2 - ORIGINAL ARTICLE
Models, Biological

Regression of gestational diabetes induced cardiomegaly in offspring of diabetic rat[1]

Regressão da miocardiopatia hipertrófica em filhotes de ratas diabéticas

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ABSTRACT
Purpose: To compare body weight and length, heart weight and length, heart-to-body weight ratio, glycemia, and morphometric cellular data of offspring of diabetic rats (ODR) and of normal rats (control). **Methods**: Diabetes was induced in 3 pregnant Wistar rats, bearing 30 rats, on the 11th day after conception by intraperitoneal injection of 50 mg/kg of streptozotocin. Six normal pregnant Wistar rats, bearing 50 rats, made up the control group. Morphometric data were obtained using a scale for the weight, length, heart and body measurements. Morphometric cellular data were obtained by a computer assisted method applied to the measurements of myocytes. Statistical analysis utilized Student's *t*-test, ANOVA and Levene test. **Results**: Control offspring had greater mean body weight and length than offspring of diabetic rats ($p < 0.001$). Heart weight and length and heart-to-body ratios of newborn rats differed between groups at birth ($p < 0.001$), but showed no difference at 21 days. Mean nuclei area and perimetric value of the myocytes decrees throughout the first 21 days of life ($p < 0.01$) in the diabetic group. **Conclusions**: Heart hypertrophy on the offspring of diabetic rats at birth was demonstrated by the significant difference between the groups. After the eleventh day, no difference was found, which confirmed regression of cardiomegaly. The significant difference between the first and the 21th day of life, for nuclei area feature, demonstrate regression of cardiac hypertrophy in the·offspring of diabetic rats.
Key words: Diabetes, Gestational. Cardiomyopathy, Hypertrophic. Rats.

RESUMO
Objetivo: Comparar as medidas cardíacas e a morfometria celular miocárdica dos filhotes de ratas diabéticas (FRD) com filhotes de ratas normais (FRN). **Métodos**: Foram estudados 30 filhotes de 3 ratas Wistar com diabetes gestacional induzido por 50mg/kg de estreptozotocina, no 11° dia após a concepção. O grupo controle foi de 50 filhotes de 6 ratas Wistar normais. As medidas de comprimento, peso corporal e peso cardíaco foram realizadas com paquímetro e balança e as medidas celulares por analisador computadorizado de imagem. A análise estatística usou o Teste *t* de Student, ANOVA e teste de Levene. **Resultados**: A média de peso e comprimento dos filhotes, desde o nascimento até os 21 dias de vida, foi significativamente maior ($p<0,001$) no grupo dos FRN. O peso, tamanho cardíaco e a proporção cardíaca dos FRD, ao nascimento, foi, significativamente, maior ($p<0,001$), regredindo ao longo dos 21 dias de vida. Os FRD apresentaram uma regressão significativa da área e perímetro nuclear ($p<0,01$) do nascimento aos 21 dias de vida, o mesmo não ocorrendo no grupo controle. **Conclusões**: Os FRD apresentaram, ao nascimento, maior tamanho cardíaco, maior peso cardíaco e maior proporção peso cardíaco-peso corporal do que FRN, havendo igualdade estatística entre os dois grupos a partir do 11° dia de vida. Houve diferença significativa entre o nascimento e o 21° dia de vida nas medidas celulares demonstrando regressão da hipertrofia miocárdica nos filhotes das ratas diabéticas.
Descritores: Diabetes Gestacional. Cardiomiopatia Hipertrófica. Ratos.

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Introduction

Gestational diabetes mellitus is defined as diabetes with onset or first diagnosis during pregnancy[1]. It is a risk factor for congenital anomalies, and 3 to 5 times more structural defects are seen in infants of diabetic mothers (IDM) than in those of normal pregnant women. Postnatal studies show that 50% of the abnormalities in IDM are congenital heart defects[2,3].

The typical phenotype of IDM - overweight, moon facies, increased fat tissue and plethora - was first described by Farquhar[4] who suggested that, when the mother had no vascular disease, greater fetal growth was due to poor control of diabetes in pregnancy.

According to Pedersen[5], hyperglycemia in pregnant women causes hyperglycemia and hyperinsulinemia in the fetus.

In the pathogenesis of this process, maternal glucose crosses the placental barrier by diffusion and induces fetal hyperglycemia, which stimulates the fetus's pancreas to increase levels of plasma insulin[6]. Therefore, the increase in fetal plasma insulin is caused by maternal hyperglycemia, and the fetus produces insulin for its own reserve and for the maternal glucose[6].

During intrauterine life, insulin acts as a growth hormone. Fetuses with high levels of insulin use excess glucose to stimulate the growth of some tissues, such as the heart, liver, spleen, thymus, adrenal glands and skeletal muscles. Fetal heart tissue has a greater growth rate than other fetal tissues[7], which is due to myocardial hypertrophy rather than to glycogen deposit. According to Pildes, fetal insulin stimulation increases the number of cells, nuclei and myocardial fibers, because fetal myocardial tissue has more insulin receptors than the adult tissue. Moreover, IDMs with high insulin levels have more insulin receptors, greater affinity for insulin and a greater capacity to use insulin[8].

Other complex mechanisms of increased fat and protein synthesis have been studied to explain the role of insulin receptors in the hyperplastic or hypertrophic fetal heart. However, experimental studies with animals have not confirmed their action in the development of fetal macrosomia[9,10].

Susa *et al.*[9] and Susa and Schwartz[10] showed, in experimental studies with monkeys, that macrosomia in IDM is due to the effect of insulin and not to an excess of available substrates. Their studies used small pumps in subcutaneous tissue to provide low or high doses of insulin to the fetus so that they developed hyperinsulinism but no hyperglycemia. Their results showed no differences between fetal plasma concentrations of amino acids or free fat acids, and no differences in fetal plasma concentrations of cortisol between study and control groups.

Cardiomegaly was described in 1937 in a necropsy study of IDM[11]. It was associated with glycogen infiltration in the cardiac muscle, later also described by Driscol[12]. Incidence of hypertrophic cardiomyopathy in IDMs is unknown, but it is estimated that 10 to 20% of IDMs with cardiopulmonary symptoms may have this condition[10]. An increase in the number of cells, nuclei and myocardial fibers is seen in fetal hypertrophic cardiomyopathy. The septum is hypertrophic, and there is loss of left ventricular function and obstruction of its exit flow, which may explain why many IDMs without congenital cardiomyopathy show signs of myocardial insufficiency[8]. This condition may resolve in 8 to 12 weeks[8]. According to Zielinsky[13], gestational diabetes has the same potential to cause fetal ventricular cardiomyopathy as previous diabetes. Therefore, metabolic control during pregnancy in women with either gestational or previous diabetes is extremely important because a poor metabolic adjustment may result in fetal heart hypertrophy.

This experimental study compared body weight and length, heart weight and length, heart-to-body weight ratio, glycemia, and morphometric cellular data of offspring of diabetic rats (ODR) and of normal rats (control).

Methods

This prospective experimental study was performed on newborn offspring of adult Wistar rats (250-300 g) that had chemically induced gestational diabetes mellitus.

Diabetes was induced in 3 pregnant Wistar rats bearing 30 fetuses on the 11th day after conception by intraperitoneal injection of 50 mg/kg of streptozotocin dissolved in buffered citrate (pH 4.5) and injected 5 minutes after preparation on the 11th day after conception. Buffered citrate was prepared with 4.2 g of citric acid dissolved in 200 ml of distilled water and mixed at a 1:1 ratio with 5.9 g of sodium citrate dissolved in 200 ml of distilled water[14]. Rats were fasted overnight before administration. Six normal pregnant Wistar rats bearing 50 fetuses made up the control group. Morphometric data were obtained using a scale for the weight and length measurements of offspring heart and body. The rat hearts were excised on birth, 11th day and 21th day after birth under CO_2 anesthesia. Each heart was sectioned for counting nucleuses and measuring nuclear areas, by light microscopy, taking sections immediately below the atrioventricular line. Morphometric cellular data were obtained by a computer assisted method applied to the measurements of myocytes. The glycemia of diabetic rat mothers was measured 48 hours after diabetic induction using a blood sample from the tail. Diabetes was defined as a glucose blood level greater than 120 mg/dl[15].

Wistar rats were housed in polycarbonate cages. They were maintained on a 12-h light-dark cycle in a temperature-controlled (22°C) colony room and had free access to food and water. The experiments were performed according to the Guide for the care and use of laboratory animals, and the Ethics Committee for Experiments on Animals approved all procedures.

To analyze the development of offspring and their hearts during 21 days of breast-feeding, two control litters were analyzed at birth, two on the 11th day and two others on the 21st day of life. Offspring were measured from snout to the base of the tail. Hearts were measured from the base to the apex with a caliper (0.001 cm) and weighted individually on a scale (Ohaus Adventurer; 0.001 g). The heart-to-body weight ratio was calculated using the following formula:[16] ratio = (heart weight/body weight) x 100.

The same schedule was used for the group of offspring of diabetic rats; one litter was analyzed at birth, one on the 11th day and one on the 21st day of life to conform to the three "Rs" principle (replace, reduce and refine) according to which the number of animals analyzed is reduced due to ethical principles[17].

The diabetic rats, as well as the rats in the control group, were kept with their respective mother while breast-feeding to analyze offspring growth and weight during their first 21 days.

Data were reported as means and standard deviations. Student's *t*-test, ANOVA and Levene test were used for statistical analysis. The level of significance was 5% (alpha = 0.05)[18].

Results

Glycemia at birth was greater (p= 0.001) in offspring of diabetic rats (ODR) than in the control group. However, on the 11th day and the 21st day of life, newborn rats in the control group had greater levels of glycemia than ODRs (Table 1).

Length was significantly greater (p= 0.001) in the control group than in ODRs from birth to the 21st day of life. This difference in growth increased during the 21 days of breast-feeding (Table 1).

Weight in the control group was significantly greater (p= 0.001) than in the ODR group from birth to the 21st day of life, and the greatest weight gain was observed during breast-feeding (Table 1).

TABLE 1 - Comparison of glycemia, body length, and weight between ODR and control group during 21 days of life in rats

Age (days)	Glicemia (SD) (mg/dL)		Length (SD) (cm)		Weight (SD) (g)	
	Control	Diabetes	Control	Diabetes	Control	Diabetes
1	*69.6 (40.1)	*342.9(90.2)	+5.25 (1.1)	+4.63 (0.8)	x6.86 (1.3)	x5.50 (0.9)
11	*120.3 (12.4)	*86.7 (16.3)	+8.56 (2.4)	+6.93 (1.3)	x24.98 (6.2)	x11.70 (4.1)
21	*115.2 (10.2)	*93.8 (18.4)	+10.55 (2.7)	+8.12 (1.8)	x42.02 (12.8)	x16.96 (8.2)

SD=standard deviation *=p<0.001 +=p<0.001 x=p<0.001

Heart weight in ODRs at birth was significantly greater than in the control group. From the 11th day on, heart weight was greater in the control group (Table 2).

Heart length was greater in ODRs at birth. From the 11th day on, heart length decreased in the ODR group (Table 2).

The heart-to-body weight ratio at birth was significantly greater in ODR, but decreased from the 11th day on. On the 11th day, ratios were similar, and on the 21st day, no statistical differences were found between groups (Table 2).

TABLE 2 - Comparison of heart weight, heart length, and heart-to-body weight ratio between ODR and control group during 21 days of life in rats

Age (days)	Heart weight (SD) (g)		Heart length (SD) (cm)		heart-to-body weight ratio (SD) (%)	
	Control	Diabetes	Control	Diabetes	Control	Diabetes
1	*0.031 (0.005)	*0.046 (0.004)	x0.47 (0.09)	x0.56 (0.08)	+0.451 (0.07)	+0.836 (0.06)
11	**0.13 (0.009)	**0.05 (0.007)	xx0.87 (0.06)	xx0.60 (0.01)	++0.520 (0.02)	++0.427 (0.08)
21	**0.21 (0.08)	**0.08 (0.002)	xx0.92 (0.03)	xx0.73 (0.02)	++0.499 (0.04)	++0.471 (0.05)

SD=standard deviation *=p<0.05 **=p<0.01 x=p<0.05 xx=p<0.01 +=p<0.001 ++=NS

Table 3 data demonstrated significant reduction on nuclear area and nuclear perimeter (p<0.01) from first to 21th day of life of ODR. Control group do not showed difference among nuclear area, nuclear perimeter and nuclei number from first to 21th day of life.

TABLE 3 - Mean nuclear area, nuclear perimeter and nuclei number of offspring of diabetic rat (ODR) and control group from first to 21th day of life

Age (days)	Nuclear area (SD) (μm^2)		Nuclear perimeter (SD) (µm)		Nuclei number ($n/10^3$ μm^2)	
	Control	Diabetes	Control	Diabetes	Control	Diabetes
1	20.02* (12.44)	25.95* (25.86)	53.85** (21.48)	69.89** (24.27)	24.00+	30.8+
11	30.30 (10.21)	24.45 (29.02)	73.45 (12.15)	66.29 (35.33)	23.60	29.20
21	17.20 (22.13)	19.06* (18.06)	49.63 (20.02)	51.27** (30.44)	23.25++	37.25++

SD=standard deviation *p<0,01; **p<0,02, +p<0,05, ++p<0,05

At birth ODR group had difference from control group on nuclear area (p<0.01) and nuclear perimeter (p<0.02), the same difference occur in ODR comparing day 1 and day 21. The nuclei number was greater in ODR than in control group (p<0.05) at 21th day.

Discussion

Offspring of diabetic rats (ODR) had a higher level of glycemia at birth than the control group. From the 11th day on, their level of glycemia decreased significantly (Table1).

Our results of offspring length and weight (Table 1) confirmed findings by Kim[14], who, in 1960, demonstrated that ODR were born smaller and lighter than animals in the control group. They are also in agreement with results reported by Menezes[19], in 2001, who studied fetuses at the end of pregnancy, and by Giglio[20] who described a fetal growth curve that confirmed that the developmental delay started in intrauterine life.

Delay of intrauterine development (DIUD) may affect up to 20% of the pregnancies with gestational diabetes mellitus[21]. Several mechanisms may cause this delay in ODR: non-chromosomal malformations, abnormalities in cell replication and decrease of cell numbers[22]. Uteroplacental insufficiency, another mechanism involved in DIUD[23], may be analyzed by measuring maternal uterine artery flow. When flow is impaired, the oxygen supply to the fetus decreases, acidosis develops, CO_2 increases, and glucose[11] and the supply of energy to the fetus also decrease.

Similarly, restriction of nutrients may cause DIUD and may explain ODR developmental delay after birth. Our findings described in table 1 showed that ODRs have a lower weight and length gain during the 21-day breast-feeding period.

Histological analyses of the hearts of ODR demonstrated decrease of cellular nuclei size confirming reduction of cardiac hypertrophy during the first 21 days of life, a feature also expected to occur in human infant[8].

In gestational diabetes mellitus, the fetus produces insulin for its own metabolism and for its mother's. A greater production of insulin, a hormone that induces growth, leads to an enlargement of organs sensitive to insulin, such as the heart[4].

It is estimated that approximately 30% of infant of diabetic mother (IDM) have cardiomegaly and that 5 to 10% have congestive heart failure. Hypertrophic cardiomyopathy may also be associated with poor diabetes control[7].

The fetal heart is one of the organs most affected by diabetes, which complicates pregnancy and poses a risk of cardiac malformations in up to 8.5% of live-born IDM[24].

In this study, ODR at birth had greater heart length, heart weight and heart-to-body weight ratios than animals in the control group, which confirms the findings by Menezes[19]. On the 11th day of life, all measures were statically equal, which suggests the regression of cardiomegaly in the ODR.

Adequate metabolic control before conception and during the first weeks of pregnancy can substantially decrease the incidence of congenital defects in IDM[21]. Several risk factors for gestational diabetes mellitus has been identified and require severe control[25,26].

The findings reported in this study, as well as the data of studies with human beings, may contribute to the advancement of knowledge about gestational diabetes mellitus and the regression of hypertrophic cardiomyopathy in infant of diabetic mother.

Conclusions

Heart hypertrophy on the offspring of diabetic rats at birth was demonstrated by the significant difference between the two groups. After the eleventh day, no difference was found, which confirmed regression of cardiomegaly. The significant difference between the first and the 21th day of life, for cellular nuclei area feature, demonstrate regression of cardiac hypertrophy in the offspring of diabetic rats.

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