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EXPOSURE TO MATERNAL DIABETES ON THE OXIDATIVE STRESS, HYPOXIA, AND PANCREATIC CHANGES IN RAT PUPS DURING THE PERINATAL PERIOD

Bruna Dallaqua [1]*; Verônyca Gonçalves Paula[2]*; Yuri Karen Sinzato[3]*; Franciane Quintanilha Gallego [4]*; Isabela Lovizutto Iessi [5]*; Tiago Rodrigues[6] ; Jaqueline de Carvalho Rinaldi [7]+; Flávia Karina Delella [8]+; Sérgio Luis Felisbino [9]+; Gustavo Tadeu Volpato[10]; José Eduardo Corrente [11]; Débora Cristina Damasceno[12]*

RESUMO

Investigamos os resultados da exposição intrauterina à hiperglicemia em parâmetros glicêmicos, estresse oxidativo, hipóxia e ilhotas pancreáticas em fetos e recém-nascidos de ratos. O diabetes foi induzido com estreptozotocina no dia 1 pós-natal – PND1, ratas não diabéticas (controle) receberam o tampão. As ratas adultas foram acasaladas, e seus descendentes foram eutanasiados no nascimento e nos períodos PND5 e 15 para análise de sangue, fígado e pâncreas. O diabetes materno levou à hiperglicemia neonatal, hiperinsulinemia, resistência à insulina (maior HOMA-IR) e estresse oxidativo no PND5. No PND15, o sistema antioxidante foi ineficiente nos tecidos sanguíneo e hepático. HIF-1α e PGC-1α dos filhotes não mostraram diferenças, confirmando uma incapacidade de responder a alterações hiperglicêmicas. As ilhotas pancreáticas mostraram uma área reduzida no PND5 e PND15. O diabetes materno influencia a plasticidade fetal e afeta a área da ilhota pancreática, causando hiperglicemia, resistência à insulina e estresse oxidativo em fetos e recém-nascidos.

Palavras-chave: Hiperglicemia, fetos, estado redox, hipóxia, ratas.

ABSTRACT

We investigated the outcomes of intrauterine exposure to hyperglycemia on glycemic parameters, oxidative stress, hypoxia, and pancreatic islet in rats' fetuses and newborns. Diabetes was induced with streptozotocin in Postnatal day 1 – PND1, nondiabetic rats (control) received the buffer. The adult rats were mated, and their offspring were euthanized at birth and PND5 and 15 periods for blood, liver, and pancreas analysis. Maternal diabetes led to neonatal hyperglycemia, hyperinsulinemia, insulin resistance (higher HOMA-IR), and oxidative stress in PND5. On PND15, the antioxidant system was inefficient in blood and liver tissues. HIF-1α and PGC-1α of the pups did not show differences, confirming an inability to respond to hyperglycemic changes. Pancreatic islets showed a reduced area in PND5 and PND15. Maternal diabetes influences fetal plasticity and affects the area of the pancreatic islet, causing hyperglycemia, insulin resistance, and oxidative stress in fetuses and newborns.

Keywords: Hyperglycemia, fetuses, redox status, hypoxia, rats.

* São Paulo State University (Unesp), Laboratory of Experimental Research on Gynecology and Obstetrics - UNIPEX, Postgraduate Course on Tocogynecology, Botucatu/SP
[1] Ph.D, bruna.jaquie@frb.edu.br
[2] Ph.D, veronyca.paula@unesp.br
[3] Ph.D, yurisinzato@gmail.com
[4] Ph.D, franciane.gallego@unesp.br
[5] Ph.D, isabelaliessi@gmail.com
[6] Federal University of ABC (UFABC), Center of Natural and Human Sciences (CCNH), Santo André, São Paulo State, Brazil Ph.D, trodrigues.ufabc@gmail.com
+ São Paulo State University (UNESP), Laboratory of Extracellular Matrix Biology, Department of Structural and Functional Biology, Institute of Biosciences of Botucatu, Botucatu, São Paulo, Brazil
[7] Ph.D, jak.rinaldi@gmail.com
[8] Ph.D, flavia.delella@unesp.br
[9] Ph.D, sergio.felisbino@unesp.br
[10] Federal University of Mato Grosso (UFMT), Laboratory of System Physiology and Reproductive Toxicology, Barra do Garças/MT, Ph.D, gtvolpato@gmail.com
[11] São Paulo State University (UNESP), Research Support Office, Botucatu Medical School, Botucatu, Brazil, Ph.D, jecorren@gmail.com
[12] Contato principal Ph.D, debora.damasceno@unesp.br

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1. INTRODUCTION

Chronic hyperglycemia observed in Diabetes mellitus promotes multiple biochemical disorders, such as oxidative stress, which plays a critical role in the onset and progression of the disease (Ornoy *et al*., 2021). Oxidative stress in diabetes occurs due to an increase of reactive oxygen species (ROS), such as superoxide anion (O2−•), hydroxyl radical (OH•), and hydrogen peroxide (H2O2), or a deficiency in the antioxidant defense system (Fernandes *et al*., 2016). The antioxidant system includes some enzymes. The most important of them are superoxide dismutase (SOD), which produces hydrogen peroxide from superoxide radicals; catalase (CAT) and glutathione peroxidase (GPx), which are related to the decomposition of H2O2 (Chelikani *et al*., 2004). In the eukaryotic cell, approximately 60–75% of enzyme activity is found within the cytoplasm and 25–40% in the mitochondria. Its most common enzymatic activity occurs in the erythrocytes and the liver.

During pregnancy, hyperglycemia is associated with short-term and long-term adverse effects for mother and child (Ornoy *et al*., 2021). Children born to diabetic pregnancies are at increased risk of morbidity and mortality, macrosomia, obesity, and type 2 diabetes later in life (Schaefer-Graf *et al*., 2019). In the embryonic period, a balance between ROS production and antioxidant systems is essential to release a low physiological level of ROS, which plays a crucial role in cell regulation and signaling (Coffman; Su, 2019). Increased levels of ROS cause oxidative damage in all mitochondrial components, leading to a disturbance in ATP production and being related to a state of hypoxia (Zorov *et al*., 2014). Hyperglycemia, oxidative stress, and hypoxia play a crucial pathophysiological role in diabetic complications due to the deficient response of tissues to low oxygen tension (Catrina; Zheng, 2021).

Hyperglycemia has been shown to alter transcriptional control of cellular energy metabolism, including oxidative metabolism and inhibition of mitochondrial biogenesis, which has been defined as the growth and division of pre-existing mitochondria (Popov, 2020), a critical nuclear receptor coactivator called PGC-1α (peroxisome proliferator-activated receptor-γ coactivator) plays a crucial role in regulating the production and extinction of ROS and its activity regulates essential functions during the embryonic period participating in fetal programming induced by diabetes in various tissues, including fetal pancreas and liver (Besseiche *et al*., 2017). Furthermore, PGC-1α-induced mitochondrial biogenesis causes increased O2 consumption, leading to a decrease in intracellular O2 availability, which may be related to a state of hypoxia, as this transcription factor acts as a coactivator of factors hypoxia-inducible (HIF)

ISSN 1984-431X

(Catrina; Zheng, 2021). HIF-1 is the primary regulator of O2 homeostasis, and under hypoxic conditions, HIF-hydroxylases are inactive, and HIF-1α is stabilized, which allows the formation of a transcriptionally active heterodimeric protein. Evidence suggests that hypoxia and inappropriate responses to hypoxia due to dysregulated HIF-1 signaling are critical pathogenic factors, occurring both in tissues central to the development of diabetes (pancreatic beta cells and adipose tissue) and in tissues susceptible to diabetes complications such as liver kidneys, heart, and blood vessels (Catrina; Zheng, 2021).

Experimental animal studies support the "developmental origins of health and disease - DOHaD" concept and demonstrate the mechanisms involved in offspring susceptibility to disease in later life. Considering that stressful maternal environmental factors such as hyperglycemia from diabetes during fetal and perinatal life can influence the proper development of organs and tissues and may impact the future health of the offspring and perpetuate the diabetogenic phenotype through generations, this study aimed to evaluate the influence of this maternal insult on glycemia, redox state and hypoxia of the offspring of mildly diabetic rats.

2. METHODOLOGY

2.1 ANIMALS

Male and female Wistar rats, weighing approximately 190 and 220 grams (g), respectively, were obtained from CEMIB (Multidisciplinary Center for Biological Research, Campinas, São Paulo State). All animals were adapted and maintained in the Vivarium of the Laboratory of Experimental Research on Gynecology and Obstetrics of UNIPEX, UNESP. During all experiments, water and food were given ad libitum in a controlled environment (room temperature: 22 ± 3oC, humidity: 50 ± 10%, and 12 h light/dark cycle). For environmental enrichment, paper balls were used in animal cages. The animals were mated to obtain offspring, which were used for nondiabetic (control) and mild diabetic groups.

The Ethics Committee on Animal Experimentation of the local institution (937/2012) approved the study protocols and followed the recommendations of the Guide for the Care and Use of Experimental Animals.

2.2 INDUCTION OF EXPERIMENTAL DIABETES

The female litter of half of the dams was used at birth (postnatal day = PND1) for receiving subcutaneously 100 mg streptozotocin/kg body weight (STZ – Sigma Chem.® Co., St. Louis, Millstone, USA) dissolved in citrate buffer (0.1 M, pH 4.5) to induce mild diabetes. The female pups of the other half of the dams received only citrate buffer (control group). At PND5, the glycemia

ISSN 1984-431X

of each newborn was determined by lancing the tail vein of the animal to obtain a drop of blood. The female pups given STZ and presenting blood glucose levels higher than 400 mg/dL were included in the mildly diabetic group; female pups that presented no glycemia equal to or lower than 400 mg/dL were excluded from the experiment. The animals were given citrate buffer, and presenting glycemia lower than 120 mg/dL was included in the control group (Bueno *et al*., 2020) (Figure 1 - Experimental design).

2.3 MATING PERIOD AND PREGNANCY

Adult female rats (control and mildly diabetic) were mated overnight with nondiabetic males. The morning from the next day, when sperm was found in the vaginal smear, was designated as day 0 of pregnancy. The mating protocol was followed for 15 consecutive days (corresponding to at least three estrous cycles). After this period, the rats that presented no positive pregnancy diagnosis were excluded from this study (Soares *et al*., 2021).

On day 21 of pregnancy, half of the rats of each group (control and mild diabetes) were anesthetized with sodium thiopental (Thiopentax®, 120 mg/kg body weight, intraperitoneal route - Cristália, Brazil) and submitted to laparotomy. The other half of the groups of dams were used for obtaining their pups by vaginal delivery. All fetuses (day of birth = day 0) and four newborns/dam on PND5 and PND15 were anesthetized with sodium thiopental and decapitated to obtain the pool of blood samples for measurement of the blood oxidative stress status. The liver was removed to evaluate oxidative stress biomarkers and HIF-1α and PGC-1α protein expression. In addition, blood glucose and insulin levels were determined for HOMA index calculation in newborns.

2.4 BLOOD GLUCOSE AND INSULIN LEVELS

A conventional glucometer assessed the fasting glucose levels of all neonates at PND5 and PND15. Fasting blood samples were collected in free-anticoagulant tubes, maintained in ice for 30 minutes (min), and then centrifuged at 263 × g for 10 min at 4°C. The supernatant was collected and stored at −80°C to analyze serum insulin levels using an Ultra-Sensitive Rat Insulin ELISA Kit (Crystal® Chemical Incorporation, Chicago, Illinois, USA).

Homeostasis model assessment-insulin resistance (HOMA-IR) and beta-cell function (HOMA-β cell function) values were calculated according to Aref *et al*. (2013): HOMA-IR = (fasting insulin x fasting glucose)/22.5 and HOMA-β cell function = (20 x fasting insulin)/ (fasting glucose – 3.5), considering the glucose levels presented in mmol/l, and serum insulin concentration in µUI/ml.

2.5 OXIDATIVE STRESS MARKERS IN WASHED ERYTHROCYTES

ISSN 1984-431X

Fetal blood samples were collected in tubes with anticoagulant, and centrifuged at 90 × g for 10 min at 4°C. The supernatant was discarded, and erythrocytes were washed with phosphate buffer saline (0.01 M, pH 7.4), followed by centrifugation at 263 × g for 1 min at 4°C. This procedure was repeated three times, and the washed erythrocytes were used for the determination of superoxide dismutase (SOD) and peroxidase glutathione (GPx) activities; thiol groups (-SH) and thiobarbituric acid reactive substances (TBARS) (Sinzato *et al*., 2023).

2.6 OXIDATIVE STRESS BIOMARKERS IN THE LIVER

Fetal liver samples were quickly collected and washed with phosphate buffer saline (0.01 M, pH 7.4) to determine oxidative stress biomarkers. Hepatic TBARS levels, SOD, and GPx activities were determined using commercialized kits (Cayman® Chemical Co., Michigan, USA). Catalase activity was determined following the decrease in the absorbance at 240 nm due to H2O2 reduction at 25°C during 120 seconds (s). One µL of supernatant isolated from rat liver homogenates was placed in a cuvette and diluted to a final volume of 1 mL with phosphate-buffered saline (0.1 M, pH 7) in the presence of 20 mM H2O2. CAT activity was calculated using a molar extinction coefficient (ε = 0.0436 mM/cm) and expressed as U/mg protein (Aebi, 1984). Reduced thiol group levels in liver homogenates were evaluated by Jollow *et al*. (1974) using a spectrophotometric assay based on the development of a yellow color when DTNB was added to compounds containing these groups. The absorbance was measured at 412 nm.

2.7 PROTEIN EXTRACTION AND EXPRESSION BY WESTERN BLOTTING FOR HIF-1A AND PGC-1A IN LIVER

Frozen liver samples from all neonates were mechanically homogenized in 50 mM Tris–HCl buffer pH 7.5, 0.25% Triton X-100, and EDTA using a Polytron homogenizer (Kinematica®, Lucerne, Switzerland) for 30 s at 4°C. After homogenate centrifugation, the protein content in the supernatant was quantified, as Bradford (1976) described. Equal amounts of protein (70 µg) from liver samples were heated at 95°C for 5 min in sample-loading buffer and then subjected to SDS–PAGE under reducing conditions followed by transfer to nitrocellulose membranes (Sigma Chemical® Co., St. Louis, Missouri, USA). The blots were blocked with 3% bovine serum albumin in TBST (10 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) for 1 hour and probed overnight with the primary antibody anti-HIF-1 α (1:800; ab51608; AbcamTM) and anti-PGC-1α (1:1000; ST1202-1SET; EMD Millipore). Mouse anti-β-actin antibody (1:1,000; sc-81178, Santa Cruz Biotechnology, Santa Cruz, California, USA) was used as the loading

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control. After incubation with the corresponding horseradish peroxidase-conjugated secondary antibodies, chemiluminescence emission was detected (Amersham ECL Select Western Blotting Detection Reagent, GE Healthcare, UK). HIF-1α, PGC-1α, and β-actin protein expression were quantified by densitometry analysis of the bands, which were expressed as integrated optical density (IOD). The HIF-1α and PGC-1α expression was normalized to the β-actin values. Normalized data are expressed as the mean ± standard deviation (SD).

2.8 MORPHOLOGICAL ANALYSIS OF THE PANCREAS OF THE OFFSPRING AT DAYS 5 AND 15 OF POSTNATAL LIFE

The pancreas was dissected, weighed, fixed in 10% formaldehyde for 24 h, dehydrated in progressive alcohol concentrations, and embedded in paraffin. A rotary microtome was obtained from the pancreatic sections of 5 µm width and stained with hematoxylin and eosin for conventional histological analysis of pancreatic endocrine cells. The pancreatic islets were identified, and the images were captured using the computerized image system (Software KS-300, version 3.0, Zeiss®), integrated with the digital camera image (CCD-IRIS/RGB, Sony®, China) microscope (DMR, Leica®, Brazil). The images were analyzed through ImageJ® software, and the area was presented in pixels2.

2.9 STATISTICAL ANALYSIS

The sample size calculation was made considering the mean glycemic area under the curve of a previous study carried out in our laboratory comparing nondiabetic (9,106 ± 1,412 mg/dL/min) and diabetic pregnant rats (15,765 ± 2,347 mg/dL/min) (Araujo-Silva *et al*., 2021). Furthermore, a power of 80% and a confidence level of 95% were considered for this calculation, and the minimum "n" was five dams/group. Data were presented as mean ± standard deviation (SD). T-test and Tukey's Multiple Comparison tests were used for data with a normal distribution. A Gamma distribution test was performed for data presenting asymmetric distribution. $P < 0.05$ was the limit of statistical significance.

Revista Eletrônica Interdisciplinar
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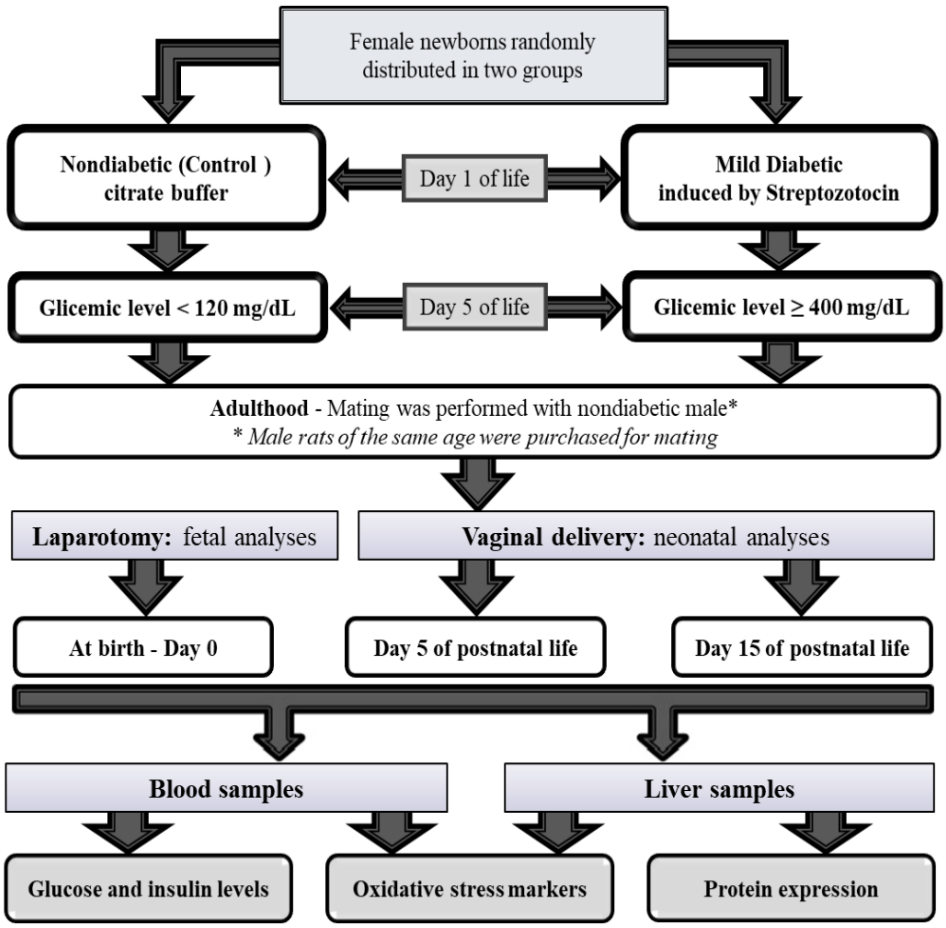
Figure 1. Experimental design.



3. RESULTS

3.1 MATERNAL DATA

Oral glucose tolerance test (OGTT) was performed on day 17 of pregnancy to confirm hyperglycemic state in rats during pregnancy. Glucose overload caused abnormal blood glucose levels. In addition, diabetic rats showed abnormal levels of oxidative stress biomarkers in blood samples at term pregnancy, as already well established in this diabetes model (Bueno *et al*., 2020).

3.2 OFFSPRING DATA

At birth, the mean weight of pups from diabetic dams (5.75 ± 0.52 grams) was higher than from control dams (5.27 ± 0.59 g), but no differences were observed in placenta weights.

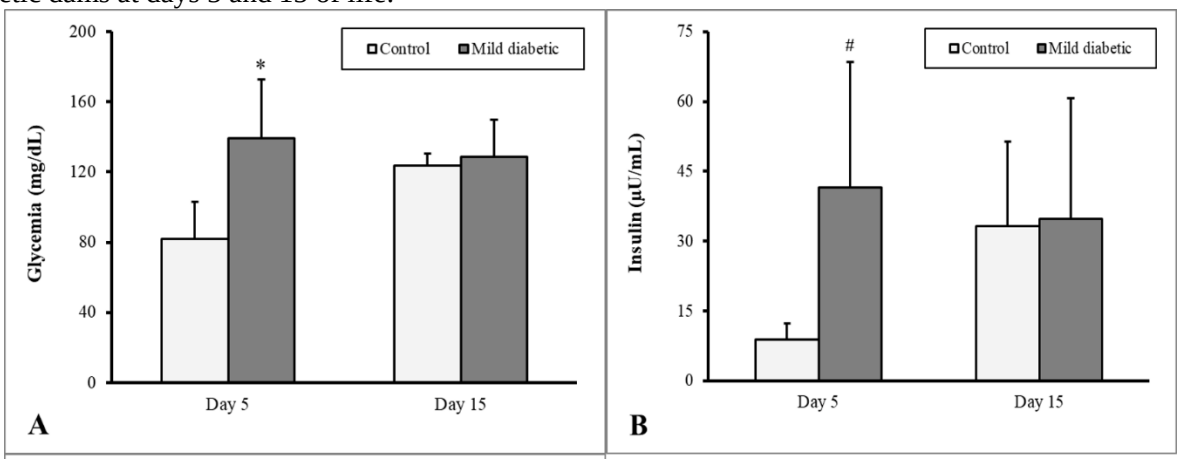
Figure 2 presents the fasting blood glucose and insulin levels. The glucose and serum insulin levels in pups on day 5 of life from mild diabetic dams were greater than those in the control pups (Figures 2A and 2B, respectively).

Our lab team calculated the HOMA-IR and HOMA-β indexes and presented them in Table 1. The HOMA-IR value of the offspring from mild diabetic rats showed a significant

Revista Eletrônica Interdisciplinar
Barra do Garças – MT, Brasil
ISSN 1984-431X
Ano: 2025 Volume: 17 Número: 1

increase at postnatal day 5 as compared with the control group.

Figure 2. Fasting blood glucose (A) and serum insulin (B) levels of the newborns from control and mild diabetic dams at days 5 and 15 of life.



Values are expressed as mean ± standard deviation (SD). n= 6 dams/each moment/group (4 newborns/dam) * $p<0.05$ – compared to the control group (Student t-test). #$p<0.05$ – compared to the control group (Gamma distribution test).

Table I. Homeostasis model assessment-insulin resistance (HOMA-IR) and beta-cell function (HOMA-β cell function) of the newborns on days 5 and 15 of life from control and mild diabetic dams.

	Neonates from Control dams (n=6)	**Neonates from Mild diabetic dams (n=6)**	**p-value**
Day 5			
HOMA-IR	1.9 ± 1.1	13.8 ± 10.9*	0.004
HOMA-β	143.1 ± 39.8	292.4 ± 188.2	NS
Day 15			
HOMA-IR	10.2 ± 5.9	12.4 ± 10.6	NS
HOMA-β	247.62 ± 213.4	163.2 ± 87.1	NS

Values are expressed as mean ± standard deviation (SD). *$p<0.05$ – compared to the control group (Gamma distribution Test). $p>0.50$ - NS – no significant.

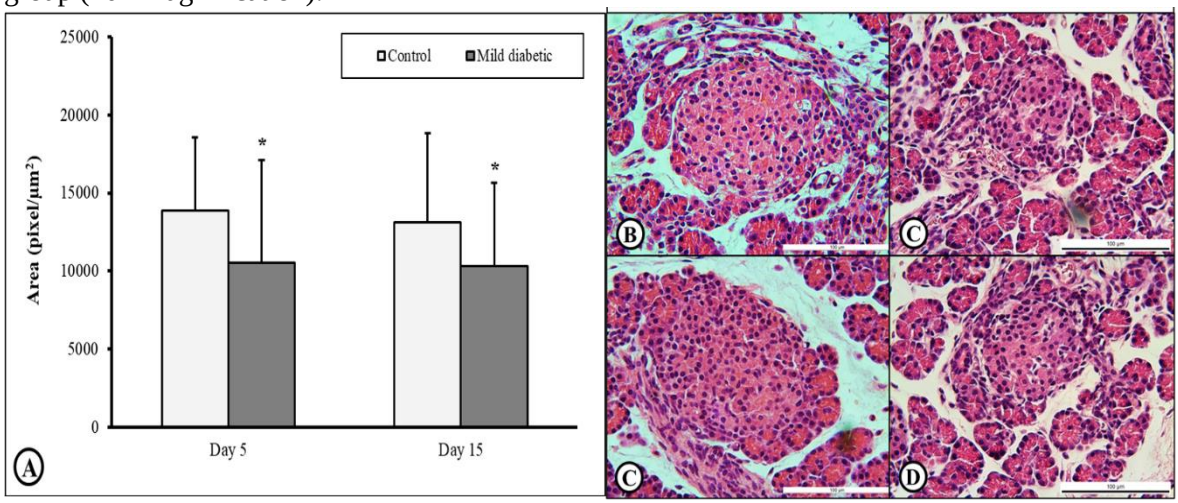
The areas of the pancreatic islets of offspring from diabetic dams were reduced compared to those from control rats at days 5 and 15 of life (Figure 3).

Considering the washed erythrocyte samples, the pups from mild diabetic dams presented greater TBARS concentrations at postnatal days (PND0, 5, and 15) of life as

Revista Eletrônica Interdisciplinar
Barra do Garças – MT, Brasil
ISSN 1984-431X
Ano: 2025 Volume: 17 Número: 1

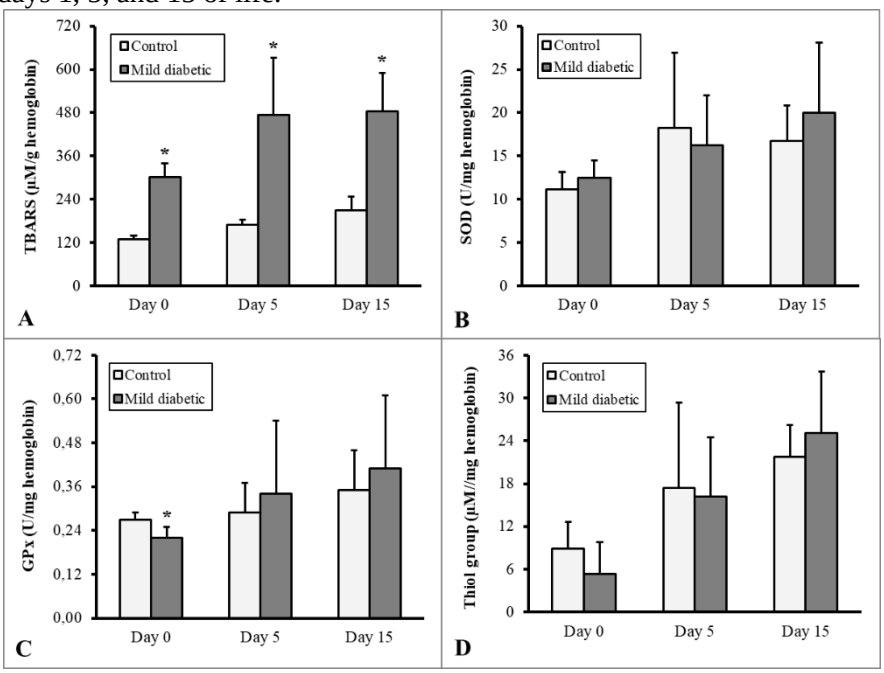
compared to the control group (Figure 4A). Regarding the antioxidant profile, the pups of mild diabetic rats presented a decreased GPx activity at birth in relation to the control group (Figure 4C).

Figure 3. Pancreatic islets of the newborns from control and mild diabetic dams at days 5 and 15 of life. (Hematoxilin/Eosin). A- Pancreatic islet in the control group (40× magnification). B. Pancreatic islet in STZ group (40× magnification).



n= 6 dams/each moment/group (4 newborns/dam)

Figure 4. Oxidative stress profile in washed erythrocyte samples of the newborns from control and mild diabetic dams at days 1, 5, and 15 of life.



Values are expressed as mean ± standard deviation (SD). n= 6 dams/each moment/group (4 newborns/dam). *p<0.05 – compared to the control group (Gamma distribution Test).

Revista Eletrônica Interdisciplinar
Barra do Garças – MT, Brasil
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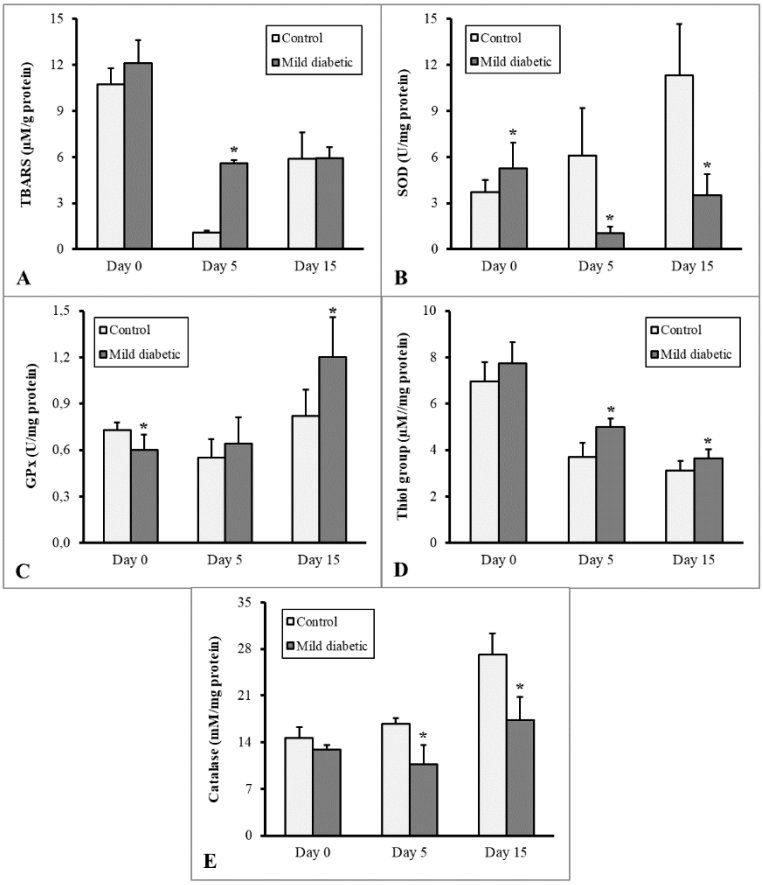
The oxidative stress status obtained from the liver samples of pups is shown in Figure 4. At birth (day 0), the pups from mild diabetic dams presented an increased SOD activity and a decreased GPx activity (Figures 5B and 5C). At PND5, the pups from mild diabetic rats showed a reduced activity of SOD and catalase (Figures 5B and 5E), while more significant levels of TBARS and thiol groups (Figures 5A and 5D). At PND15, the pups from mild diabetic rats had lower SOD and catalase activities (Figures 4B and 4E) and an increased GPx activity and thiol group level when compared to the control groups (Figures 5C and 5D).

HIF-1α and PGC-1α protein expression presented no difference in the liver samples of offspring from mild diabetic dams compared to those from control rats in the different moments of life that were analyzed (Table 2).

Figure 5. Oxidative stress profile in liver sampled of the newborns from control and mild diabetic dams at days 1, 5, and 15 of life.



Values are expressed as mean ± standard deviation (SD). n= 6 dams/each moment/group (4 newborns/dam) *p<0.05 – compared to the control group (Gamma distribution Test).

Revista Eletrônica Interdisciplinar
Barra do Garças – MT, Brasil
ISSN 1984-431X
Ano: 2025 Volume: 17 Número: 1

Table II. Protein relative expression of HIF-1α and PGC-1α by Western blotting of the newborns on days 0, 5, and 15 of life from control and mild diabetic dams.

	Neonates from Control dams (n=6)	Neonates from Mild diabetic dams (n=6)	p-value
HIF-1α			
Day 0	0.81 ± 0.37	0.64 ± 0.36	NS
Day 5	0.92 ± 0. 73	0.92 ± 0.22	NS
Day 15	0.68 ± 0.25	0.85 ± 0.48	NS
PGC-1α			
Day 0	0.69 ± 0.31	0.55 ± 0.32	NS
Day 5	0.59 ± 0.41	0.40 ± 0.15	NS
Day 15	0.40 ± 0.06	0.49 ± 0.22	NS

Values are expressed as mean ± standard deviation (SD). p>0.05 – NS – no significance.

4. DISCUSSION

Maternal hyperglycemia caused an increase in blood glucose and insulin levels on day 5 of life, leading to a high lipoperoxidation in the blood and liver and lower antioxidant activity in the liver, reflecting oxidative stress. At this time, the glycemic increase, oxidative stress, and insulin resistance contributed to the decrease in pancreatic islet areas. On day 15 of life, blood glucose, and insulin changes did not occur, but there was a commitment to an antioxidant defense system. Furthermore, a reduction in the area of the pancreatic islets was evident. However, no changes were observed in markers of hypoxia and mitochondrial biogenesis, suggesting that the decrease in islets is related to hyperglycemia and oxidative stress on day 5 and to the abnormal antioxidant system on day 15.

The newborns of rat diabetic mothers had higher levels of blood glucose and insulin near birth, which might be related to maternal intrauterine hyperglycemia (Hill, 2021). In addition, these newborns showed higher body weight at birth. The glucose promoted higher energetic levels in these newborns, and the insulin acted as a growth hormone, helping with the increased weight (Hill, 2021) on day 5 of postnatal life. Nilsen *et al*. (2014) demonstrated that fetal beta cell mass was also increased, and hyperinsulinemia was maintained, leading to

ISSN 1984-431X

macrosomia and insulin resistance (high HOMA-IR index), corroborating our findings. In our study, the pups presented no high levels of blood glucose after the first two weeks (day 15 postnatal of life). This result might be related to decreased beta cell response to glucose stimulation, beta cell degranulation, and decreased pancreatic islet size (Sinzato *et al*., 2019), leading to reduced blood glucose and insulin concentrations, as evidenced in the present study. However, these neonates of diabetic dams at PND 15 presented 167 mg/dL of circulating blood glucose level in the area under the curve of the oral glucose tolerance test (OGTT) after the glucose overload (unpublished data). This finding confirms that maternal hyperglycemia caused adverse outcomes for these offspring since intrauterine life.

Adequate metabolic status during pregnancy is crucial for fetal development. Our research team demonstrated that streptozotocin-induced maternal hyperglycemia promotes oxidative stress in term pregnancy (Sinzato *et al*., 2019). Furthermore, maternal diabetes and oxidative stress induce hypoxia and alter placental development, which may be the mechanism related to abnormal placental function and fetal programming (Myatt, 2006). Here, we showed that elevated maternal blood glucose levels led to higher placental exchange of glucose to the fetus in development, contributing to an abnormal redox state during the postnatal period. The oxidative stress status in pups at a different age in early life (at birth, PND5, and PND15) was confirmed by lipid peroxidation (higher TBARS levels) and lower GPx activity in blood samples.

In addition, abnormal antioxidant biomarkers (-SH level, SOD, GPx, and catalase activities) and lipoperoxidation were also verified in liver samples of newborns. These results confirmed oxidative stress status, whereas GPx was increased, perhaps as a compensatory mechanism to fight the hydrogen peroxide. According to Houstis *et al*. (2006), oxidative stress can induce insulin resistance by impairing insulin signal transduction. Another study showed that excess free radicals suppress GLUT-4 localization in cell membranes by impairing insulin signaling (Hurrle *et al*., 2017). The neonatal tissues are also sensitive to damage induced by oxidative stress (Kloppel *et al*., 2023).

Although the precise mechanisms for this impairment are not completely clear, the hyperglycemic intrauterine environment and the presence of neonatal oxidative stress might have influenced the plasticity of the development of the pancreas of these offspring. The maternal hyperglycemia led to fetal and neonatal hyperglycemia, contributing to a lower level of antioxidant defenses in the liver and an increased lipoperoxidation in blood tissue, confirming the state of oxidative stress in these neonates. Gallego *et al*. (2019) corroborate our findings as they confirmed the decrease in immunostaining

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of the antioxidant enzyme superoxide dismutase (SOD) in the pancreatic islets in neonates at 5 and 15 days of postnatal life, reducing insulin synthesis. The pups of diabetic rats also showed insulin resistance at 5 days of life. Literature shows that a failure of the ability of the β-cells to compensate for insulin resistance may cause progressive hyperglycemia, glucose toxicity, and overt diabetes (Christensen; Gannon, 2019).

Hyperglycemia alters the metabolism of the rat embryonic tissue, inhibiting oxygen uptake, which may lead to hypoxia (Ornoy *et al*., 2010). HIF-1α and PGC-1α are good markers associated with hypoxia (Catrina; Zheng 2021) and may be related. Since PGC-1α acts as a transcriptional coactivator of HIF-1α during hypoxia, we evaluated both these biomarkers. Still, we found no alteration in the protein expression of these factors in the liver of the offspring (at birth, postnatal days 5 and 15 of life) of diabetic rat mothers. These results did not corroborate other authors who found hyperglycemia interference in these proteins' expression. However, literature has shown conflicting evidence of the hyperglycemia on HIF-1α in different tissues or cells. Some studies showed hyperglycemia promotes higher HIF-1α expression, and others report the role of hyperglycemia on the degradation of HIF-1α in some tissues (Kasputin *et al*., 2021; Li *et al*., 2023). Diabetic animals have been induced with fatty liver disease and presented a higher HIF-1α level in the liver, which was associated with hypoxia in these animals (Bai *et al*., 2017). On the other hand, hepatocytes isolated from PGC-1α-deficient mice exhibited decreased mitochondrial respiration rates (Szalardy *et al*., 2016), providing a mechanistic explanation for the reduced ability to oxidize hepatic fatty acids. However, these studies did not assess the expression of these factors during the perinatal period, which might explain why we did not find changes in the studied periods of our model.

An important fact is that diabetes compromises HIF-1α signaling, leading to inefficient responses to hypoxia and increasing the risk of tissue damage (Cerychova; Pavlinkova, 2018). Furthermore, the increase in hyperglycemia-induced ROS leads to an increased expression of PGC-1α. Investigation has demonstrated that PGC-1α limits ROS accumulation, protects against oxidative damage, and acts as a coactivator of HIF-1α. Then, it can exert its regulatory role in hypoxia (Mohammadi *et al*., 2022). Therefore, we expected that puppies from diabetic mothers, when presenting oxidative stress both in the liver and systematically, would have an increased protein expression of PGC-1α and HIF-1α to try to compensate for establishing a hypoxic condition in the presence of oxidative stress. However, these newborns present no protein expression changes in the biomarkers of hypoxia and mitochondrial biogenesis. These findings are interesting because the newborns of diabetic dams presented hyperglycemia and oxidative

ISSN 1984-431X

stress regardless of the neonatal period, and this condition favors the appearance of late hypoxia. Then, no alteration in PGC-1α and HIF-1α expression is not compatible. Mitochondria isolated from the livers of diabetic rats at 9 weeks showed lower respiratory chain activity, and those isolated from animals at 6 months showed increased respiratory control (Palmeira *et al*., 2007). This finding confirms our suggestion the neonates had no modification in the mitochondrial hypoxic biomarkers near birth, which could present long-term changes due to maternal diabetes-induced fetal programming.

Our investigation's strong point is evaluating the influence of maternal hyperglycemia on metabolic complications in offspring, which favors a better understanding of diabetes's effects on future generations. As a limitation, assessing gene expression of factors related to hypoxia and mitochondrial biogenesis could better explain the absence of protein expression changes.

5. CONCLUSION

Therefore, this study shows that mild maternal diabetes influences fetal plasticity and affects the area of the pancreatic islet, causing hyperglycemia, insulin resistance, and oxidative stress in fetuses and newborns. However, no hypoxia or abnormal mitochondrial biogenesis is verified in these offspring at this stage of life studied.

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