

## Evaluation of placental glycogen storage in mild diabetic rats<sup>1</sup>

### Avaliação dos estoques de glicogênio placentário em ratas com diabetes moderado

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#### ABSTRACT

**Purpose:** To evaluate the placental glycogen storage and fetal development in the pregnancy of neonatally streptozocin-induced diabetic rats and to establish relation with glycemia and insulin levels. **Methods:** At the birth day, 147 female rats were randomly distributed in two experimental groups: 1) Non-diabetic Group (Control, n=45) - received the vehicle; 2) Diabetic Group (STZ, n=102) - received 100 mg streptozocin/kg in neonatal period. At day 0 of pregnancy, adult female rats were included in the control group when presented glycemia below 120 mg/dL and, in the group STZ with glycemia between 120 and 300 mg/dL. At day 21 of pregnancy, blood samples were collected for glycemia and insulin determination, and placentas withdrawn for placental glycogen determination. The newborns (NB) were classified in small (SGA), appropriate (AGA) and large (LGA) for gestational age. **Results:** Rats STZ presented higher glycemia at days 0 and 14 of pregnancy. At end of pregnancy, rats STZ showed higher proportion of NB SGA and LGA; reduced rate of NB AGA and unaltered glycemia, insulin and placental glycogen determinations. **Conclusion:** Mild diabetes altered the maternal glycemia in the early pregnancy, impairing future fetal development, but it caused no alteration on insulin and placental glycogen determination, confirming that this glycemic intensity was insufficient to change glycogen metabolism.

**Key words:** Diabetes Mellitus. Insulin. Pregnancy, Animal. Glycogen. Streptozocin. Glycemia. Rats.

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#### RESUMO

**Objetivo:** Avaliar os estoques de glicogênio placentário e o desenvolvimento fetal na prenhez de ratas com diabetes moderado induzido no período neonatal e relacionar com glicemia e níveis de insulina. **Métodos:** No dia de nascimento, foram distribuídas aleatoriamente 147 ratas em dois grupos experimentais: 1) Grupo Não-diabético (Controle, n=45) - recebeu o veículo; 2) Grupo Diabético (STZ, n=102) - recebeu 100 mg streptozocin/kg peso corpóreo. No dia 0 de prenhez, foram incluídas ratas controle que apresentassem glicemia baixo de 120 mg/dL e, no grupo STZ, com glicemia entre 120 e 300 mg/dL. No 21º dia de prenhez, amostras de sangue foram coletadas para glicemia e determinação de insulina e as placentas foram retiradas para determinação de glicogênio placentário. Os recém-nascidos (RN) foram classificados em pequeno (PIP), adequado (AIP) e grande (GIP) para idade de prenhez. **Resultados:** As ratas STZ apresentaram glicemias maiores nos dias 0 e 14 de prenhez. No final da prenhez, as ratas STZ mostraram maior proporção de RN PIP e GIP, taxa reduzida de RN AIP e inalteração em glicemia, insulina e na determinação de glicogênio placentário. **Conclusão:** O diabetes moderado alterou a glicemia materna no início da prenhez, prejudicando o futuro desenvolvimento placentário e fetal, mas não causou nenhuma alteração na determinação de insulina e de glicogênio placentário, confirmando que esta intensidade de glicêmica foi insuficiente para modificar o metabolismo de glicogênio.

**Descritores:** Diabetes Mellitus. Insulina. Prenhez. Glicogênio. Streptozocin. Glicemia. Ratos.

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#### Introduction

The maternal glucose is considered important energy substrate for the development fetal<sup>1</sup>. It almost all the energy source of the fetal-placental unit, making the placenta and therefore the fetus totally dependent on maternal source of glucose and its metabolic reserve for the supply of multiple functions of the placenta<sup>2</sup>.

After the meal, the blood glucose is removed by the liver through the portal vein and the systemic circulation, and temporarily stored as glycogen<sup>3</sup>. The glycogen storage polysaccharide is the most important existing in animal cells. Each branch of glycogen ends with a non-reducing sugar, so it is not reducing how many terminals ramifications, but with a single terminal reducer. When it is used as an energy source, its units of glucose are removed one

by one from terminals not reducing. The enzymes can act in many terminals, making this polysaccharide is reduced to a monosaccharide<sup>4</sup>. The synthesis of glycogen is the process by which glucose is polymerized the glycogen, which is accumulated in the cells in amounts varying according to the cell type, working there as a deposit of energy accessible to the cell. The metabolism of glycogen is controlled predominantly by the coordinated action of two enzymes, glycogen synthase (promotes the glycogen synthesis) and glycogen phosphorylase (promotes the glycogen lysis)<sup>5</sup>.

Insulin and glucose may inhibit the glycogenolysis network, stimulating the enzymatic flow of glycogen synthase and/or inhibiting the enzymatic flow of the enzyme glycogen phosphorylase<sup>4</sup>. Insulin is the hormone responsible for stimulating the glycogen synthesis. It was observed in at term human placentas that the activity of glycogen phosphorylase increases while the activity of glycogen synthase decreases<sup>5</sup>.

In a normal pregnancy the placental glycogen level is increased, decreasing gradually as pregnancy following the term<sup>6</sup>. But when there is any endocrine change the glycogen storage may change. Among the changes that occur during pregnancy can include diabetes. *Diabetes mellitus* is a chronic disorder caused by the lack of insulin production by pancreatic beta ( $\beta$ ) cells or the defect in the receptor for insulin in the cells target, resulting in metabolic hyperglycemic disease. This syndrome alters lipid, carbohydrate and protein metabolism<sup>7</sup>.

Many experimental models using laboratory animals are made to enhance the understanding of the pathophysiological mechanisms involved in diabetic syndrome, such as the use of  $\beta$ -cytotoxic chemical agents (streptozocin and alloxan). The hyperglycemia induced by streptozocin (STZ) is dose-dependent, when low doses of STZ are administered at the beginning of the pregnancy, there is appearance of the gestational diabetes (mild diabetes) occurs with presence of fetal macrosomia, while high doses administered in adulthood of the animal induce insulin deficient diabetes associated with intrauterine growth restriction of uterine<sup>8</sup>.

The glycogen placental increases both in women and in diabetic animals since diabetes results in the glycogen lysis. The enzymatic mechanism that can result in the glycogen accumulation in the placental diabetes, favoring the glycogen synthesis or decreasing its degradation has been studied in rats presenting STZ-induced diabetes<sup>9</sup>.

The placenta is glucose-dependent related with of energy. Maternal glucose transfer by maternal placenta is determined by the glucose concentration and glucose amount stored as glycogen<sup>10</sup>. In late pregnancy the insulin concentration in the fetal circulation is high<sup>11</sup>, particularly in the presence of maternal hyperglycaemia<sup>12</sup>. As the placenta depends on its insulin with regard to the glycogen metabolism, hyperglycemia is a major factor in the change of its storage. Then, according to previous studies and interest to investigate the relationship between mild diabetes and placental glycogen in rats, the aim of this study was to evaluate the placental glycogen storage and fetal development in the pregnancy of neonatally streptozocin-induced diabetic rats and to establish relation with glycemia and insulin levels.

## Methods

Nine-week-old male and female Wistar rats (n=10), weighting approximately 180g and 220g were kept in collective cages in controlled conditions of temperature of ( $22 \pm 3^\circ \text{C}$ ), light (12h light/dark cycle) and relative humidity ( $60 \pm 5\%$ ). The animals were fed with laboratory chow and tap water *ad libitum* and cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals. The local Committee of Ethics in Animal Experimentation approved all experimental procedures of this study. Parental female rats were mated to obtain offspring. The female newborns (NB) received streptozocin in dose of 100 mg/kg body weight, subcutaneous route dissolved in citrate buffer (0.1M, pH 4.5) at day 0 of birth, according to modified procedures<sup>13,14</sup>. Control NB received citrate buffer only. NB rats remained with their mothers until the end of the weaning period (day 21 of post-natal life) and were maintained under similar conditions to those of their mothers. In the month 3 of life, rats were mated overnight with non-diabetic male Wistar rats. The morning when sperm was found in the vaginal smear was designated Gestational Day 0. At days 0, 7, 14 and 21 of pregnancy, maternal body weights were determined and blood samples were collected from the tail vein in order to evaluate glucose levels using glucose oxidase reagents strips by glucose oxidase method<sup>15</sup>. Criteria inclusion for this study was rats presenting glycemic level between 120-300 mg/dL classified as mild diabetics rats. Rats with glycemia below 120 mg/dL were considered control. All female rats were mated overnight to non-diabetic male. The morning when sperm was found in the vaginal smear was designated gestational day 0. At day 21 of pregnancy, fed rats were anesthetized with sodium thiopental. Following trichotomy of the abdominal region, the animal was placed in dorsal decubitus, and its limbs were fixed to the surgery table. The laparoscopy procedure was carried out by an incision in the medium line beginning in the xiphoid cartilage and ending in the pubis. The intestinal loops were moved cranially for uterus exposure. The hysterectomy was carried out by ligament, artery and ovarian vein section and incision of the body uterine above the cervix. Afterward, the uterus and his content were weighed using analytical scale. Incisions were performed in the whole extension of the uterine horns, on their free margin and on the most avascular area. The fetus, amniotic sac and placenta were removed by slight traction. The umbilical cord was clamped by using Halstead-type hemostatic clamps. Next, each cord was sectioned and each placenta was isolated weighed on an analytical scale to obtain 250mg of tissue. These placental samples were stored in potassium hydroxide (KOH - 30%) at  $-80^\circ \text{C}$ . The tissue sample was digested in hot concentrated KOH, precipitated with ethanol, hydrolyzed with phenol and sulphuric acid and so performed by a spectrophotometer with a wavelength of 490 nm according to modified procedure from Nomura *et al.*<sup>16</sup>.

The results were reported as mean  $\pm$  standard error of mean (SEM). All data were statistically analyzed using Two-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test.  $p < 0.05$  was taken to be statistically significant.

**Results**

*Maternal weight gain during pregnancy*

During whole pregnancy, it was observed increased body

weight in mild diabetic rats (STZ group). At day 0, 7, 14 and 21 of pregnancy, these animals presented lower ( $p < 0.05$ ) body weight and maternal weight gain (day 21 – 0) compared to non-diabetic rats (Table 1).

**TABLE 1** - Body weight at days 0, 7, 14, 21 and maternal weight gain (day 21 – 0) from pregnant mild diabetic (STZ) and non-diabetic (control) rats

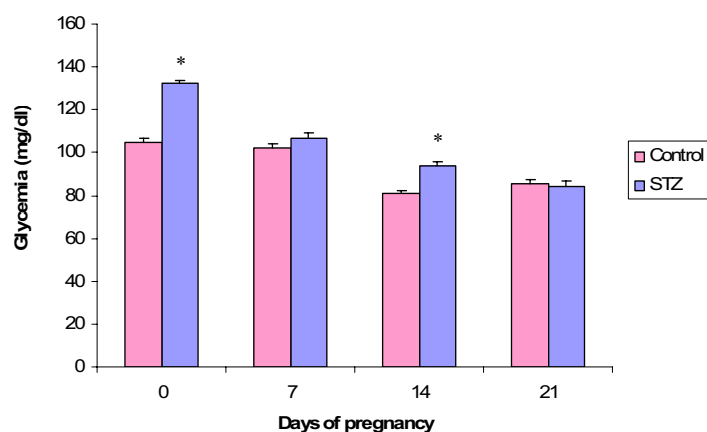
	<b>Control</b> (n=28)	<b>STZ</b> (n=28)
Day 0	284.19 ± 4.66	260.71 ± 7.99*
Day 7	305.29 ± 5.09	278.81 ± 7.90*
Day 14	330.96 ± 5.36	303.26 ± 8.71*
Day 21	407.22 ± 6.43	357.86 ± 11.67*
Maternal weight gain (day 21-0)	123.03 ± 2.91	97.81 ± 4.91

Data are reported as mean ± standard error of mean (SEM)

\* $p < 0.05$  –significant statistically difference compared to control group (Student Newman KeulsTest).

*Glycemia during pregnancy*

In control rats, normoglycemia was confirmed with mean glucose values bellow 100 mg/dL (day 0, 7, 14 and 21 of pregnancy); while in rats STZ the higher ( $p < 0.05$ ) glycemia was observed at day 0 and 14 of pregnancy compared to control rats (Figure 1).



**FIGURE 1** - Glycemic levels on days 0, 7, 14 and 21 of pregnancy from mild diabetic (STZ, n=28) and non-diabetic (control, n=28) rats

Data are reported as mean ± standard error of mean (SEM)

\* $p < 0.05$  –significant statistically difference compared to control group (Student Newman KeulsTest).

*Insulin levels*

At end of pregnancy, rats STZ presented no significant difference ( $p > 0.05$ ) on the insulin levels compared to control group (Table 2).

**TABLE 2** - Insulin determination from mild diabetic (STZ) and non-diabetic (control) rats at day 21 of pregnancy

	<b>Control</b> (n=20)	<b>STZ</b> (n=28)
Insulin (ug/L)	0.56 ± 0.50	0.70 ± 0.49

Data are reported as mean ± standard error of mean (SEM)

$p > 0.05$  – non significant

*Placental glycogen levels*

There was no difference on the levels of placental glycogen in rats STZ in the at term pregnancy compared to control rats ( $p > 0.05$ ) (Table 3).

**TABLE 3** - Placental glycogen levels from mild diabetic (STZ) and non-diabetic (control) rats at day 21 of pregnancy

	Control (n=15)	STZ (n=15)
Placental glycogen (mg/100mg)	0.29 ± 0.03	0.29 ± 0.02

Data are reported as mean ± standard error mean (SEM)  
p>0.05 – non significant

### Discussion

In this study, the rats with streptozocin-induced mild diabetes showed weight gain during pregnancy. However, such animals began their pregnancy with lower weights than those in the control group, and these weights remained lower until the end of pregnancy. The smaller body-weight gain of STZ rats during pregnancy might be related to alterations in the growth hormone.

STZ rats began pregnancy with a glycemia which was compatible with that of mild diabetes, that is, between 120 and 300 mg/dL. These results are in accordance with Portha *et al.*<sup>13</sup>, who first described the experimental model for mild-intensity diabetes (100 ≤ glicemia ≤ 300mg/dL) in adulthood. Similarly, Bonner-Weir *et al.*<sup>17</sup> reported that neonatal rats which received STZ on the second day of life showed transitory hyperglycemia followed by normal glycemia until the sixth week of life. After this period, the animals developed diabetes with the absence of ketonic bodies and without the need for insulin treatment, which suggest that these hyperglycemic rats presented a selective defect in insulin secretion when it was stimulated by glucose

Movassat *et al.*<sup>18</sup> showed that regeneration of pancreatic β-cells between the 4<sup>th</sup> and the 7<sup>th</sup> postnatal days of rats with diabetes induced in the neonatal period contributed to the development of normoglycemic as well as hyperglycemic rats. Nevertheless, various studies in the literature report that STZ administration during the neonatal period causes the onset of mild-intensity diabetes in these animals adulthood<sup>13,19</sup>, thus confirming our findings. In this study, the glycemic means were higher at days 0 and 14 of pregnancy of rats with mild diabetes. Additionally, they showed glucose intolerance and insulin resistance (data not shown) in the period similar to that presented by women with gestational diabetes, which suggest that both in gestation and in pregnancy pancreatic β-cells develop a sensitive mechanism to respond to glycemic signs. These alterations may be mediated by hormones, such as placental lactogen, estrogen and progesterone.

Our results did not show alterations in the placental glycogen rates of diabetic rats in at-term pregnancy, thus differing from Shafir and Barash<sup>20</sup>, leading to the unalteration of glycemic levels at the end of pregnancy, which could be related to the lack of modification of placental glycogen levels. Glycogen accumulation in the placenta, in face of maternal insulin deficiency and the fact that hyperinsulinemia on the fetal side is not more accentuated in diabetic than in control rats, indicates that insulin has no direct influence on glycogen metabolism in this tissue<sup>10</sup>. Glucose flow across the placental is substantially reduced in diabetic hyperglycemia, and there is no convincing demonstration, as yet, of an insulin-dependent, rate-limiting locus in the placenta<sup>20</sup>.

### Conclusion

Mild diabetes altered the maternal glycemia in the early pregnancy, impairing future fetal development, but it caused no alteration on insulin and placental glycogen determination, confirming that this glycemic intensity was insufficient to change glycogen metabolism.

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