal originate from? *Diabetes Res Clin Pract*. 2013;99(3):260-271. doi:10.1016/j.diabres.2012.11.003
65. Poulsen MW, Hedegaard R V., Andersen JM, et al. Advanced glycation endproducts in food and their effects on health. *Food Chem Toxicol*. 2013;60:10-37. doi:10.1016/j.fct.2013.06.052
66. Allaman I, Bélanger M, Magistretti PJ. Methylglyoxal, the dark side of glycolysis. *Front Neurosci*. 2015;9(FEB). doi:10.3389/fnins.2015.00023
67. 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(42):31038-31045. doi:10.1074/jbc.M704703200
68. 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. doi:10.1016/j.fct.2016.03.028

69. Bose T, Bhattacherjee A, Banerjee S, Chakraborti AS. Methylglyoxal-induced modifications of hemoglobin: Structural and functional characteristics. *Arch Biochem Biophys*. 2013;529(2):99-104. doi:10.1016/j.abb.2012.12.001
70. Guerin-Dubourg A, Catan A, Bourdon E, Rondeau P. Structural modifications of human albumin in diabetes. *Diabetes Metab*. 2012;38(2):171-178. doi:10.1016/j.diabet.2011.11.002
71. 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(7):1973-1980. doi:10.2337/db11-0085
72. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: Sparking the development of diabetic vascular injury. *Circulation*. 2006;114(6):597-605. doi:10.1161/CIRCULATIONAHA.106.621854
73. Pedchenko VK, Chetyrkin S V., 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(10):2952-2960. doi:10.2337/diabetes.54.10.2952
74. Bento CF, Fernandes R, Matafome P, Sena C, Seiça R, Pereira P. 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(9):955-970. doi:10.1113/expphysiol.2010.053561
75. Bento CF, Marques F, Fernandes R, Pereira P. Methylglyoxal Alters the Function and Stability of Critical Components of the Protein Quality Control. Cotterill S, ed. *PLoS One*. 2010;5(9):e13007. doi:10.1371/journal.pone.0013007
76. Padival AK, Crabb JW, Nagaraj RH. Methylglyoxal modifies heat shock protein 27 in glomerular mesangial cells. *FEBS Lett*. 2003;551(1-3):113-118. doi:10.1016/S0014-5793(03)00874-3
77. Palsamy P, Bidasee KR, Ayaki M, Augusteyn RC, Chan JY, Shinohara T. 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. doi:10.1016/j.freeradbiomed.2014.04.010
78. 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. doi:10.1016/j.yjmcc.2015.05.016

79. Chang T, Wang R, Wu L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. *Free Radic Biol Med*. 2005;38(2):286-293. doi:10.1016/j.freeradbiomed.2004.10.034
80. Ward RA, McLeish KR. Methylglyoxal: A stimulus to neutrophil oxygen radical production in chronic renal failure? *Nephrol Dial Transplant*. 2004;19(7):1702-1707. doi:10.1093/ndt/gfh271
81. Skrha J, Gáll J, Buchal R, Sedláčková E, Pláteník J. Glucose and its metabolites have distinct effects on the calcium-induced mitochondrial permeability transition. *Folia Biol (Praha)*. 2011;57(3):96-103. http://www.ncbi.nlm.nih.gov/pubmed/21888832.
82. 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(11):1460-1471. doi:10.1016/j.bbadis.2011.06.017
83. 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(5):369-372. doi:10.1007/BF01973710
84. 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(1):20-30. doi:10.1016/S0891-5849(01)00550-0
85. Uriuhara A, Miyata S, Liu BF, et al. Methylglyoxal induces prostaglandin E 2 production in rat mesangial cells. *Kobe J Med Sci*. 2007;53(6):305-315.
86. Seo K, Ki SH, Shin SM. Methylglyoxal induces mitochondrial dysfunction and cell death in liver. *Toxicol Res*. 2014;30(3):193-198. doi:10.5487/TR.2014.30.3.193
87. Suh KS, Choi EM, Rhee SY, Kim YS. Methylglyoxal induces oxidative stress and mitochondrial dysfunction in osteoblastic MC3T3-E1 cells. *Free Radic Res*. 2014;48(2):206-217. doi:10.3109/10715762.2013.859387
88. Di Loreto S, Caracciolo V, Colafarina S, Sebastiani P, Gasbarri A, Amicarelli F. 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(2):157-167. doi:10.1016/j.brainres.2004.01.066
89. Dafre AL, Goldberg J, Wang T, Spiegel DA, Maher P. Methylglyoxal, the foe and friend of glyoxalase and Trx/TrxR systems in HT22 nerve cells. *Free Radic Biol Med*.

2015;89:8-19. doi:10.1016/j.freeradbiomed.2015.07.005

90. Kikuchi S, Shinpo K, Moriwaka F, Makita Z, Miyata T, Tashiro K. 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(2):280-289. doi:10.1002/(SICI)1097-4547(19990715)57:2<280::AID-JNR14>3.0.CO;2-U
91. 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(8):856-871. doi:10.1016/S0891-5849(03)00438-6
92. Morgan PE, Sheahan PJ, Pattison DI, Davies MJ. Methylglyoxal-induced modification of arginine residues decreases the activity of NADPH-generating enzymes. *Free Radic Biol Med*. 2013;61:229-242. doi:10.1016/j.freeradbiomed.2013.03.025
93. Suravajjala S, Cohenford M, Frost LR, Pampati PK, Dain JA. Glycation of human erythrocyte glutathione peroxidase: Effect on the physical and kinetic properties. *Clin Chim Acta*. 2013;421:170-176. doi:10.1016/j.cca.2013.02.032
94. 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. doi:10.1016/j.freeradbiomed.2013.11.025
95. Paget C, Lecomte M, Ruggiero D, Wiernsperger N, Lagarde M. Modification of enzymatic antioxidants in retinal microvascular cells by glucose or advanced glycation end products. *Free Radic Biol Med*. 1998;25(1):121-129. doi:10.1016/S0891-5849(98)00071-9
96. Di Loreto S, Zimmitti V, Sebastiani P, Cervelli C, Falone S, Amicarelli F. Methylglyoxal causes strong weakening of detoxifying capacity and apoptotic cell death in rat hippocampal neurons. *Int J Biochem Cell Biol*. 2008;40(2):245-257. doi:10.1016/j.biocel.2007.07.019
97. 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(3):235-247. doi:10.1007/s00109-009-0439-2
98. Negre-Salvayre A, Salvayre R, Augé N, Pamplona R, Portero-Otín M. Hyperglycemia and Glycation in Diabetic Complications. *Antioxid Redox Signal*. 2009;11(12):3071-3109. doi:10.1089/ars.2009.2484
99. Yan SF, Ramasamy R, Naka Y, Schmidt AM. Glycation, Inflammation, and RAGE. *Circ Res*. 2003;93(12):1159-1169. doi:10.1161/01.res.0000103862.26506.3d

100. Xue J, Rai V, Singer D, et al. Advanced glycation end product recognition by the receptor for AGEs. *Structure*. 2011;19(5):722-732. doi:10.1016/j.str.2011.02.013

101. Xue J, Ray R, Singer D, et al. The receptor for advanced glycation end products (RAGE) specifically recognizes methylglyoxal-derived AGEs. *Biochemistry*. 2014;53(20):3327-3335. doi:10.1021/bi500046t

102. 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(3):947-957. doi:10.1046/j.1523-1755.2003.00829.x

103. Du J, Cai S, Suzuki H, et al. Involvement of MEKK1/ERK/P21 Waf1/Cip1 signal transduction pathway in inhibition of IGF-I-mediated cell growth response by methylglyoxal. *J Cell Biochem*. 2003;88(6):1235-1246. doi:10.1002/jcb.10478

104. 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(1):249-255. doi:10.2337/db09-0801

105. 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(2):431-436. doi:10.1016/j.atherosclerosis.2010.04.006

106. Thangarajah H, Yao D, Chang EI, et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. *Proc Natl Acad Sci*. 2009;106(32):13505-13510. doi:10.1073/pnas.0906670106

107. 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 (Lond)*. 2005;109(1):83-95. doi:10.1042/CS20050026

108. Diez-Sampedro A, Lenz O, Fornoni A. Podocytopathy in diabetes: A metabolic and endocrine disorder. *Am J Kidney Dis*. 2011;58(4):637-646. doi:10.1053/j.ajkd.2011.03.035

109. Giacco F, Du X, D'Agati VD, et al. Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. *Diabetes*. 2014;63(1):291-299. doi:10.2337/db13-0316

110. 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(1-2):35-42. doi:10.1007/s11010-007-9422-9

111. Kim J, Kim OS, Kim CS, Sohn E, Jo K, Kim JS. 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(2):167-175. doi:10.3858/emm.2012.44.2.012

112. Kim J, Kim OS, Kim CS, Kim NH, Kim JS. 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(1):86-93. doi:10.1016/j.cbi.2010.07.002

113. Kim OS, Kim J, Kim CS, Kim NH, Kim JS. KIOM-79 prevents methyglyoxal-induced retinal pericyte apoptosis in vitro and in vivo. *J Ethnopharmacol*. 2010;129(3):285-292. doi:10.1016/j.jep.2010.03.027

114. Kim J, Son JW, Lee JA, Oh YS, Shinn SH. Methylglyoxal Induces Apoptosis Mediated by Reactive Oxygen Species in Bovine Retinal Pericytes. *J Korean Med Sci*. 2004;19(1):95-100. doi:10.3346/jkms.2004.19.1.95

115. 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(6):926-933. doi:10.1038/nm.2750

116. 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(3):262-267. doi:10.1016/j.jdiacomp.2012.12.002

117. 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. doi:10.1016/j.redox.2015.03.005

118. Li W, Maloney RE, Circu ML, Alexander JS, Aw TY. 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. doi:10.1016/j.freeradbiomed.2012.10.552

119. Chun HJ, Lee Y, Kim AH, Lee J. Methylglyoxal Causes Cell Death in Neural Progenitor Cells and Impairs Adult Hippocampal Neurogenesis. *Neurotox Res*. 2016;29(3):419-431. doi:10.1007/s12640-015-9588-y

120. 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(3):429-438. doi:10.1007/s11011-013-9379-1

121. Li XH, Du LL, Cheng XS, et al. Glycation exacerbates the neuronal toxicity of β-

amyloid. *Cell Death Dis*. 2013;4(6). doi:10.1038/cddis.2013.180

122. Lovestone S, Smith U. Advanced glycation end products, dementia, and diabetes. *Proc Natl Acad Sci U S A*. 2014;111(13):4743-4744. doi:10.1073/pnas.1402277111
123. 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(11):950-956. doi:10.1093/abbs/gmu094
124. 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(3):361-366. doi:10.1016/j.bbrc.2015.02.069
125. 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(3):736-742. doi:10.3892/ijmm.2013.1604
126. 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(1):101-112. doi:10.1007/s10741-013-9384-9
127. 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(12):1223-1230. doi:10.1016/j.numecd.2013.01.005
128. Almeida F, Santos-Silva D, Rodrigues T, et al. Pyridoxamine reverts methylglyoxal-induced impairment of survival pathways during heart ischemia. *Cardiovasc Ther*. 2013;31(6). doi:10.1111/1755-5922.12039
129. Molgat ASD, Tilokee EL, Rafatian G, et al. Hyperglycemia inhibits cardiac stem cell-mediated cardiac repair and angiogenic capacity. *Circulation*. 2014;130(11):S70-S76. doi:10.1161/CIRCULATIONAHA.113.007908
130. Su Y, Qadri SM, Wu L, Liu L. Methylglyoxal modulates endothelial nitric oxide synthase-associated functions in EA.hy926 endothelial cells. *Cardiovasc Diabetol*. 2013;12(1). doi:10.1186/1475-2840-12-134
131. Su Y, Qadri SM, Hossain M, Wu L, Liu L. Uncoupling of eNOS contributes to redox-sensitive leukocyte recruitment and microvascular leakage elicited by methylglyoxal. *Biochem Pharmacol*. 2013;86(12):1762-1774. doi:10.1016/j.bcp.2013.10.008
132. Mukohda M, Yamawaki H, Nomura H, Okada M, Hara Y. Methylglyoxal Inhibits Smooth Muscle Contraction in Isolated Blood Vessels. *J Pharmacol Sci*. 2009;109(2):305-310. doi:10.1254/jphs.08300fp

133. Semba RD, Najjar SS, Sun K, Lakatta EG, Ferrucci L. Serum carboxymethyl-lysine, an advanced glycation end product, is associated with increased aortic pulse wave velocity in adults. *Am J Hypertens*. 2009;22(1):74-79. doi:10.1038/ajh.2008.320

134. Dhar I, Dhar A, Wu L, Desai KM. Methylglyoxal, a reactive glucose metabolite, increases renin angiotensin aldosterone and blood pressure in male sprague-dawley rats. *Am J Hypertens*. 2014;27(3):308-316. doi:10.1093/ajh/hpt281

135. Dhar I, Dhar A, Wu L, Desai KM. Increased Methylglyoxal Formation with Upregulation of Renin Angiotensin System in Fructose Fed Sprague Dawley Rats. Ashton N, ed. *PLoS One*. 2013;8(9):e74212. doi:10.1371/journal.pone.0074212

136. Rabbani N, Chittari MV, Bodmer CW, Zehnder D, Ceriello A, Thornalley PJ. Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin. *Diabetes*. 2010;59(4):1038-1045. doi:10.2337/db09-1455

137. Beisswenger PJ, Howell SK, Touchette AD, Lal S, Szwergold BS. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes*. 1999;48(1):198-202. doi:10.2337/diabetes.48.1.198

138. 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(11):3915-3925. doi:10.2337/db13-0932

139. Hadas K, Randriamboavonjy V, Elgheznawy A, Mann A, Fleming I. Methylglyoxal Induces Platelet Hyperaggregation and Reduces Thrombus Stability by Activating PKC and Inhibiting PI3K/Akt Pathway. *PLoS One*. 2013;8(9). doi:10.1371/journal.pone.0074401

140. 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(1):58-64. doi:10.1038/aps.2013.122

141. Dhar A, Dhar I, Jiang B, Desai KM, Wu L. Chronic methylglyoxal infusion by minipump causes pancreatic β-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes*. 2011;60(3):899-908. doi:10.2337/db10-0627

142. Jia X, Wu L. Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats. *Mol Cell Biochem*. 2007;306(1-2):133-139. doi:10.1007/s11010-007-9563-x

143. Engelbrecht B, Stratmann B, Hess C, Tschoepe D, Gawlowski T. Impact of GLO1 Knock Down on GLUT4 Trafficking and Glucose Uptake in L6 Myoblasts. *PLoS One*.

2013;8(5). doi:10.1371/journal.pone.0065195

144. Dhar A, Desai KM, Wu L. Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in Sprague-Dawley rats. *Br J Pharmacol*. 2010;159(1):166-175. doi:10.1111/j.1476-5381.2009.00469.x
145. 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(7):2082-2089. doi:10.2337/diabetes.51.7.2082
146. Rodrigues T, Matafome P, Santos-Silva D, Sena C, Seiça R. Reduction of methylglyoxal-induced glycation by pyridoxamine improves adipose tissue microvascular lesions. *J Diabetes Res*. 2013;2013:690650. doi:10.1155/2013/690650
147. Wei Y, Wang D, Moran G, Estrada A, Pagliassotti MJ. Fructose-induced stress signaling in the liver involves methylglyoxal. *Nutr Metab*. 2013;10(1). doi:10.1186/1743-7075-10-32
148. Gaens KHJ, Niessen PMG, Rensen SS, et al. Endogenous formation of Nε-(carboxymethyl)lysine is increased in fatty livers and induces inflammatory markers in an in vitro model of hepatic steatosis. *J Hepatol*. 2012;56(3):647-655. doi:10.1016/j.jhep.2011.07.028
149. Uribarri J, Cai W, Woodward M, et al. Elevated serum advanced glycation endproducts in obese indicate risk for the metabolic syndrome: A link between healthy and unhealthy obesity? *J Clin Endocrinol Metab*. 2015;100(5):1957-1966. doi:10.1210/jc.2014-3925
150. Kong X, Ma M zhe, Huang K, et al. Increased plasma levels of the methylglyoxal in patients with newly diagnosed type 2 diabetes. *J Diabetes*. 2014;6(6):535-540. doi:10.1111/1753-0407.12160
151. 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(7):1610-1616. doi:10.2337/dc11-0091
152. De Courten B, de Courten MPJP, 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(6):1426-1433. doi:10.3945/ajcn.115.125427
153. Macías-Cervantes MH, Rodríguez-Soto JMD, Uribarri J, Díaz-Cisneros FJ, Cai W, Garay-Sevilla ME. Effect of an advanced glycation end product-restricted diet and exercise on metabolic parameters in adult overweight men. *Nutrition*. 2015;31(3):446-

451. doi:10.1016/j.nut.2014.10.004

154. Kellow NJ, Coughlan MT. Effect of diet-derived advanced glycation end products on inflammation. *Nutr Rev*. 2015;73(11):737-759. doi:10.1093/nutrit/nuv030
155. 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(7):1-12. doi:10.1371/journal.pone.0159175
156. Šebeková K, Brouder Šebeková K. Glycated proteins in nutrition: Friend or foe? *Exp Gerontol*. 2019;117(February):76-90. doi:10.1016/j.exger.2018.11.012
157. Guosheng L, Hongmei S, Chuan N, Haiying L, Xiaopeng Z, Xianqiong L. The relationship of serum AGE levels in diabetic mothers with adverse fetal outcome. *J Perinatol*. 2009;29(7):483-488. doi:10.1038/jp.2009.12
158. Lappas M. Activation of inflammasomes in adipose tissue of women with gestational diabetes. *Mol Cell Endocrinol*. 2014;382(1):74-83. doi:10.1016/j.mce.2013.09.011
159. 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(10):2232-2237. doi:10.2337/dc10-1058
160. 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(9):2156-2162. doi:10.1093/humrep/deh389
161. Hao L, Noguchi S, Kamada Y, et al. Adverse effects of advanced glycation end products on embryonal development. *Acta Med Okayama*. 2008;62(2):93-99. doi:10.18926/AMO/30960
162. Haucke E, Santos AN, Simm A, et al. Accumulation of advanced glycation end products in the rabbit blastocyst under maternal diabetes. *Reproduction*. 2014;148(2):169-178. doi:10.1530/REP-14-0149
163. Ejdesjö A, Brings S, Fleming T, Fred RG, Nawroth PP, Eriksson UJ. Receptor for advanced glycation end products (RAGE) knockout reduces fetal dysmorphogenesis in murine diabetic pregnancy. *Reprod Toxicol*. 2016;62:62-70. doi:10.1016/j.reprotox.2016.04.015
164. 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(4):383-396. doi:10.1007/s10654-019-00480-y
165. WHO/UNICEF. *Global Strategy for Infant and Young Child Feeding.* Geneva: World

Health Organization; 2003. http://apps.who.int/gb/archive/pdf_files/WHA54/ea54id4.pdf?ua=1&ua=1.

166. Vieira Borba V, Sharif K, Shoenfeld Y. Breastfeeding and autoimmunity: Programing health from the beginning. *Am J Reprod Immunol*. 2018;79(1). doi:10.1111/aji.12778
167. Donovan SM, Odle J. Growth Factors in Milk as Mediators of Infant Development. *Annu Rev Nutr*. 1994;14(1):147-167. doi:10.1146/annurev.nu.14.070194.001051
168. Shehadeh N, Shamir R, Berant M, Etzioni A. Insulin in human milk and the prevention of type 1 diabetes. *Pediatr Diabetes*. 2001;2(4):175-177. doi:10.1034/j.1399-5448.2001.20406.x
169. Plagemann A, Harder T, Schellong K, Schulz S, Stupin JH. Early postnatal life as a critical time window for determination of long-term metabolic health. *Best Pract Res Clin Endocrinol Metab*. 2012;26(5):641-653. doi:10.1016/j.beem.2012.03.008
170. Knowles JA. Breast Milk: A Source of More than Nutrition for the Neonate. *Clin Toxicol*. 1974;7(1):69-82. doi:10.3109/15563657408987978
171. Schwarzenbolz U, Hofmann T, Sparmann N, Henle T. 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(24):5071-5078. doi:10.1021/acs.jafc.6b01375
172. Peppa M, He C, Hattori M, McEvoy R, Zheng F, Vlassara H. Fetal or neonatal low-glycotoxin environment prevents autoimmune diabetes in NOD mice. *Diabetes*. 2003;52(6):1441-1448. doi:10.2337/diabetes.52.6.1441
173. 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(2):477-486. doi:10.1007/s00394-016-1330-x
174. Dittrich R, Hoffmann I, Stahl P, Müller A, Beckmann MW, Pischetsrieder M. Concentrations of N ε -Carboxymethyllysine in Human Breast Milk, Infant Formulas, and Urine of Infants. *J Agric Food Chem*. 2006;54(18):6924-6928. doi:10.1021/jf060905h
175. Han L, Li L, Li B, et al. Review of the characteristics of food-derived and endogenous ne-carboxymethyllysine. *J Food Prot*. 2013;76(5):912-918. doi:10.4315/0362-028X.JFP-12-472
176. 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(3):211-248. doi:10.1080/10408398909527499

177. Zamora R, Hidalgo FJ. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Crit Rev Food Sci Nutr*. 2005;45(1):49-59. doi:10.1080/10408690590900117

178. 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(6):911-916.e12. doi:10.1016/j.jada.2010.03.018

179. Prosser CG, Carpenter EA, Hodgkinson AJ. N ε -carboxymethyllysine in nutritional milk formulas for infants. *Food Chem*. 2019;274(June 2018):886-890. doi:10.1016/j.foodchem.2018.09.069

180. Šebeková K, Saavedra G, Zumpe C, Somoza V, Klenovicsová K, Birlouez-Aragon I. Plasma Concentration and Urinary Excretion of N ε -(Carboxymethyl)lysine in Breast Milk- and Formula-fed Infants. *Ann N Y Acad Sci*. 2008;1126(1):177-180. doi:10.1196/annals.1433.049

181. Elmhiri G, Mahmood DFDD, 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(5):939-947. doi:10.1002/mnfr.201400722

182. Firmin S, Elmhiri G, Crepin D, Souidi M, Taouis M, Abdennebi-Najar L. 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(6):566-572. doi:10.1017/S2040174417001015

183. Elliott RB. Diabetes - A man made disease. *Med Hypotheses*. 2006;67(2):388-391. doi:10.1016/j.mehy.2005.11.047

184. Klenovics KS, Boor P, Somoza V, Celec P, Fogliano V, Šebeková K. Advanced Glycation End Products in Infant Formulas Do Not Contribute to Insulin Resistance Associated with Their Consumption. Müller M, ed. *PLoS One*. 2013;8(1):e53056. doi:10.1371/journal.pone.0053056

185. Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H. 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. doi:10.1196/annals.1333.052

186. Uribarri J, Cai W, Peppa M, et al. Circulating Glycotoxins and Dietary Advanced Glycation Endproducts: Two Links to Inflammatory Response, Oxidative Stress, and

Aging. *Journals Gerontol Ser A Biol Sci Med Sci*. 2007;62(4):427-433. doi:10.1093/gerona/62.4.427

187. Koschinsky T, He C-J, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): An environmental risk factor in diabetic nephropathy. *Proc Natl Acad Sci*. 1997;94(12):6474-6479. doi:10.1073/pnas.94.12.6474
188. 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(5):1220-1226. doi:10.3945/ajcn.2009.28737
189. 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(1):88-95. doi:10.2337/dc13-0842
190. He C, Sabol J, Mitsuhashi T, Vlassara H. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. *Diabetes*. 1999;48(6):1308-1315. doi:10.2337/diabetes.48.6.1308
191. Hellwig M, Geissler S, Peto A, Knütter I, Brandsch M, Henle T. Transport of Free and Peptide-Bound Pyrraline at Intestinal and Renal Epithelial Cells. *J Agric Food Chem*. 2009;57(14):6474-6480. doi:10.1021/jf901224p
192. 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(8):1270-1279. doi:10.1002/cbic.201000759
193. 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. doi:10.1016/j.foodres.2017.10.002
194. Snelson M, Coughlan M. Dietary Advanced Glycation End Products: Digestion, Metabolism and Modulation of Gut Microbial Ecology. *Nutrients*. 2019;11(2):215. doi:10.3390/nu11020215
195. 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(10):1700118. doi:10.1002/mnfr.201700118
196. Seiquer I, Rubio LA, Peinado MJ, Delgado-Andrade C, Navarro MP. Maillard reaction products modulate gut microbiota composition in adolescents. *Mol Nutr Food Res*. 2014;58(7):1552-1560. doi:10.1002/mnfr.201300847

197. 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. Barretti P, ed. *PLoS One*. 2017;12(9):e0184789. doi:10.1371/journal.pone.0184789

198. Harada E, Syuto B. Precocious cessation of intestinal macromolecular transmission and sucrase development induced by insulin in adrenalectomized suckling rat. *Comp Biochem Physiol A Comp Physiol*. 1991;99(3):327-331. http://www.ncbi.nlm.nih.gov/pubmed/1678330.

199. 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(3-4):369-374. doi:10.1016/S0147-6513(78)80009-8

200. Toop CR, Muhlhausler BS, O'Dea K, Gentili S. 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(13):4379-4398. doi:10.1113/JP274066

201. 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(6):3432-3446. doi:10.1039/c8fo00183a

202. 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(1):10-24. doi:10.1080/19382014.2017.1405189

203. 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(5):1222-1228. doi:10.2337/db07-1016

204. Kawaharada R, Masuda H, Chen Z, Blough E, Kohama T, Nakamura A. 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(8):2701-2712. doi:10.1007/s00394-017-1536-6

205. 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(6):1361-1363. doi:10.2337/dc11-2302

206. Chandna AR, Kuhlmann N, Bryce CA, Greba Q, Campanucci VA, Howland JG.

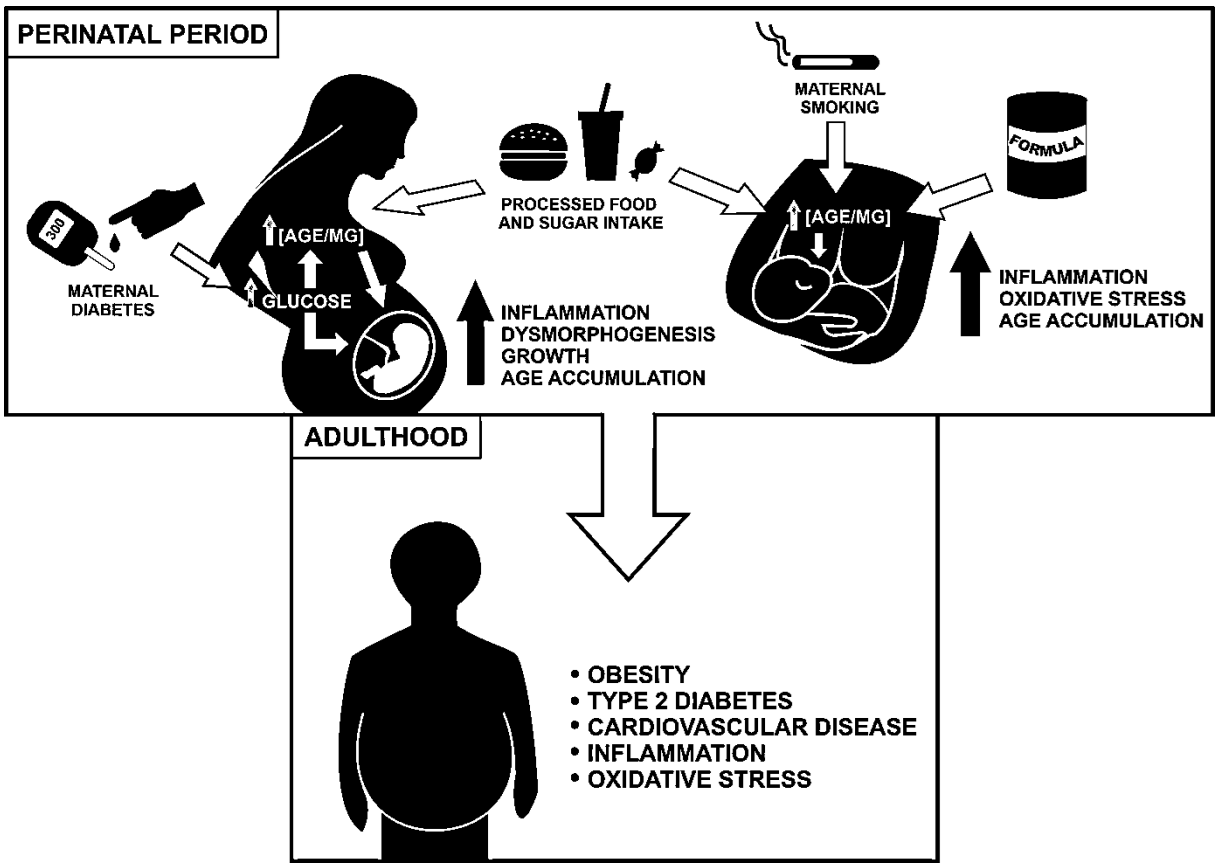
Chronic maternal hyperglycemia induced during mid-pregnancy in rats increases RAGE expression, augments hippocampal excitability, and alters behavior of the offspring. *Neuroscience*. 2015;303:241-260. doi:10.1016/j.neuroscience.2015.06.063

207. 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(4):1022-1036. doi:10.1016/j.celrep.2016.09.067
208. Shimizu I, Hiramatsu Y, Omori Y, Nakabayashi M. 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(6):639-646. doi:10.1177/0004563218763695
209. Sugawara D, Sato H, Ichihashi K, Nagai K, Kawano A. Glycated albumin level during late pregnancy as a predictive factor for neonatal outcomes of women with diabetes. *J Matern Neonatal Med*. 2018;31(15):2007-2012. doi:10.1080/14767058.2017.1333103
210. 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(15):1874-1881. doi:10.1080/14767058.2016.1228061
211. Kinsky OR, Hargraves TL, Anumol T, et al. Metformin Scavenges Methylglyoxal to Form a Novel Imidazolinone Metabolite in Humans. *Chem Res Toxicol*. 2016;29(2):227-234. doi:10.1021/acs.chemrestox.5b00497
212. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: From mechanisms of action to therapies. *Cell Metab*. 2014;20(6):953-966. doi:10.1016/j.cmet.2014.09.018

FIGURE CAPTION

Figure 01.

Scheme of main sources and potential mechanisms by which exposure to glycotoxins during perinatal life (eg. gestation and lactation), may potentially program to cardiometabolic diseases during adult-life.



ATTACHMENT 02:

ARTICLE 02:

Postnatal early methylglyoxal exposure induces oxidative stress and inflammatory state leading to metabolic dysfunction in adult rat offspring

Postnatal early methylglyoxal exposure induces oxidative stress and inflammatory state leading to metabolic dysfunction in adult rat offspring

Flávio Andrade Francisco[1]; Lucas Paulo Jacinto Saavedra[1]; Veridiana Mota Moreira[1]; Kelly Valério Prates[1]; Sandra da Silva Silveira[1]; Stephanie Carvalho Borges[2]; Nilza Cristina Buttow[2]; Marcos Divino Ferreira Junior[3]; Keilah Valéria Naves Cavalcante[3]; Tatiane Aparecida Ribeiro[1]; Paulo Cezar de Freitas Mathias[1] and Rodrigo Mello Gomes[3]

[1]Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringá, Maringá, PR, Brazil
[2]Department of Morphological Sciences, State University of Maringá, Maringá, PR, Brazil
[3]Department of Physiological Sciences, Federal University of Goiás, Goiânia, GO, Brazil

Short title: Postnatal early methylglyoxal induces metabolic programming

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Keywords: Metabolic programming; Diabetes; AGEs; Methylglyoxal; Dyslipidemia; Lactation.

ABSTRACT

Methylglyoxal (MG) is a precursor for the generation of endogenous AGEs that are commonly found in the processed foods and infant formulas due to industrial processing applied in their production. Postnatal (PN) early nutritional disorders are critical for the developmental origins of health and disease. This work aimed to investigate the effects of early MG exposure in progeny programming for metabolic dysfunction later in life. At delivery (PN1), the animals were divided into two groups: control group (CO), treated with saline and methylglyoxal group (MG), treated with MG (20 mg/kg of BW; i.p.) during the first two weeks of the lactation period. Throughout experimental period, food intake and body weight were evaluated daily. We evaluated CO and MG offspring at adulthood. At PN90 MG treatment decreased body weight, adipose tissue, liver and kidney masses. Differentially, MG increased food intake, blood fructosamine, insulin levels and HOMA-IR, evidencing insulin resistance. Besides, MG animals presented dyslipidemia, increased oxidative stress and inflammation. Likewise, MG offspring showed steatosis and perivascular fibrosis in the liver, increased in adipocyte, pancreatic islets, glomerular area and pericapsular fibrosis, but reduced capsular space. In conclusion, postnatal early MG exposure induces oxidative stress and inflammatory state leading to morphological disruptions. In this sense, lactation is an important trigger for health or disease programming.

1. INTRODUCTION

Modern western diets are composed of highly processed foods that are rich not only in fat, sugar and salt but also contain potentially pathogenic compounds known as advanced glycation end products (AGEs). In addition, food preparation methods that uses high temperatures (frying, baking, grilling) potentiate the production of AGEs [1, 2].

Methylglyoxal (MG) is a highly reactive dicarbonyl compound, being the important precursor in the formation of AGEs, playing an important role in its synthesis [3, 4]. Specifically, MG originates as a byproduct of glycolysis [3, 5], in a non-enzymatic reaction, by degradation process of glyceraldehyde-3-phosphate and dihydroxyketone-posphate, intermediates of the glycolytic pathway [3], may also originate in specific reactions on lipid metabolism [6, 7].

In physiological conditions, endogenously formed MG is metabolized, detoxified and converted into D-lactate by the glyoxalase system [8]. This system was first described by Darkin and Dudley in 1913, being identified in tissues such as pancreas, liver, muscle tissue, heart, kidney, blood, spleen and brain [9]. In this sense, glyoxalase system is a critical defense mechanism against the glycation of proteins, lipids and nucleic acids [10-20].

Postnatal (PN) early environmental and nutritional disorders are critical for the developmental origins of health and disease. Clinical and experimental studies have demonstrated that acute and chronic effects of these disturbances on growth and metabolism in the progeny, with long-term consequences [21-27]. There is a great interest in the possible adverse human health outcomes associated with exposure to chemical products present in common diet that leads to development of chronic non-communicable diseases, such as obesity, diabetes and hypertension. Several works evidenced that these metabolic diseases can be “programmed” during critical stages of development, such as pregnancy and lactation [26, 27].

Studies have shown that different types of stress during lactation can induce metabolic programming, leading to obese phenotype in adult offspring. In this way, maternal under- or overnutrition during PN early period promotes deleterious metabolic outcomes in the offspring [28, 29]. Besides, studies have been demonstrated that PN early overnutrition rat offspring by small litter (SL), is an animal model of obesity during suckling period. SL offspring show increased body mass from the seventh postnatal day onward, associated with development of the metabolic syndrome from weaning (PN21) until adulthood [30]. Further, SL rats show hyperphagia, hypothalamic leptin resistance and hepatic insulin resistance associated with oxidative stress in the liver [31, 32]. On the other hand, it is very important to

note that some studies have been shown an expressive protective effect of breastfeeding against obesity and diabetes in later life [33-35]. Recently, our group showed that cross-fostering during lactation normalized body weight, food intake and leptin signaling in the offspring from monosodium L-glutamate (MSG)-obese dams suckled by lean dams [36]. These findings show the great importance of adequate nutrition in the lactation period, in addition to describing this phase as a "programming window".

However, it is important to emphasize, that infant formulas are used worldwide as an important substitute for breast milk. Unfortunately, studies have demonstrated that infant formulas exhibit high levels of MG and AGEs [37-39]. In this sense, exposing infants to these nutritional contaminants during PN early in life contributes to the development of cardiometabolic disorders at adulthood.

Recently, we demonstrated that maternal treatment with MG leads to dyslipidemia and disruption on glucose homeostasis in adult rat offspring, programmed the adult offspring to present type 2 diabetic phenotype [40]. Nevertheless, we cannot attribute the effects observed in the aforementioned work to the direct effect of MG that may have passed through the milk. Thus, considering that infant formulas contain MG, it is important to evaluate the direct effect of MG administration during suckling period on the offspring. However, few studies have shown the effects of offspring MG exposure during PN early in life. Thus, our hypothesis is that the offspring MG exposure, such as present in infant formulas, in the first two weeks of lactation, leads to homeostasis impairment in adult life. Thereby, our aim was to evaluate long-term effects of postnatal early MG exposure on metabolic parameters in adult rat offspring.

2. MATERIALS AND METHODS

2.1. Ethical approval

The handling of animals and experimental procedures were done according to the rules of National Council of Animal Experiments Control (CONCEA) and the Brazilian Society of Science in Laboratory Animals (SBCAL) and approved by the Ethics Committee on Animal Use of Universidade Estadual de Maringa – CEUA/UEM (protocol number 3830171215).

2.2. Experimental design and treatment

Wistar rats (70-day-old) were housed in the Animal Facility of the Laboratory of Cell Secretion Biology, Department of Biotechnology, Genetic and Cell Biology of State

University of Maringa, in polypropylene cages (45 x 30 x 15 cm) maintained on a 12:12h light- dark cycle (0700 lights on) and controlled temperature (22.0 ± 2°C). After one week of adaptation, the animals were mated in a ratio of three females (*n* = 24) to each male (*n* = 8). Pregnant rats were accommodated in individual cages throughout the pregnancy and nursing period. At delivery (PN1), animals were divided into two groups: control group (CO; *n* = 48) offspring treated with saline (0.9 % NaCl, 1 ml/kg of BW i.p.) and MG group (MG; *n* = 48) offspring treated with MG (20 mg/kg/day of BW i.p., Sigma-Aldrich®, São Paulo, São Paulo, Brazil). Litter size was standardized for 8 pups per mother (preferentially male) to maximize lactation efficiency. Treatment started at delivery and occurred at 04:00 - 05:00 p.m. throughout the first two weeks of the suckling period. From PN14 until weaning the offspring remained with their mothers who received standard chow (Nuvital®, Curitiba, Paraná, Brazil) and had unlimited access to food and water throughout lactation period. Throughout the experimental period food intake and BW were evaluated daily.

2.3. Experimental procedures

At weaning, male offspring were housed in polypropylene cages (3 – 4 rats per cage), under same conditions of their mothers. The offspring from both groups received standard chow (Nuvital®, Curitiba, Paraná, Brazil), and had unlimited access to food and water until PN90. Body weight was evaluated throughout experimental period. At PN90 batch offspring (*n* = 12 – 15 / group) were 12-h fasted, anesthetized with sodium thiopental (45 mg/kg of BW, i.p., Thiopentax®, Cristália, Itapira, São Paulo, Brazil) and euthanized for blood, WAT, liver, pancreas and kidney sample collection. For each experimental procedure, offspring from least three litters per group were used to avoid litter effects, as previously described [41, 42].

2.4. Intravenous Glucose Tolerance Test (ivGTT)

At PN90 other batch of adult offspring (*n* = 10 – 12 / group), from both experimental groups, were anesthetized (ketamine – xylazine, 75 mg + 15 mg/kg of BW, i.m.) and then submitted to the implantation of a silicone cannula (Silastic®, Dow Corning, Midland, MI, USA) in the right jugular vein for intravenous glucose tolerance test (ivGTT). The animals were overnight fasted and then ivGTT was performed in conscious rats, as previously described [43]. Blood samples were centrifuged at (10,000 rpm for 5 min) for plasma collection and stored at -20 ºC for subsequent quantification of glucose and insulin. Animals used for the ivGTT were not used in any other experimental procedures.

2.5. Biochemical Analyses

Blood samples were centrifuged (10,000 rpm for 5 min) and plasma was used for the measurements of glucose, total cholesterol, HDL cholesterol, triglycerides and fructosamine by enzymatic-colorimetric method with specific commercial kits (Gold Analisa®, Belo Horizonte, Minas Gerais, Brazil), according to the manufacturer's instructions [40, 44]. The LDL cholesterol was calculated according to the Friedewald equation: LDL = Total cholesterol − (HDL + triglycerides/5) [45]. HOMA-IR was calculated using the formula: serum insulin (mmol/L) × (blood glucose (mmol/L)/22.5 [46].

2.6. Radioimmunoassay

Plasma insulin was measured by radioimmunoassay in gamma counter (Wizard2 Automatic Gamma Counter, TM-2470, PerkinElmer®, Shelton, CT, USA). It was used as standard human insulin and anti-rat insulin antibody (Sigma-Aldrich®, St. Louis, MO, USA) and recombinant human insulin labeled I^{125} (PerkinElmer®, Shelton, CT, USA). The intra-assay coefficients of variation were in the range 8–10 %. The limit of detection was 0.006 ng/ml [48]. The measurements were taken in a single assay.

2.7. Histological preparations

Retroperitoneal white adipose tissue (rWAT), liver, kidney and pancreas samples were fixed in 10% formalin and embedded in histological paraffin. Nonserial histological sections (5 μm thick) were performed using a Leica RM2145 microtome (Leica Biosystems, Richmond, USA). rWAT, liver and pancreas sections were stained with hematoxylin and eosin. Kidney sections were stained with Picrosirius Red and counter-stained with Hematoxylin. Digital images (TIFF 24-bit color, 2560 x 1920 pixels) were obtained with a light microscope (Olympus BX41, Tokyo, Japan) coupled to a QColor 3 Olympus camera through 40X objective lens.

2.7.1. Morphometric adipose tissue analysis

Adipocytes area were measured using 20 digital images (×400 magnification) from each animal (n = 5 animals/group). Analyses were performed using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA).

2.7.2. Morphologic liver analyses

Cross sections of the liver were performed to analyze the percentage of steatosis. Stereological analysis was performed, with a mesh of 594 points, using the Image-Pro Plus software (version 6.0, Media Cybernetics, São Paulo). The mean and standard error of mean were calculated and the results were compared between groups.

2.7.3. Morphologic kidney analyses

Coronal sections, where glomeruli with well-defined renal capillaries can be seen, were used to assess the glomerulus area and capsular space. For analysis of glomerulus count by area, 3 micrographs per field of each animal were used and then the count was performed, the result was expressed in number/field. The analysis was performed using the ICY software (Institut Pasteur, Paris, France. <http://icy.bioimageanalysis.org/>), and the mean and standard error of mean of each structure, per animal, were calculated and the results of the glomerular area and capsular space were expressed in μm^2, and compared between groups.

2.7.4. Morphometric endocrine pancreas analyses

Analyses of the pancreatic islet area were performed using 20 digital images (×400 magnification) from each animal (n = 5 animals/group). Analyses were performed using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA).

2.8. Biochemical assays of Oxidative Stress and Myeloperoxidase

Liver, kidney and pancreas samples were collected, fractionated, and processed for evaluate the biochemical markers of oxidative stress and inflammatory parameters. After being weighed, tissue portions were homogenized separately in 200 mM potassium phosphate buffer (pH 6.5). Part of the homogenate was used for quantification of reduced glutathione (GSH) levels. The other part was centrifuged for 20 minutes at 9.000 g, and the supernatant was used for catalase (CAT), superoxide dismutase (SOD), and lipid hydroperoxide (LOOH) measurements. The precipitate was used for analysis of the myeloperoxidase enzyme activity (MPO). The biochemical assays were performed as previously described [49].

2.8.1. Non-protein sulfhydryl groups levels (GSH)

Tissue GSH levels were determined according to the adapted method of Sedlak and Lindsay [50]. The reaction was performed in a 96-well plate containing the supernatant and 0.4 M TRIS-HCL buffer (pH 8.9). The reaction was started by the addition of 5,5'-dithiobis-2-

nitrobenzoic acid (DTNB). Readings were performed at 212nm using a spectrophotometer. Individual values were interpolated based on a GSH standard curve and are expressed as μg of GSH/g of tissue.

2.8.2. Catalase enzymatic activity (CAT)

CAT enzymatic activity was performed as previously described [49]. The supernatant was homogenized in potassium phosphate buffer (pH 6.5). The reaction was performed in a 96-well plate containing 5mM Tris/EDTA Buffer (pH8.0), 30% hydrogen peroxide and distilled water. Readings were performed at 240nm using a spectrophotometer.

2.8.3. Superoxide dismutase enzymatic activity (SOD)

The enzymatic assay for SOD is based on the ability of SOD to inhibit the autoxidation of pyrogallol [51]. Readings were performed at 405 nm using a spectrophotometer. The results are expressed as U of SOD/mg of protein.

2.8.4. Lipid hydroperoxide levels (LOOH)

Lipid hydroperoxide (LOOH) levels was determined, as previously described [52]. Readings were performed at 560nm using a spectrophotometer. LOOH concentrations were determined using an extinction coefficient of 4.3 mmol/mg, and the results are expressed as mmol/mg of tissue.

2.8.5. Myeloperoxidase enzyme activity (MPO)

The precipitate from liver, kidney and pancreas centrifugation were resuspended in 80mM potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium (HTAB). The reaction was performed in a 96-well plate using tetramethylbenzidine (TMB). Enzymatic activity was determined at 620 nm using a spectrophotometer. The results are expressed as units of optical density (OD)/min/mg of protein.

2.9. Statistical analyses

Statistical analysis of the data and the construction of the graphics were performed using GraphPad Prism® version 6.01 (GraphPad software, Inc., La Jolla, CA, USA). All data were submitted to D'Agostino-Pearson omnibus K2 normality test and analyzed using unpaired Student's *t* test. Results were expressed as the mean ± standard error of means (SEM) and $p<0.05$ was considered significantly different.

3. RESULTS

3.1. Effect of postnatal early MG exposure on body composition and food intake

MG offspring presented less body weight from PN10 until PN90-day-old, compared with the CO offspring ($p<0.05$; Fig. 1A – 1D). After weaning, MG offspring were hyperphagic, compared to CO offspring ($p<0.05$; Fig. 1E - 1H). MG treatment decreased naso-anal length ($p<0.01$; Fig. 1I). In addition, MG group had 2-fold ($p<0.0001$; Fig. 1J), 37% ($p<0.01$; Fig. 1K) and 5-fold ($p<0.0001$; Fig. 1L) less periepididymal, retroperitoneal and mesenteric fat mass, respectively as compared to CO. In addition, they presented adipocytes with approximately 2-fold size than the CO group ($p <0.0001$; Fig.1M and 1N).

3.2 Effect of postnatal early MG exposure on glucose homeostasis and lipid profile

Figure 2 illustrates the effect of postnatal early MG exposure on glucose homeostasis of rat offspring. Plasma glucose did not change during the ivGTT as well as in blood fasting glucose, compared to CO group (Fig. 2A, 2B and 2E). However, animals from MG group showed increased plasma insulin levels at 0, 5, 15 and 30 min, during the ivGTT ($p<0.05$; Fig 2C) leading to one-fold greater AUC in these animals, when compared with CO group ($p<0.01$; Fig. 2D). We also observed higher basal insulin levels (57%) and increased HOMA index (50%) in MG offspring ($p<0.05$; Fig 2F and 2G). Besides, blood fructosamine was higher in the MG group (+9%; $p<0.05$; Fig 2H).

In addition, Figure 2 shows the lipid profile of MG offspring in adult life. Postnatal early MG exposure did not change total cholesterol (Fig 2I). However, HDL-cholesterol and triglycerides were reduced in the MG group compared with the control group ($p<0.01$; Fig. 2J and 2K) besides showed higher LDL-cholesterol ($p<0.001$; Fig. 2L).

3.3 Effect of postnatal early MG exposure on liver oxidative stress, inflammation and metabolism

As shown in Fig. 3, adult MG offspring presented lower liver mass ($p<0.01$; Fig. 3A). Significant increases in the enzymatic activity of SOD ($p<0.01$; Fig. 3B) and CAT ($p<0.05$; Fig. 3C) were observed in the liver in MG compared to CO group. A significant decrease in GSH level was observed in the liver of MG, compared with that in the CO ($p<0.05$; Fig. 3D).That is why, liver LOOH levels was greater in MG than CO group ($p<0.01$; Fig. 3E) as well as the MPO activity ($p<0.0$; 1Fig. 3F). In addition, MG group showed elevated levels of hepatic steatosis and perivascular fibrosis ($p<0.05$; Fig. 3G – 3J).

3.4 Effect of postnatal early MG exposure on kidney oxidative stress, inflammation and metabolism

As shown in Fig. 4, adult MG offspring showed lower kidney mass ($p<0.05$; Fig. 4A). Significant increases in the enzymatic activity of SOD ($p<0.001$; Fig. 4B) and CAT ($p<0.01$; Fig. 4C) were observed in the kidney from MG compared to CO group. As depicted in Fig. 4D, renal GSH levels was lower in MG than CO group ($p<0.05$) and a significant increase in LOOH level was observed in the kidney from MG, compared with that from CO ($p<0.05$; Fig. 4E) besides MPO activity was increase in MG group when compare to CO group ($p<0.01$; Fig. 4F).

Regarding to glomerulus, we did not found any difference between the groups (Fig. 4G) but glomerular area in MG group is lager then the CO group ($p<0.05$; Fig.4H) with reduced capsular space ($p<0.01$; Fig. 4I). In addition, MG animals presented highest pericapsular fibrosis ($p<0.01$; Fig. 4J and 4K).

3.5 Effect of postnatal early MG exposure on pancreas oxidative stress, inflammation and islet area

There is no difference in pancreas weigh between the groups (Fig. 5A). However, an increase in the enzymatic activity of SOD ($p<0.05$; Fig. 5B) was observed in MG compared to CO group. Still, pancreas GSH levels was lower in MG than CO group ($p<0.05$; Fig. 5C) and a significant increase in LOOH level was observed in the pancreas of MG, compared with that in the CO ($p<0.05$; Fig. 5D) with higher MPO activity ($p<0.001$; Fig. 5E) and larger area in the islet pancreatic ($p<0.01$; Fig. 5F and 5G).

4. DISCUSSION

In this manuscript, we demonstrated that PN early MG exposure, during first two thirds of lactation, leads to development of metabolic syndrome, with glycemic and lipid dyshomeostasis. To evaluate the possibility of metabolic programming, both CO and MG groups were investigated at adult life. Interestingly, MG offspring had hyperphagia, although we observed less body weight combined with decreased fat stores. Furthermore, MG animals showed insulin resistance, dyslipidemia, disorders in oxidative stress parameters and inflammatory state at adulthood. Thus, we show for the first time, that the PN early MG exposure leads to metabolic syndrome in adult rat offspring.

At PN90 MG group presented, less body weight and body length, it is possible that MG causes a deleterious effect on growth hormone (GH) release or inhibition of their action on tissue development, such as type 1 diabetic patient, as previously suggested [45]. This reduced body weight in MG animals can also be caused by impairment of adipose tissue development, as well as in the other tissues. Rodrigues *et al*. observed that MG further impairs adipose tissue metabolism by the decrease of blood supply [53]. In this sense, studies have shown the role of hypoxia on adipose tissue dysfunction and consequent decrease of adipocytokine secretion. Normal expansion of adipocytes leads to increased cell volume, making the distance between central and peripheral adipocytes greater than the maximum oxygen diffusion distance. Thus, disturbance in tissue oxygenation, inefficient angiogenesis and vascular network impairment may probably be the basis of questions related to adipose tissue dysfunctions with regard to adequate adipocyte growth and accumulation of fat stores [54-60]. However, the mechanisms directly involved in the microvascularization of adipose tissue are still unknown. Perhaps, protein glycation contributes directly to vascular dysfunction in adipose tissue during chronic treatment with MG [53]. MG has a direct effect on neo-angiogenesis in adipose tissue, compromising its healthy growth due to the decrease in tissue vascularization. It has been observed that MG reduced HIF-2α expression, which compromises angiogenesis and consequent tissue expansivity [61]. These previous findings reflect the effects on adipose tissue impairment caused by MG exposure during 14 days, as well as the presented here in our study. However, we show for the first time, that precocious MG exposure leads to lower body weight, despite a higher food intake throughout life. Decreased adipose tissue mass, as expected, can be linked with to lower production of leptin, which can be the key to understanding the increased food intake. Unfortunately, we do not investigate this parameter in this work.

As opposed to the work of Rodrigues et al, 2017, we observed in our study that adipocytes in the MG group are larger than in the CO group, although the adipose tissue is reduced in MG animals as a whole. It is well known that MG leads to a lower expression of PPAR gamma, which is an important factor for adipogenesis [61], and that a decrease in the expression of PPAR gamma leads to a decrease in adipogenesis [86]. Thus, angiogenic dysfunction leads to a decrease in adipose tissue, and a decrease in the expression of PPAR gamma leads to an increase in adipocytes.Therefore, what leads to a decrease in adipose tissue as a whole is angiogenic dysfunction and what leads to an increase in adipocytes is a decrease in the expression of PPAR gamma.

In this study, we clearly demonstrate, by ivGTT and HOMA-IR, that MG animals were insulin resistant. Previously studies have already demonstrated the effects of MG on insulin pathway and its intimate relationship between high blood MG levels and insulin resistance in humans [62], rodents [63] and cell culture [64].

It is known that dicarbonyl stress has an important role in the development of type 2 diabetes, as well as in insulin resistance, mainly due to its toxicity to the pancreatic beta cell [69]. This toxic effect can lead to pancreatic islets hypertrophy, caused by the decrease in beta cell function and increased reactive oxygen species production [87].

Several studies have shown that the quantification of the levels of fructosamine is relevant to evaluate the level of total glycated proteins [65, 66]. In our study, MG animals have increased blood fructosamine levels. In a previous study, we demonstrated that maternal MG treatment, during lactation, increased blood and milk fructosamine levels; further, their pups have increased blood fructosamine levels at adult life [40]. In the present study, we show that direct exogenous MG i.p. injections in offspring also leads to increased blood fructosamine levels.

Among the factors that characterize the metabolic syndrome, dyslipidemia is one of them and as it is known is strongly related to cardiovascular diseases. Furthermore, it is also known that lipids are an important source of protein modifying, in the formation of advanced lipoxidation agents (ALEs), directly related to the formation of atherosclerosis plaques [67-69]. Our study clearly shows that the MG animals have dyslipidemia, which is consistent with other studies demonstrating that MG is associated with dyslipidemia, showing decrease HDL cholesterol, mainly by changes in the methionine and tyrosine of apolipoprotein A1 (ApoA1) [70]. In addition, another study shows that glycated HDL has a lower content of sphingosine-1-phosphate, contributing to structural and functional modification of HDL [71]. Thus, MG animals present an increased risk to development cardiovascular disease [47].

Elevated MG levels are found parallel to oxidative stress as well as AGEs; however, not always these data are accompanied by high blood glucose levels [72-75]. In this study, MG animals showed higher activity of SOD and CAT in the liver and kidney, and only SOD in the pancreas, which can indicate an increase of reactive oxygen species, and an increase in AGE levels in the liver, that is closely related to the development of hepatic steatosis at an early stage of the development of non-alcoholic fatty liver disease [88, 89], as well as inflammatory infiltrates and the development of fibrosis [89]. However, all the studies in the literature related similar data due to MG exposure at adult life, while our study for the first time show this due to exposure in early period with effects at adulthood.

Lipid peroxidation is one of the outcomes of reactions with unstable molecules, and LOOH levels are an indirect measure of damage that is caused by oxidative stress [76]. In the current study, we observed a significant increase in LOOH in the liver, kidney and pancreas. These findings are consistent with previous studies, showing a clear relationship between dicarbonyl stress and the glycation of membrane phospholipids [69]. Moreover, studies have shown that HDL cholesterol is an important protective agent against oxidative stress [71].

Berlanga *et al*. demonstrated that there is an intimate relationship between high levels of MG and AGEs and renal diseases [45]; however, more studies are needed to confirm whether these diseases are directly related to kidney mass loss. We found in this study that the early exposure to MG leads to morphological kidney modification. Development of chronic kidney disease, renal fibrosis and the onset of kidney failure is directly related to the increased levels of MG [90], leading to whole organ injury, which includes lower glomerular capsule and filtration rate, in addition to the well stablished role in the reactive oxygen species generation [91].

When AGEs binding to their respective receptors (RAGE) it causes numerous modifications in cell cycle, with significant alteration in the translation of target genes responsible for the expression of adhesion molecules, endothelial growth factors and inflammatory cytokines [77-80]. In addition, there is a suppression of GSH activity, which decreases the activity of glyoxalase system and impairs detoxification of MG, leading to increasing levels of MG and circulating AGEs [81]. Moreover, in our study, MG group showed decreased GSH, which is also involved in elevated oxidative stress in these animals.

Our results clearly demonstrate that the MG group presents higher levels of MPO in the liver, kidney and pancreas indicating inflammatory process already established in these tissues. The relationship of oxidative stress, inflammation, AGEs and their precursors, and their relevant role in the development of renal diseases, is already known [82]. In addition, studies have shown that a diet with low levels of AGEs had a significant reducing effect on inflammatory markers, oxidative stress and improved insulin sensitivity in resistant patients [83-85].

In conclusion, the present study shows that postnatal early MG exposure, induces oxidative stress and inflammation state, which can induces metabolic dysfunction onset, such as dyslipidemia, hyperinsulinemia and insulin resistance, increasing the risk of cardiovascular disease. All together, these observations confirm lactation as an important period for health or disease programming, and suggest the careful use of infant formulas in the newborn diets.

Declaration of interest

The authors declare no conflict of interest.

Author contributions

F.A.F., P.C.F.M. and R.M.G. designed research. F.A.F., L.P.J.S., V.M.M., K.V.P., S.S.S. and T.A.R., animal treatment, sample collection and measurements. S.C.B and N.C.B. biochemical assays of oxidative stress and myeloperoxidase. F.A.F., M.D.F.J., R.M.G., analysis and interpretation of data. F.A.F. and R.M.G. wrote the manuscript. All authors reviewed the manuscript.

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REFERENCES

[1] Melo AM, Benatti RO, Ignacio-Souza LM, Okino C, Torsoni AS, Milanski M, et al. Hypothalamic endoplasmic reticulum stress and insulin resistance in offspring of mice dams fed high-fat diet during pregnancy and lactation. Metabolism. 2014;63:682-92.

[2] Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Maternal and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. Endocrinology. 2008;149:5348-56.

[3] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414:813-20.

[4] Giardino I, Edelstein D, Brownlee M. Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity. A model for intracellular glycosylation in diabetes. J Clin Invest. 1994;94:110-7.

[5] Phillips SA, Thornalley PJ. The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. Eur J Biochem. 1993;212:101-5.

[6] Miyata T, Inagi R, Asahi K, Yamada Y, Horie K, Sakai H, et al. Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids. FEBS Lett. 1998;437:24-8.

[7] Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW. Alterations in nonenzymatic biochemistry in uremia: origin and significance of "carbonyl stress" in long-term uremic complications. Kidney Int. 1999;55:389-99.

[8] McLellan AC, Phillips SA, Thornalley PJ. The assay of S-D-lactoylglutathione in biological systems. Anal Biochem. 1993;211:37-43.

[9] Dakin H, Dudley H. An enzyme concerned with the formation of hydroxy acids from ketonic aldehydes. Journal of Biological Chemistry. 1913;14:155-7.

[10] Maessen DE, Stehouwer CD, Schalkwijk CG. The role of methylglyoxal and the glyoxalase system in diabetes and other age-related diseases. Clin Sci (Lond). 2015;128:839-61.

[11] Neuberg C. Uber die zerstorung von milch saurealdehyd und methylglyoxal durch tierische organe. Biochem Z. 1913;49:502-6.

[12] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615-25.

[13] Nishikawa T, Edelstein D, Brownlee M. The missing link: a single unifying mechanism for diabetic complications. Kidney Int Suppl. 2000;77:S26-30.

[14] Monnier VM, Taniguchi N. Advanced glycation in diabetes, aging and age-related diseases: editorial and dedication. Glycoconj J. 2016;33:483-6.

[15] Monnier VM, Sun W, Sell DR, Fan X, Nemet I, Genuth S. Glucosepane: a poorly understood advanced glycation end product of growing importance for diabetes and its complications. Clin Chem Lab Med. 2014;52:21-32.

[16] Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation - a mini-review. Gerontology. 2012;58:227-37.

[17] Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, Lyons TJ, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem. 2000;275:39027-31.

[18] Wells-Knecht MC, Thorpe SR, Baynes JW. Pathways of formation of glycoxidation products during glycation of collagen. Biochemistry. 1995;34:15134-41.

[19] Lal S, Szwergold BS, Taylor AH, Randall WC, Kappler F, Wells-Knecht K, et al. Metabolism of fructose-3-phosphate in the diabetic rat lens. Arch Biochem Biophys. 1995;318:191-9.

[20] Murata-Kamiya N, Kamiya H. Methylglyoxal, an endogenous aldehyde, crosslinks DNA polymerase and the substrate DNA. Nucleic Acids Res. 2001;29:3433-8.

[21] Barker CJ, Berggren PO. Inositol hexakisphosphate and beta-cell stimulus-secretion coupling. Anticancer Res. 1999;19:3737-41.

[22] Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35:595-601.

[23] Lesage J, Sebaai N, Leonhardt M, Dutriez-Casteloot I, Breton C, Deloof S, et al. Perinatal maternal undernutrition programs the offspring hypothalamo-pituitary-adrenal (HPA) axis. Stress. 2006;9:183-98.

[24] Martins PA, Sawaya AL. Evidence for impaired insulin production and higher sensitivity in stunted children living in slums. Br J Nutr. 2006;95:996-1001.

[25] Vieau D, Sebaai N, Leonhardt M, Dutriez-Casteloot I, Molendi-Coste O, Laborie C, et al. HPA axis programming by maternal undernutrition in the male rat offspring. Psychoneuroendocrinology. 2007;32 Suppl 1:S16-20.

[26] Moura AS, Carpinelli AR, Barbosa FB, Gravena C, Mathias PC. Undernutrition during early lactation as an alternative model to study the onset of diabetes mellitus type II. Res Commun Mol Pathol Pharmacol. 1996;92:73-84.

[27] de Oliveira JC, Scomparin DX, Andreazzi AE, Branco RC, Martins AG, Gravena C, et al. Metabolic imprinting by maternal protein malnourishment impairs vagal activity in adult rats. J Neuroendocrinol. 2011;23:148-57.

[28] de Oliveira JC, de Moura EG, Miranda RA, de Moraes AMP, Barella LF, da Conceicao EPS, et al. Low-protein diet in puberty impairs testosterone output and energy metabolism in male rats. J Endocrinol. 2018;237:243-54.

[29] Ribeiro TA, Tofolo LP, Martins IP, Pavanello A, de Oliveira JC, Prates KV, et al. Maternal low intensity physical exercise prevents obesity in offspring rats exposed to early overnutrition. Scientific reports. 2017;7:7634.

[30] de Almeida DL, Fabricio GS, Trombini AB, Pavanello A, Tofolo LP, da Silva Ribeiro TA, et al. Early overfeed-induced obesity leads to brown adipose tissue hypoactivity in rats. Cell Physiol Biochem. 2013;32:1621-30.

[31] Rodrigues AL, de Moura EG, Passos MC, Dutra SC, Lisboa PC. Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. J Physiol. 2009;587:2647-61.

[32] Conceição EP, Franco JG, Oliveira E, Resende AC, Amaral TA, Peixoto-Silva N, et al. Oxidative stress programming in a rat model of postnatal early overnutrition--role of insulin resistance. J Nutr Biochem. 2013;24:81-7.

[33] von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V, et al. Breast feeding and obesity: cross sectional study. Bmj. 1999;319:147-50.

[34] Grunewald M, Hellmuth C, Demmelmair H, Koletzko B. Excessive weight gain during full breast-feeding. Annals of nutrition & metabolism. 2014;64:271-5.

[35] Rodekamp E, Harder T, Kohlhoff R, Franke K, Dudenhausen JW, Plagemann A. Long-term impact of breast-feeding on body weight and glucose tolerance in children of diabetic mothers: role of the late neonatal period and early infancy. Diabetes Care. 2005;28:1457-62.

[36] Miranda RA, da Silva Franco CC, de Oliveira JC, Barella LF, Tofolo LP, Ribeiro TA, et al. Cross-fostering reduces obesity induced by early exposure to monosodium glutamate in male rats. Endocrine. 2016.

[37] Federico G, Gori M, Randazzo E, Vierucci F. Skin advanced glycation end-products evaluation in infants according to the type of feeding and mother's smoking habits. SAGE open medicine. 2016;4:2050312116682126.

[38] Kutlu T. Dietary glycotoxins and infant formulas. Turk pediatri arsivi. 2016;51:179-85.

[39] Pischetsrieder M, Henle T. Glycation products in infant formulas: chemical, analytical and physiological aspects. Amino Acids. 2012;42:1111-8.

[40] Francisco FA, Barella LF, Silveira SDS, Saavedra LPJ, Prates KV, Alves VS, et al. Methylglyoxal treatment in lactating mothers leads to type 2 diabetes phenotype in male rat offspring at adulthood. Eur J Nutr. 2018;57:477-86.

[41] Malta A, Souza AA, Ribeiro TA, Francisco FA, Pavanello A, Prates KV, et al. Neonatal treatment with scopolamine butylbromide prevents metabolic dysfunction in male rats. Scientific reports. 2016;6:30745.

[42] Gomes RM, de Paulo LF, Bonato Panizzon C, Neves CQ, Cordeiro BC, Zanoni JN, et al. Anti-Diabetic Effects of the Ethyl-Acetate Fraction of Trichilia catigua in Streptozo-tocin-Induced Type 1 Diabetic Rats. Cell Physiol Biochem. 2017;42:1087-97.

[43] Gomes RM, Tofolo LP, Rinaldi W, Scomparin DX, Grassiolli S, Barella LF, et al. Moderate exercise restores pancreatic beta-cell function and autonomic nervous system activity in obese rats induced by high-fat diet. Cell Physiol Biochem. 2013;32:310-21.

[44] Degen J, Vogel M, Richter D, Hellwig M, Henle T. Metabolic transit of dietary methylglyoxal. J Agric Food Chem. 2013;61:10253-60.

[45] Berlanga J, Cibrian D, Guillen I, Freyre F, Alba JS, Lopez-Saura P, et al. Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. Clin Sci (Lond). 2005;109:83-95.

[46] Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Archives of endocrinology and metabolism. 2016;60:138-42.

[47] Guarda DS, Lisboa PC, de Oliveira E, Nogueira-Neto JF, de Moura EG, Figueiredo MS. Flaxseed oil during lactation changes milk and body composition in male and female suckling pups rats. Food Chem Toxicol. 2014;69:69-75.

[48] Fagundes AT, Moura EG, Passos MC, Santos-Silva AP, de Oliveira E, Trevenzoli IH, et al. Temporal evaluation of body composition, glucose homeostasis and lipid profile of male rats programmed by maternal protein restriction during lactation. Horm Metab Res. 2009;41:866-73.

[49] Borges SC, Ferreira PEB, da Silva LM, de Paula Werner MF, Irache JM, Cavalcanti OA, et al. Evaluation of the treatment with resveratrol-loaded nanoparticles in intestinal injury model caused by ischemia and reperfusion. Toxicology. 2018;396:13-22.

[50] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25:192-205.

[51] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-74.

[52] Jiang ZY, Woollard AC, Wolff SP. Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids. 1991;26:853-6.

[53] Rodrigues T, Matafome P, Seica R. Methylglyoxal further impairs adipose tissue metabolism after partial decrease of blood supply. Arch Physiol Biochem. 2013;119:209-18.

[54] Gaens KH, Ferreira I, van der Kallen CJ, van Greevenbroek MM, Blaak EE, Feskens EJ, et al. Association of polymorphism in the receptor for advanced glycation end products (RAGE) gene with circulating RAGE levels. J Clin Endocrinol Metab. 2009;94:5174-80.

[55] Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9:367-77.

[56] Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes. 2007;56:901-11.

[57] Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol. 2010;314:1-16.

[58] Regazzetti C, Peraldi P, Gremeaux T, Najem-Lendom R, Ben-Sahra I, Cormont M, et al. Hypoxia decreases insulin signaling pathways in adipocytes. Diabetes. 2009;58:95-103.

[59] Rutkowski JM, Davis KE, Scherer PE. Mechanisms of obesity and related pathologies: the macro- and microcirculation of adipose tissue. FEBS J. 2009;276:5738-46.

[60] Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Br J Nutr. 2008;100:227-35.

[61] Rodrigues T, Matafome P, Sereno J, Almeida J, Castelhano J, Gamas L, et al. Methylglyoxal-induced glycation changes adipose tissue vascular architecture, flow and expansion, leading to insulin resistance. Scientific reports. 2017;7:1698.

[62] Biswas UK, Kumar A. Study on the changes of carbonic anhydrase activity in insulin resistance and the effect of methylglyoxal. J Pak Med Assoc. 2012;62:417-21.

[63] Sheader EA, Benson RS, Best L. Cytotoxic action of methylglyoxal on insulin-secreting cells. Biochem Pharmacol. 2001;61:1381-6.

[64] Riboulet-Chavey A, Pierron A, Durand I, Murdaca J, Giudicelli J, Van Obberghen E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. Diabetes. 2006;55:1289-99.

[65] Lima Nda S, de Moura EG, Passos MC, Nogueira Neto FJ, Reis AM, de Oliveira E, et al. Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. Br J Nutr. 2011;105:1405-13.

[66] Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovasc Diabetol. 2008;7:29.

[67] Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J. 1990;269:1-11.

[68] Franco JG, Fernandes TP, Rocha CP, Calvino C, Pazos-Moura CC, Lisboa PC, et al. Maternal high-fat diet induces obesity and adrenal and thyroid dysfunction in male rat offspring at weaning. J Physiol. 2012;590:5503-18.

[69] Rabbani N, Xue M, Thornalley PJ. Dicarbonyls and glyoxalase in disease mechanisms and clinical therapeutics. Glycoconj J. 2016;33:513-25.

[70] Godfrey L, Yamada-Fowler N, Smith J, Thornalley PJ, Rabbani N. Arginine-directed glycation and decreased HDL plasma concentration and functionality. Nutrition & diabetes. 2014;4:e134.

[71] Brinck JW, Thomas A, Lauer E, Jornayvaz FR, Brulhart-Meynet MC, Prost JC, et al. Diabetes Mellitus Is Associated With Reduced High-Density Lipoprotein Sphingosine-1-Phosphate Content and Impaired High-Density Lipoprotein Cardiac Cell Protection. Arterioscler Thromb Vasc Biol. 2016;36:817-24.

[72] Wang X, Desai K, Clausen JT, Wu L. Increased methylglyoxal and advanced glycation end products in kidney from spontaneously hypertensive rats. Kidney Int. 2004;66:2315-21.

[73] Wang X, Desai K, Chang T, Wu L. Vascular methylglyoxal metabolism and the development of hypertension. J Hypertens. 2005;23:1565-73.

[74] Chen C, Hosokawa H, Bumbalo LM, Leahy JL. Mechanism of compensatory hyperinsulinemia in normoglycemic insulin-resistant spontaneously hypertensive rats. Augmented enzymatic activity of glucokinase in beta-cells. J Clin Invest. 1994;94:399-404.

[75] Sechi LA, Griffin CA, Giacchetti G, Zingaro L, Catena C, Bartoli E, et al. Abnormalities of insulin receptors in spontaneously hypertensive rats. Hypertension. 1996;27:955-61.

[76] Borges SC, Ferreira PEB, da Silva LM, de Paula Werner MF, Irache JM, Cavalcanti OA, et al. Evaluation of the treatment with resveratrol-loaded nanoparticles in intestinal injury model caused by ischemia and reperfusion. Toxicology. 2018;396-397:13-22.

[77] Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation. 2006;114:597-605.

[78] Bierhaus A, Humpert PM, Morcos M, Wendt T, Chavakis T, Arnold B, et al. Understanding RAGE, the receptor for advanced glycation end products. Journal of molecular medicine. 2005;83:876-86.

[79] Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001;108:949-55.

[80] Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, et al. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. Diabetes. 2001;50:2792-808.

[81] Thornalley PJ. Glutathione-dependent detoxification of alpha-oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. Chem Biol Interact. 1998;111-112:137-51.

[82] Uribarri J, del Castillo MD, de la Maza MP, Filip R, Gugliucci A, Luevano-Contreras C, et al. Dietary advanced glycation end products and their role in health and disease. Advances in nutrition. 2015;6:461-73.

[83] Uribarri J, Cai W, Ramdas M, Goodman S, Pyzik R, Chen X, 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;34:1610-6.

[84] Mark AB, Poulsen MW, Andersen S, Andersen JM, Bak MJ, Ritz C, 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.

[85] Vlassara H. Advanced glycation in health and disease: role of the modern environment. Ann N Y Acad Sci. 2005;1043:452-60.

[86] Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. [The adipose tissue as a regulatory center of the metabolism]. Arq Bras Endocrinol Metabol. 2006;50:216-29.

[87] Franco JG, Dias-Rocha CP, Fernandes TP, Albuquerque Maia L, Lisboa PC, Moura EG, et al. Resveratrol treatment rescues hyperleptinemia and improves hypothalamic leptin signaling programmed by maternal high-fat diet in rats. Eur J Nutr. 2016;55:601-10.

[88] Cha SH, Hwang Y, Heo SJ, Jun HS. Indole-4-carboxaldehyde Isolated from Seaweed, Sargassum thunbergii, Attenuates Methylglyoxal-Induced Hepatic Inflammation. Marine drugs. 2019;17.

[89] Neves C, Rodrigues T, Sereno J, Simoes C, Castelhano J, Goncalves J, et al. Dietary Glycotoxins Impair Hepatic Lipidemic Profile in Diet-Induced Obese Rats Causing Hepatic Oxidative Stress and Insulin Resistance. Oxidative medicine and cellular longevity. 2019;2019:6362910.

[90] Markova I, Huttl M, Oliyarnyk O, Kacerova T, Haluzik M, Kacer P, et al. The effect of dicarbonyl stress on the development of kidney dysfunction in metabolic syndrome - a transcriptomic and proteomic approach. Nutr Metab (Lond). 2019;16:51.

[91] Rodrigues L, Matafome P, Crisostomo J, Santos-Silva D, Sena C, Pereira P, et al. Advanced glycation end products and diabetic nephropathy: a comparative study using diabetic and normal rats with methylglyoxal-induced glycation. J Physiol Biochem. 2014;70:173-84.

FIGURE CAPTIONS

Fig 1. Effect of postnatal early MG exposure on body composition and food intake. Body weight evolution before weaning (A), body weight at PN21 (B), body weight evolution after weaning (C), body weight at PN90 (D), food intake (E), AUC food intake (F), relative food intake (G) and AUC relative food intake (H), body length (I), periepididymal fat (J), retroperitoneal fat (K) and mesenteric fat (L), adipocyte area (M) and representative histological image of adipose tissue (N). Data are presented as mean ± SEM (n = 12 – 15). To compare the experimental groups Student's t test was used, where *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Fig 2. Effect of postnatal early MG exposure on glucose homeostasis and lipid profile. Blood glucose during ivGTT (A), AUC of blood glucose (B), blood insulin during ivGTT (C), AUC of insulin ivGTT (D), fasting blood glucose (E), fasting blood insulin (F), HOMA-IR (G), blood fructosamine (H), total cholesterol (I), HDL cholesterol (J), triglycerides (K), LDL cholesterol (L). Data are presented as mean ± SEM (n = 10 – 15). To compare the experimental groups Student's t test was used, where *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Fig 3. Effect of postnatal early MG exposure on liver oxidative stress, inflammation and metabolism. Liver mass (A), hepatic SOD (B), hepatic CAT (C), hepatic GSH (D), hepatic LOOH (E), hepatic MPO (F), hepatic steatosis (G), representative histological images of hepatic steatosis (H), perivascular hepatic fibrosis (I) and representative histological images of perivascular hepatic fibrosis (J). Data are presented as mean ± SEM (n = 8 – 10). To compare the experimental groups Student's t test was used, where *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

Fig 4. Effect of postnatal early MG exposure on kidney oxidative stress, inflammation and metabolism. Kidney mass (A), kidney SOD (B), kidney CAT (C), kidney GSH (D), kidney LOOH (E), kidney MPO (F), glomeruli per field (G), glomerular area (H), capsular space (I), pericapsular fibrosis (J) and representative histological pericapsular fibrosis (K). Data are presented as mean ± SEM (n = 8 – 10). To compare the experimental groups Student's t test was used, where *p<0.05, **p<0.01 and ***p<0.001.

Fig 5. Effect of postnatal early MG exposure on pancreas oxidative stress, inflammation and islet area. Pericapsular mass (A), pancreas SOD (B), pancreas GSH (C), pancreas LOOH (D), pancreas MPO (E), islet area (F), and representative histological image of islet area (G). Data are presented as mean ± SEM (n = 8 – 10). To compare the experimental groups Student's t test was used, where *p<0.05, **p<0.01 and ***p<0.001.

Figure 1

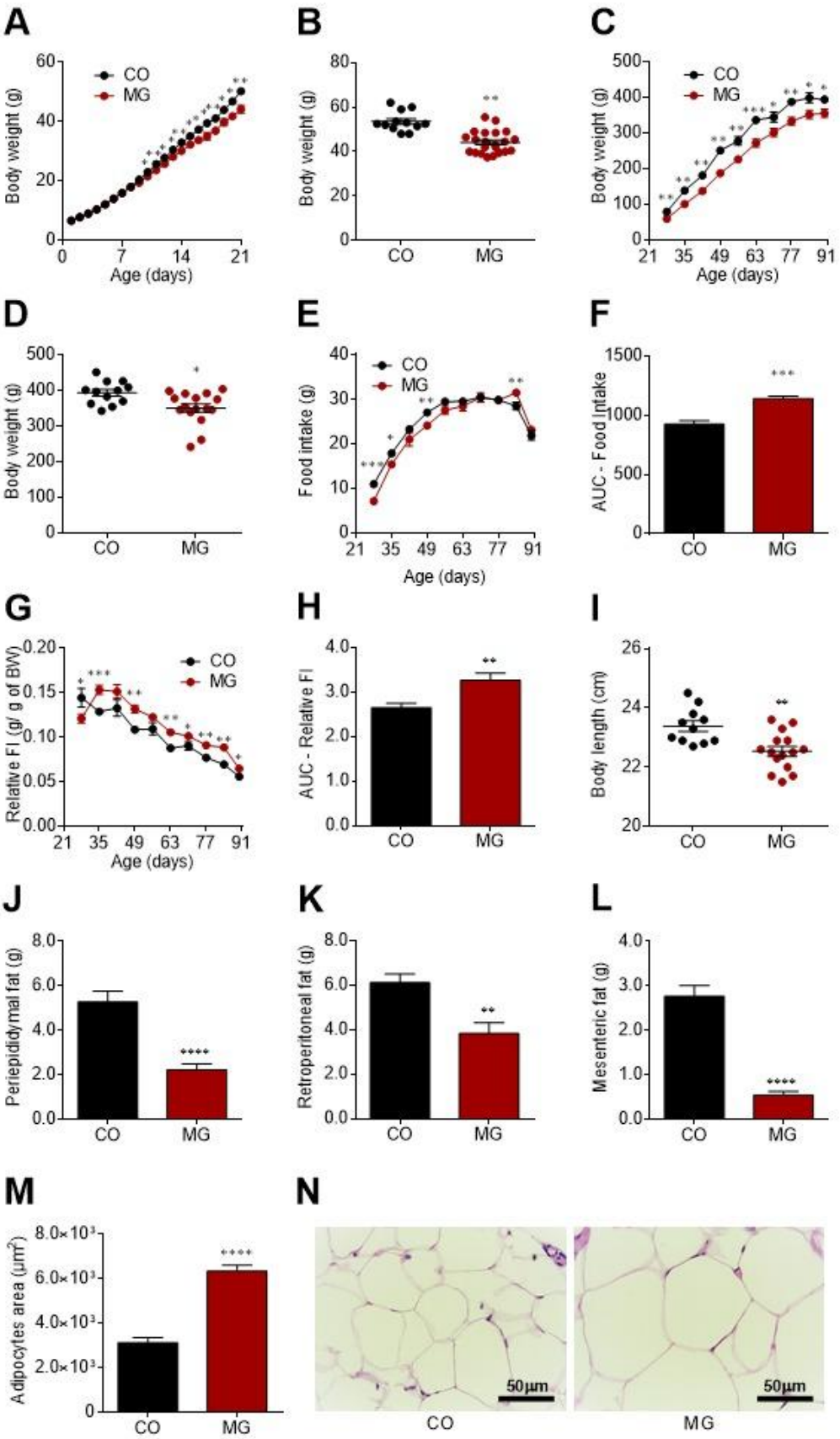

A
Body weight (g)
CO
MG
Age (days)
B
Body weight (g)
CO
MG
C
Body weight (g)
CO
MG
Age (days)
D
Body weight (g)
CO
MG
E
Food intake (g)
CO
MG
Age (days)
F
AUC - Food Intake
CO
MG
G
Relative FI (g/ g of BW)
CO
MG
Age (days)
H
AUC - Relative FI
CO
MG
I
Body length (cm)
CO
MG
J
Periepididymal fat (g)
CO
MG
K
Retroperitoneal fat (g)
CO
MG
L
Mesenteric fat (g)
CO
MG
M
Adipocytes area (µm²)
CO
MG
N
50µm
50µm
CO
MG

Figure 2

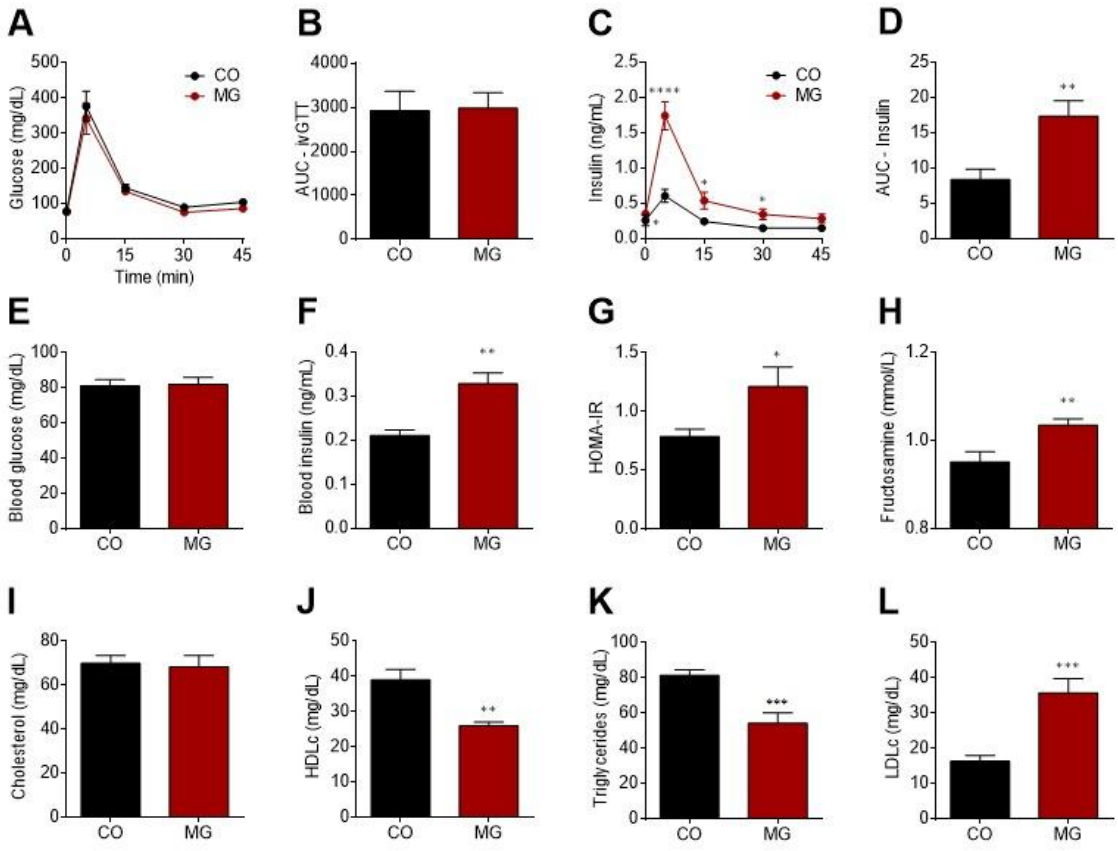

A
Glucose (mg/dL)
Time (min)
CO
MG
B
AUC - IVGTT
C
Insulin (ng/mL)
D
AUC - Insulin
E
Blood glucose (mg/dL)
F
Blood insulin (ng/mL)
G
HOMA-IR
H
Fructosamine (mmol/L)
I
Cholesterol (mg/dL)
J
HDLc (mg/dL)
K
Triglycerides (mg/dL)
L
LDLc (mg/dL)

Figure 3

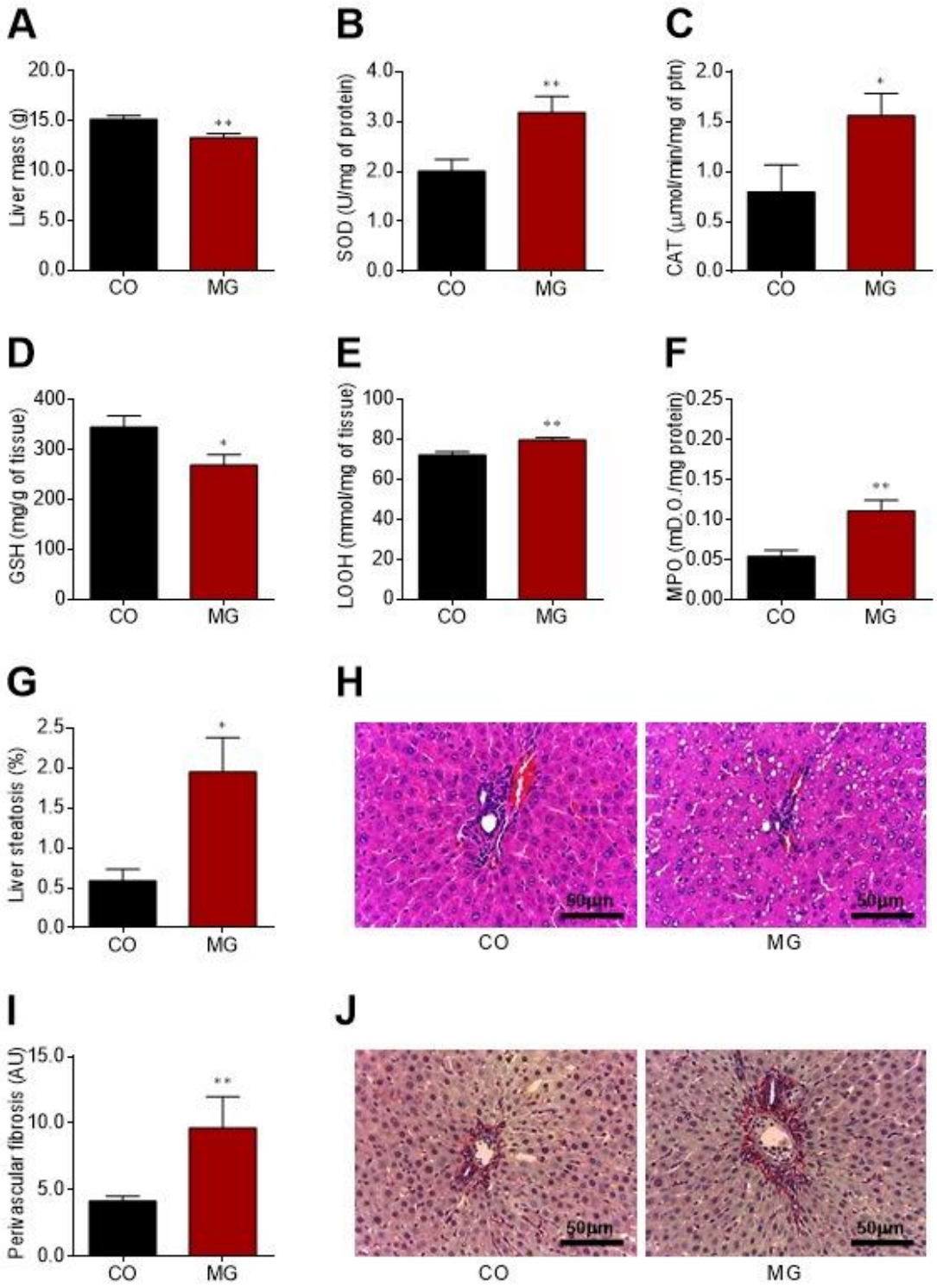

A
Liver mass (g)
20.0
15.0
10.0
5.0
0.0
++
CO
MG
B
SOD (U/mg of protein)
4.0
3.0
2.0
1.0
0.0
**
CO
MG
C
CAT (µmol/min/mg of ptn)
2.0
1.5
1.0
0.5
0.0
*
CO
MG
D
GSH (mg/g of tissue)
400
300
200
100
0
*
CO
MG
E
LOOH (mmol/mg of tissue)
100
80
60
40
20
0
**
CO
MG
F
MPO (mD.O./mg protein)
0.25
0.20
0.15
0.10
0.05
0.00
**
CO
MG
G
Liver steatosis (%)
2.5
2.0
1.5
1.0
0.5
0.0
*
CO
MG
H
50µm
50µm
CO
MG
I
Perivascular fibrosis (AU)
15.0
10.0
5.0
0.0
**
CO
MG
J
50µm
50µm
CO
MG

Figure 4

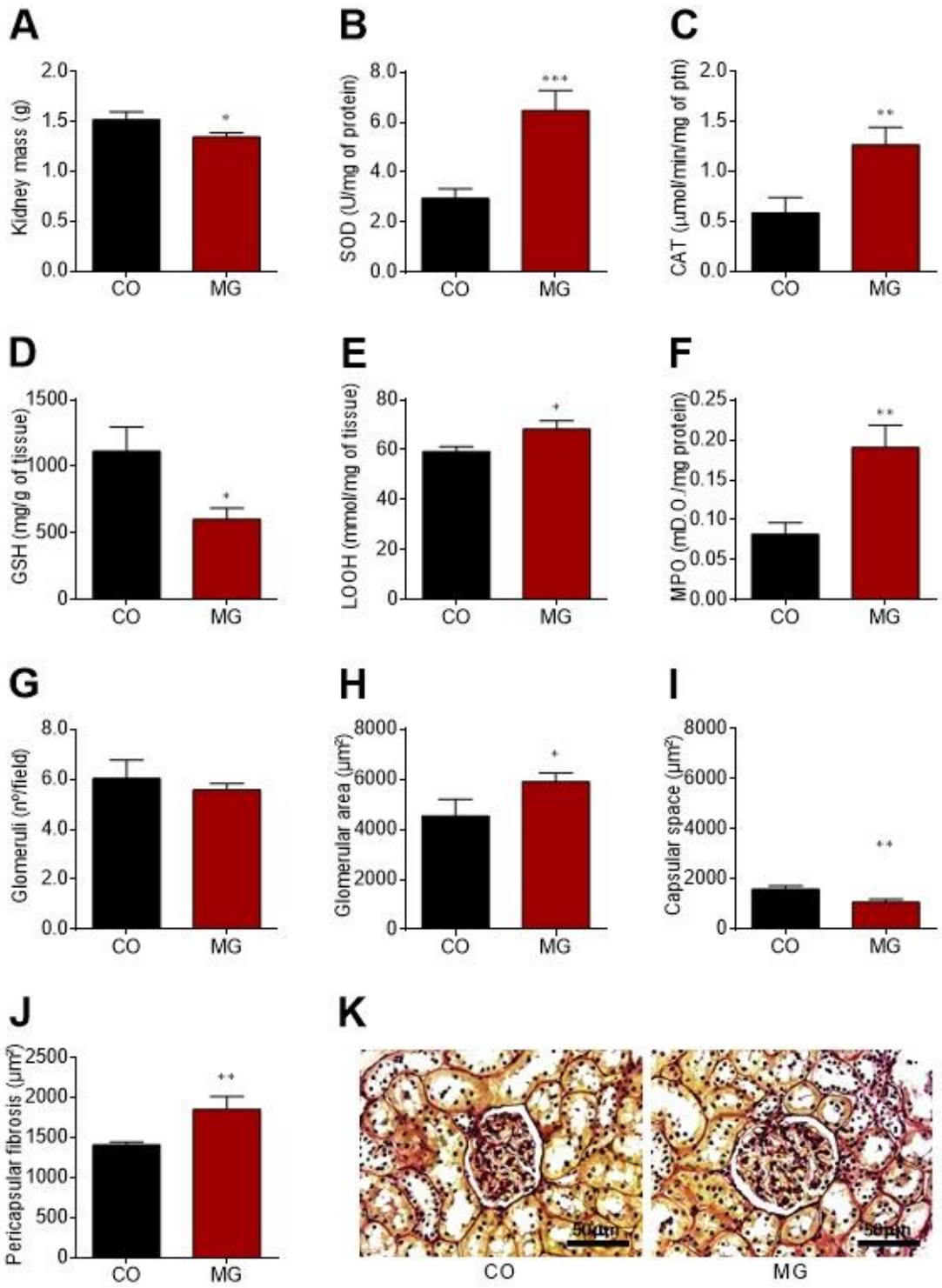
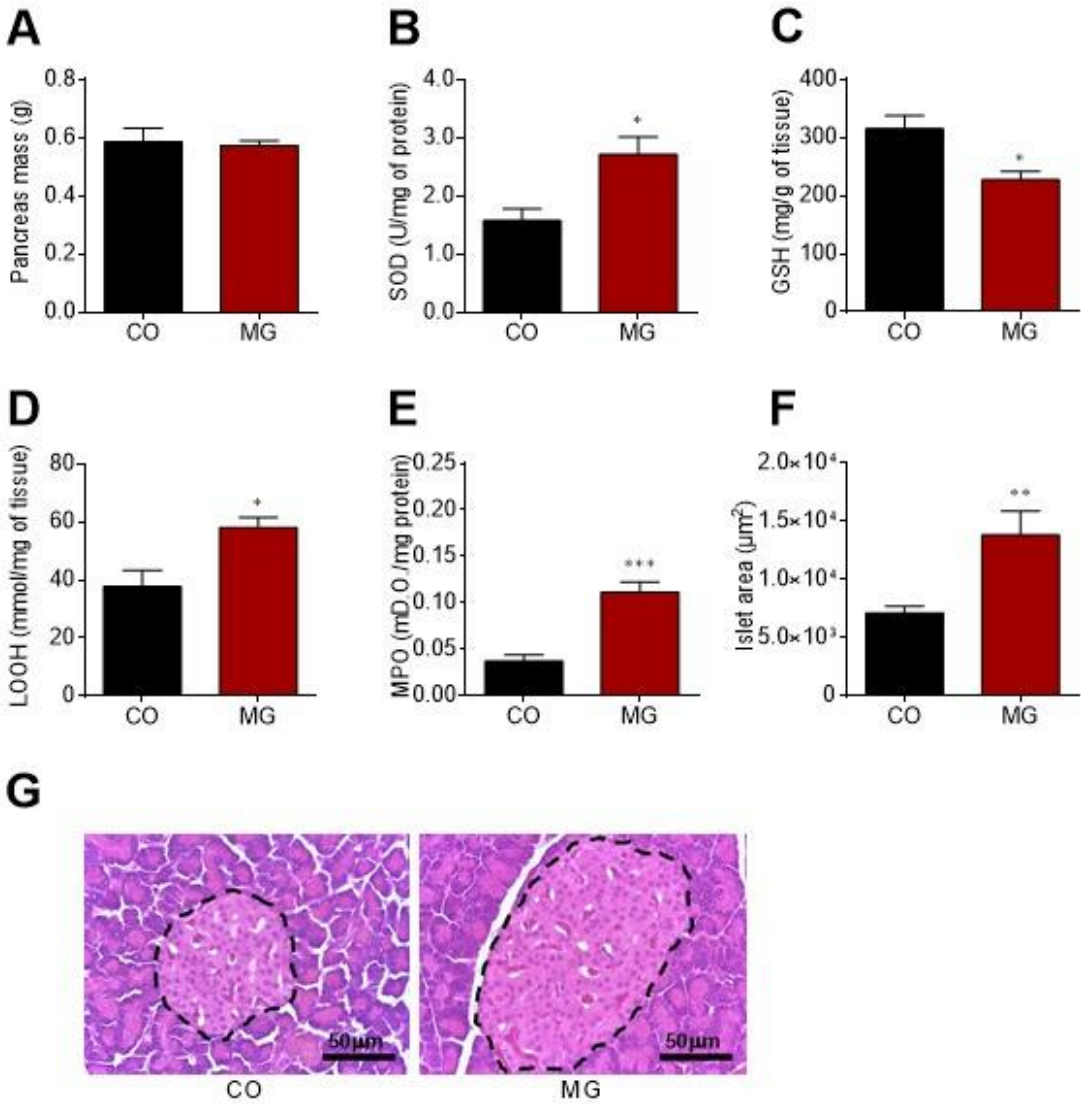

A
Kidney mass (g)
2.0
1.5
1.0
0.5
0.0
*
CO
MG
B
SOD (U/mg of protein)
8.0
6.0
4.0
2.0
0.0
***
CO
MG
C
CAT (µmol/min/mg of ptn)
2.0
1.5
1.0
0.5
0.0
**
CO
MG
D
GSH (mg/g of tissue)
1500
1000
500
0
*
CO
MG
E
LOOH (mmol/mg of tissue)
80
60
40
20
0
*
CO
MG
F
MPO (mD.O./mg protein)
0.25
0.20
0.15
0.10
0.05
0.00
**
CO
MG
G
Glomeruli (nº/field)
8.0
6.0
4.0
2.0
0.0
CO
MG
H
Glomerular area (µm²)
8000
6000
4000
2000
0
*
CO
MG
I
Capsular space (µm²)
8000
6000
4000
2000
0
++
CO
MG
J
Pericapsular fibrosis (µm²)
2500
2000
1500
1000
500
0
++
CO
MG
K
50µm
50µm
CO
MG

Figure 5


A
Pancreas mass (g)
0.8
0.6
0.4
0.2
0.0
CO
MG
B
SOD (U/mg of protein)
4.0
3.0
2.0
1.0
0.0
*
CO
MG
C
GSH (mg/g of tissue)
400
300
200
100
0
*
CO
MG
D
LOOH (mmol/mg of tissue)
80
60
40
20
0
*
CO
MG
E
MPO (mD.O./mg protein)
0.25
0.20
0.15
0.10
0.05
0.00
***
CO
MG
F
Islet area (μm²)
2.0×10⁴
1.5×10⁴
1.0×10⁴
5.0×10³
0
**
CO
MG
G
50μm
50μm
CO
MG

ATTACHMENT 03:

University Comitee of Ethics in Animals Use Approval

CERTIFICADO

Certificamos que o Projeto intitulado "EFEITOS ENDÓCRINOS E METABÓLICOS PROLONGADOS DO TRATAMENTO NEONATAL COM METILGLIOXAL EM FILHOTES DE RATAS WISTAR", protocolado sob o CEUA nº 3830171215, sob a responsabilidade de **Paulo Cezar De Freitas Mathias** *e equipe; Flávio Andrade Francisco; Caroline Ribeiro; Claudinéia Conationi Da Silva Franco; Lucas J. Saavedra; Luiz Henrique Schimitt ; Maroly Valentin Alves Pinto; Rodrigo Mello Gomes; Vander Silva Alves; Veridiana M. Mreira* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, com o Decreto 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais da Universidade Estadual de Maringá (CEUA/UEM) em reunião de 25/01/2016.

We certify that the proposal "EFFECTS ENDOCRINE AND METABOLIC EXTENDED THE NEWBORN TREATMENT IN RATS METHYLGLYOXAL WISTAR PUPPIES", utilizing 320 Heterogenics rats (260 males and 60 females), protocol number CEUA 3830171215, under the responsibility of **Paulo Cezar De Freitas Mathias** *and team; Flávio Andrade Francisco; Caroline Ribeiro; Claudinéia Conationi Da Silva Franco; Lucas J. Saavedra; Luiz Henrique Schimitt ; Maroly Valentin Alves Pinto; Rodrigo Mello Gomes; Vander Silva Alves; Veridiana M. Mreira* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes (or teaching) - it's in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the State University of Maringá (CEUA/UEM) in the meeting of 01/25/2016.

Vigência da Proposta: de 03/2016 a 12/2018 Laboratório: Depto De Biotecnologia, Genética E Biologia Celular

Procedência:	Biotério Setorial do Laboratório de Biologia Celular da Secreção - (LBCS, PRONEDO)				
Espécie:	Rato heterogênico	Gênero: Machos	idade:	21	N: 120
Linhagem:	Wistar		Peso:	~50	
Procedência:	Biotério Central da UEM				
Espécie:	Rato heterogênico	Gênero: Machos	idade:	70-80	N: 20
Linhagem:	Wistar		Peso:	~300	
Procedência:	Biotério Setorial do Laboratório de Biologia Celular da Secreção - (LBCS, PRONEDO)				
Espécie:	Rato heterogênico	Gênero: Machos	idade:	90	N: 120
Linhagem:	Wistar		Peso:	~400	
Procedência:	Biotério Central da UEM				
Espécie:	Rato heterogênico	Gênero: Fêmeas	idade:	60-70	N: 60
Linhagem:	Wistar		Peso:	~250	

Resumo: É crescente a observação de que insultos perinatais têm consequências de curto- e longo-prazo no crescimento e metabolismo dos animais como indicam alguns dados epidemiológicos e experimentais. Com base nessas observações, podemos dizer que doenças metabólicas tais como o diabetes tipo 2, obesidade e hipertensão podem ser “programadas” durante estágios críticos de desenvolvimento, como gestação e lactação. Entre as diversas pesquisas para explicar os diferentes agentes causadores e seus papéis na instalação e manutenção do diabetes e disfunções das ilhotas pancreáticas, a formação dos produtos de glicação avançada, chamados de AGEs (do inglês, Advanced Glycation End Products) tem ganhado significativa importância entre os pesquisadores. Além da formação de AGEs ocorrer normalmente sob condições fisiológicas esses produtos também podem ser introduzidos no organismo por fontes exógenas, como o fumo e a dieta. O objetivo deste trabalho será estudar os efeitos da administração de metilglioxal em filhotes de ratos Wistar durante a lactação, sobre o metabolismo e função pancreática tanto aos 21 quanto aos 90 dias de vida. Os resultados serão expressos como a média ± erro padrão da média (M ± EPM). Para avaliar diferenças entre os grupos será usado one-way ANOVA, e pós-teste de Tukey, nível de significância p<0,05.

Maringá, 27 de janeiro de 2016

Prof. Dr. Alexandre Ribas de Paulo
Coordenador da Comissão de Ética no Uso de Animais
Universidade Estadual de Maringá

ATTACHMENT 04:

Completion Certificate

Universidade Estadual de Maringá
Centro de Ciências Biológicas
Programa de Pós-Graduação em Ciências Biológicas

CERTIDÃO DE CONCLUSÃO N.º 035/2020-PBC

Declaramos, para os devidos fins, que FLÁVIO ANDRADE FRANCISCO obteve o título de "DOUTOR EM CIÊNCIAS BIOLÓGICAS", área de concentração em Biologia Celular e Molecular, com a tese intitulada: "The early glycotoxins exposure and metabolic programming: Inflammation, oxidative stress and metabolic dysfunctions at adult life", orientado pelo Prof. Dr. Paulo Cezar de Freitas Mathias.

A banca examinadora designada para avaliar a tese e a defesa foi composta pelos professores doutores: Paulo Cezar de Freitas Mathias como Presidente, Rodrigo Mello Gomes (Universidade Federal de Goiás), Paulo Nuno Centeio Matafome (Universidade de Coimbra), Alex Rafacho (Universidade Federal de Santa Catarina), Paul David Taylor (Royal College of London) como membros, sendo o pós-graduando aprovado em 08 de junho de 2020.

Declaramos que o Programa está reconhecido pela Portaria 609/2019-MEC, de 14/03/2019, publicado no Diário Oficial da União em 18/03/2019, Edição: 52, Seção 1, página: 63, e o curso é credenciado junto à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES com o conceito 5 (quadriênio 2013-2016).

Por ser a expressão da verdade, firmamos a presente.

Maringá, 18 de junho de 2020.

Prof. Dr. Max Jean de Ornelas Toledo,
Coordenador Adjunto do Programa de Pós-Graduação
em Ciências Biológicas

Av. Colombo, 5.790 • Câmpus Universitário • CEP 87.020-900 • Maringá – PR
Fones: (44) 3011-4908 •• E-mail: sec-pbc@uem.br • Internet: www.pbc.uem.br