

# Impact of experimental diabetes and chronic hypoxia on rat fetal body weight

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## ABSTRACT

**Objectives:** The aim of the study is to determine the impact of the experimental diabetes and the chronic hypoxia on pregnancy development and rat fetal body weight.

**Material and methods:** The experiment was performed on female Wistar rats. Animals were divided into the experimental groups. I — Controls, II — Untreated diabetes, III — Insulin-treated diabetes, IV — No diabetes with chronic hypoxia, V — Untreated diabetes and chronic hypoxia, VI — Insulin-treated diabetes and chronic hypoxia. Diabetes was induced in groups II, III, V and VI with intraperitoneal injection of streptozocin (STZ) at a dose of 40 mg/kg. Chronic hypoxia was induced by placing dams (groups IV, V and VI) in conditions of 10.5% oxygen and 89.5%. Insulin was administered subcutaneously at the dose of 9 IU/kg. Starting from the 6<sup>th</sup> day after STZ injection and chronic hypoxia conditions animals were caged together for 12 hours for 3 consecutive days to ensure fertilization. On day 21 of gestation the animals were decapitated, the fetuses were removed and weighted.

**Results:** Mean fetal body weight in separate groups were: I — 5.38 g, II — 6.04g, III — 5.32g, IV— 5.56 g, V — 3.45 g, VI — 6.23 g.

**Conclusions:** Pre-existing type 1 diabetes does not affect fetal body weight compared to healthy newborn control rats. Prolonged hypoxia does not impact on fetal body weight. Chronic hypoxia during pregnancy complicated with untreated type 1 diabetes mellitus leads to significant reduction of fetal body weight. Insulin treatment reversed the detrimental effect of chronic hypoxia on fetal development.

**Key words:** diabetes, hypoxia, body weight, fetus

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## INTRODUCTION

Diabetes is a multifactorial disease. It causes several maternal and fetal vascular anomalies and may lead to the impaired uteroplacental blood flow resulting with fetal hypoxia. Gestational diabetes has been confirmed to cause the intrauterine growth restriction. It is connected with an elevated incidence of congenital diseases and may lead to postpartum neurological disorders. Mild and moderate intellectual deficiencies are reported in offspring of diabetic mothers as well as mothers suffering from asthma, renal

disorders or epilepsy. It appears that the severity of these disorders is correlated with a degree of glycemic control during pregnancy. An inadequate metabolic balance in pregnant women with diabetes seems to account for worse results in intelligence (IQ) tests in offspring at school age. Metabolic disorders during the first and third trimester of gestation seem to be most prognostically relevant [1]. Children born to mothers with well-controlled diabetes score similarly in intelligence tests to children born to healthy mothers. Their motor functions, ability of attention can be,

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however, adversely affected, which may result in psychomotor hyperactivity [2]. The relationship between ketonemia in mothers with gestational diabetes and lower IQ score in offspring has been extensively described in literature [1]. The age of pregnant diabetic mothers is also a relevant factor, a negative correlation has been demonstrated between maternal age and offspring IQ scores. This association has not been observed in women with normal carbohydrate metabolism [3–5]. Studies in animal models of gestational diabetes in rats and their offspring have demonstrated a reduced level of neurotransmitters such as taurine and  $\gamma$ -amino-butyric acid (GABA), both critical for normal brain development and L-carnosine production, known for its neuroprotective function. This is unlikely to have no repercussions on the structural and functional development of central nervous system (CNS) [6]. Fetal CNS is most probably impaired during cell differentiation as a result of hyperglycemia-induced oxidative stress during gestation [7]. In experimental models of diabetes, a substantial decrease in the activity of endogenous antioxidant enzymes with significantly lower levels of vitamin C and E in fetal cells has been observed, which may be one of the potential factors responsible for CNS abnormalities. Therefore, vitamin C, E, carotenoids and folic acid supplementation may have a positive effect on anti-oxidative protective functions by reducing the defects caused by metabolic disorders in diabetes [8].

Hypoxia induced by gestational diabetes affects fetal CNS development and is connected with an altered sexual behavior both reproductive and non-reproductive in future. Likewise children exposed prenatally to hypoxia may suffer from abnormalities such as suction dysfunction, mother-infant bonding disorders, attention deficit disorders, motor deficits, encephalopathy and cerebral palsy. Hypoxia leads to neurochemical and structural brain injury at the cellular level, disrupting the maintenance of brain homeostasis. Extensive and severe damages of the brain may occur during neural transformation, differentiation, migration, synapse and nerve fibre formation. Moreover, during the development of receptors, hypoxia influence on the elevated level of endogenous monoamines, opioid peptides and excitatory aminoacids, inhibiting normal fetal brain development. Hypoxia is potentially accountable for the onset of autism and autoaggressive behavior in children and could also be a causative factor in adulthood for Alzheimer's disease, may have a detrimental effect on the quality of life and social situation of the affected individuals [9, 10].

## OBJECTIVES

The aim of the study is to determine the impact of the experimental diabetes and the coexistent hypoxia on pregnancy development and rat fetal body weight.

## MATERIAL AND METHODS

The experiment was carried out on female Wistar rats. The animals were obtained from laboratory animal facility (Laboratory animals cultivation, Lipiec Zbigniew, Brwinów, Poland). The animals were on average 120 days old and their body weight ranged from 200 to 250 g. For experimental purposes, the female rats were selected and randomly allocated to groups, each comprising 6 dams. Male Wistar rats weighing 240–260 g were used for fertilization. There were two times less males than females (2:1 ratio). The animals were housed in standard laboratorial conditions, under 12 hour day/night cycle, with an access to food and water ad libitum and in strictly controlled temperature 23°C and air humidity (45–50%). The controls were housed in the same container under the same temperature and humidity conditions. Experimental procedures have been approved by the Local Ethical Committee in Lublin and have been in agreement with European Communities Council Directive on the use of animals in experimental studies.

Experimental diabetes was induced with streptozocin (STZ) (Sigma-Aldrich, St. Louis, USA). Diabetes in animals was treated with NPH insulin and Gensulin N (Bioton, Poland). All other reagents were obtained from POCH (Gliwice, Poland). The analyzed substances were diluted directly prior to administration in a sterile solution 0.9% NaCl and injected intraperitoneally to experimental groups. Controls were injected intraperitoneally with the same volume of 0.9% NaCl solution in a proportion to kilogram of body weight.

Animals were divided into the following experimental groups. Ultimately, on the day of decapitation, each group consisted of 8 dams.

- I — Controls;
- II — Group with untreated diabetes;
- III — Group with insulin-treated diabetes;
- IV — Group without diabetes with chronic hypoxia;
- V — Group with untreated diabetes and chronic hypoxia;
- VI — Group with insulin treated diabetes and chronic hypoxia.

Diabetes was induced in groups II, III, V and VI with a single intraperitoneal injection of streptozocin dissolved in 0.05M citrate buffer, pH = 4.2 at a dose of 40 mg/kg body weight in 5 mL of volume. The presence of glucosuria was established with a semiquantitative method with Ketodiastix (Bayer) test straps 7 days after injection and it confirmed the diagnosis of diabetes. Control group (I and IV) was treated with 0.05 M citrate buffer of pH 4.2 without STZ. Diabetes was diagnosed in 85% of treated female rats.

Insulin treatment of diabetic rats was provided to groups III and VI 7 days after STZ injection and was continued until the end of experiment. NPH insulin was administered subcutaneously at the dose of 9 IU/kg body weight. The

group with untreated diabetes received 0.9% NaCl injection instead of insulin. The control group, which previously had been treated only with citrate buffer without STZ, was treated with injections of 0.9% NaCl. Injections were performed every day at the same time, with 24 hour intervals between injections. The treatment was conducted over 22 days.

Prior to insulin administration, dams from all groups were weighed, on daily basis, to determine the proper dose of insulin based on the stage of pregnancy and the current weight of the pregnant dam.

Starting from the 6<sup>th</sup> day after streptozotocin injection, the experimental females and males were caged together (2 females per 1 male) for 12 hours for 3 consecutive days to ensure fertilization. Conception was confirmed on the 12<sup>th</sup> day after the last coitus.

Chronic hypoxia was induced by placing pregnant rat dams (groups IV, V and VI) in a gas mixture consist of 10.5% oxygen and 89.5% nitrogen 24 hours a day from 15 to 20 day of gestation. Animals were provided with food and water ad libidum during hypoxic exposure.

On day 21 of gestation (29 days after STZ injection) the animals were decapitated. Immediately after pregnant dams were decapitated, the fetuses were removed, decapitated, their sex determined and tissues were taken for further analysis.

### The statistical analysis

The obtained experimental data was statistically analyzed. The default margin of error probability of 5% was used and the threshold for statistical significance was set at  $p < 0.05$ . The study results were presented graphically in the Table 1 and Figure 1. All statistical analyses were performed using Statistica 5.0 Software.

## RESULTS

The obtained results indicate that, pre-existing type 1 diabetes mellitus, whether insulin-treated or untreated, does not affect fetal body weight compared to healthy newborn control rats. Similarly, prolonged hypoxia did not impact fetal body weight. However, chronic hypoxia during pregnancy complicated with untreated type 1 diabetes

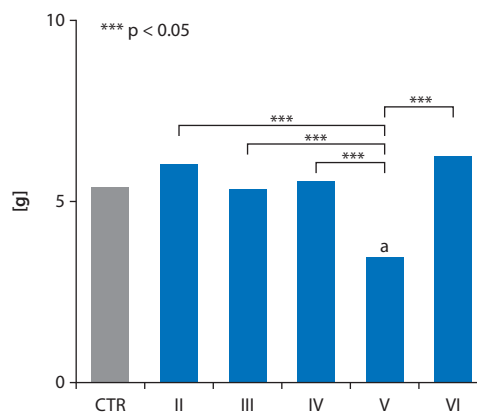


Figure 1. Fetal body weights in each experimental group

mellitus leads to significant reduction of fetal body weight, affecting 65% of control group (Fig.1). Insulin treatment reversed the detrimental effect of chronic hypoxia on fetus development.

## DISCUSSION

Intrauterine growth restriction (IUGR) is defined as a condition in which the estimated weight of the fetus is below the 10<sup>th</sup> percentile for the gestational age and whose abdominal circumference is below the 2.5<sup>th</sup> percentile or birth weight is below 2500 g [11]. It has been proved that either genetic, placental or maternal factors may lead to IUGR. Diabetes mellitus and intrauterine hypoxia are well-known causative factors. IUGR may have lasting consequences on postnatal growth and development; in some instances the development of metabolic syndrome can be attributed to IUGR [12].

Gestational hypoxia may occur in different conditions and may trigger disorders affecting not only fetus development but also its future life. Fetal hypoxia may develop in course of multiple pathologies. Placental insufficiency, pulmonary diseases (e.g. asthma, chronic obstructive pulmonary disease), smoking, maternal anemia, chronic maternal diseases may lead to fetal decreased oxygen supply and the development of IUGR [12]. In the model of rat chronic intermittent hypoxia, an asymmetrical growth restriction of newborn rats was reported. Moreover, increased body fat

Table 1. Fetal body weights — males and females in the experimental groups

Group	Mean fetal weight [g]	Standard deviation (SD)
Controls — CTR	5.38	1.09
Untreated diabetes — II	6.04	0.45
Insulin-treated diabetes — III	5.32	0.81
Chronic hypoxia — IV	5.56	1.22
Untreated diabetes and chronic hypoxia — V	3.45	1.07
Insulin treated diabetes and chronic hypoxia — VI	6.23	0.70

deposition, hyperglycemia and hyperinsulinemia occurred during adolescence [13].

Nowadays, diabetes in pregnant women is widespread. It is estimated that pre-gestational diabetes complicates about 4% and gestational diabetes affects even 8% of pregnancies [14]. Depending on the type of diabetes, different complications may be expected. Untreated or uncontrolled diabetes type 1 results inter alia in ketoacidosis and hyperglycemia. The risk of spontaneous miscarriage, congenital malformations, stillbirth, fetal growth restriction, fetal macrosomia, preeclampsia, early mortality or preterm delivery is increased [15, 16]. Pre-gestational diabetes affects also placental weight and function, causing placental circulatory disorders, vasculopathy and placental insufficiency [17]. Gestational diabetes may create conditions likely to induce inflammation and placental hypoxia [18].

Diabetic placentas may present histological changes similar to placentas from large-for-gestational-age infants [19]. Placental structural dysfunctions are dependent on the duration of diabetes and can lead to chronic fetal hypoxia [20]. Pregnancy planning for patients with type 1 diabetes reduces the risk of adverse pregnancy outcomes [21]. Proper metabolic control both before and during pregnancy is crucial to reduce the risk of complication. It is known that proper glycemic control results in normal fetal weight. Moderately increased values of glycated hemoglobin are related with macrosomia and very high glycated hemoglobin values are related with IUGR [22]. However, in some clinical studies, despite the increased risk of fetal abnormalities, perinatal complications and perinatal morbidity, there was no direct correlation between some of those factors and glycated hemoglobin [15, 23]. In vitro studies have shown that the growth-retarding and teratogenic effect of even lower doses of glucose and ketone bodies acting simultaneously, is greater than when each substance acted separately [24]. In the course of uncontrolled type 1 diabetes mellitus both hyperglycemia and elevated ketone bodies occur. In our experiment, untreated diabetic rats exhibited an extremely high glucose level and presence of ketone bodies. Treatment with insulin reversed those anomalies. Surprisingly, pre-gestational diabetes mellitus type 1 did not affect fetal body weight and we did not observe significant differences between fetuses from insulin treated and untreated females. However, coexistence of untreated type 1 diabetes and chronic hypoxia during pregnancy lead to significant reduction of fetal weight. Those results may suggest that the combination of harmful factors exert stronger effect than each factor acting separately. The insignificant changes in fetal body weight in the group of untreated diabetic rats may be due to the short duration of diabetes and lack of late complications. Insulin treatment reduced glucose and ketone bodies level to virtually normal, and reversed the

detrimental effects of type 1 diabetes. In treated animals hypoxia was not a sufficient factor to disturb fetal growth.

## CONCLUSIONS

The obtained data suggests that disturbances of rat fetal growth is dependent on combinations of harmful factors which escalate their own effects on fetal development. Optimal treatment of each irregularity during pregnancy decreases the risk of their overlap.

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