

Association of diabetes and cigarette smoke exposure on the glycemia and liver glycogen of pregnant Wistar rats¹

Associação entre diabetes e exposição à fumaça de cigarro sobre a glicemia e glicogênio hepático de ratas Wistar prenhes

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ABSTRACT

Purpose: To evaluate cigarette smoke exposure and/or diabetes association effects on the glycemia and liver glycogen levels of pregnant Wistar rats. **Methods:** 60 adult rats were randomly distributed into (n=10/group): non-diabetic exposed to filtered air (G1); non-diabetic exposed to cigarette smoke only before pregnancy (G2); non-diabetic exposed to cigarette smoke before and during pregnancy (G3); diabetic exposed to filtered air (G4); diabetic exposed to cigarette smoke only before pregnancy (G5), and diabetic exposed to cigarette smoke before and during pregnancy (G6). Glycemia was determined at days 0 and 21 of pregnancy. Liver samples were collected for liver glycogen determinations. **Results:** At day 21 of pregnancy, glycemia was higher in G5 and G6 compared to G4 group. G2 (2.43±0.43), G3 (3.20±0.49), G4 (2.62±0.34), G5 (2.65±0.27) and G6 groups (1.94±0.35) presented decreased liver glycogen concentrations compared to G1 (4.20±0.18 mg/100mg liver tissue) (p<0.05). G5 and G6 groups presented decreased maternal weight gain and litter weight. **Conclusions:** Severe diabetes and cigarette smoke exposure, alone or associated, caused impairment in liver glycogen storage at term pregnancy. Due to the fact that liver glycogen storages were considered determinant for glucose tolerance, it is relevant to point out a rigid clinical glycemic control and to stop smoking so earlier in pregnancy programming.

Key words: Diabetes Mellitus, Experimental. Pregnancy. Glycogen. Tobacco Smoke Pollution. Glycemia. Rats.

RESUMO

Objetivo: Avaliar a associação da exposição à fumaça de cigarro e/ou diabete sobre a glicemia e concentrações de glicogênio hepático em ratas Wistar prenhes. **Métodos:** 60 ratas adultas foram distribuídas aleatoriamente em seis grupos (n=10/grupo): não-diabético exposto ao ar filtrado (G1); não-diabético exposto à fumaça de cigarro antes da prenhez (G2); não-diabético exposto à fumaça de cigarro antes e durante a prenhez (G3); diabético exposto ao ar filtrado (G4); diabético exposto à fumaça de cigarro antes da prenhez (G5); diabético exposto à fumaça de cigarro antes e durante a prenhez (G6). A glicemia foi determinada nos dias 0 e 21 de prenhez. Foram coletadas amostras de fígado para dosagens de glicogênio. **Resultados:** No 21º dia de prenhez, a glicemia foi maior nos grupos G5 e G6 comparados ao grupo G4. Os grupos G2 (2,43±0,43), G3 (3,20±0,49), G4 (2,62±0,34), G5 (2,65±0,27) e G6 (1,94±0,35) apresentaram concentrações de glicogênio diminuídas comparados ao grupo G1 (4,20±0,18 mg/100mg) (p <0.05). Os grupos G5 e G6 apresentaram ganho de peso materno e peso da ninhada diminuídos. **Conclusões:** O diabete grave e a exposição à fumaça de cigarro, sozinhos ou associados, causaram prejuízo no armazenamento de glicogênio na prenhez a termo. Devido ao fato dos estoques de glicogênio serem determinantes para a tolerância à glicose, é imprescindível indicar um rígido controle glicêmico e deixar de fumar antes da gestação.

Descritores: Diabetes Mellitus Experimental. Gravidez. Glicogênio. Poluição por Fumaça de Tabaco. Glicemia. Ratos.

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Introduction

Pregnancy has been characterized as producing a varied group of physiological and metabolic effects to both the mother

and the developing fetus. The principal metabolic nutrients in the fetus are glucose and amino acids. Glucose (including its metabolic product lactate) plays as the principal substrate in the fetus for maintenance energy production and expenditure, energy

storage in glycogen and adipose tissue, and energy requirements of protein synthesis and growth^{1,2}. The rate of glucose transfer from maternal to fetal plasma and the net rate of fetal glucose uptake are directly related to the maternal glucose concentration³. The exponential growth of the embryo and/or fetus places an increasing demand on the mother, which is reflected by increased maternal food intake during the course of gestation and the development of a state of maternal insulin resistance⁴.

A major determinant of glucose tolerance is the efficiency of hepatic glycogen synthesis. A long-standing concept holds that, after ingestion of a carbohydrate or a mixed meal, a large fraction of glucose is taken up by the liver and converted into glycogen by a direct process⁵. The relative contributions of these pathways to glycogen repletion may be determined by physiological changes or pathological abnormalities⁴, such as diabetes and cigarette smoke exposure. Insulin is the hormone responsible for glycogen synthesis stimulation in the liver and skeletal muscle. From the total amount of ingested carbohydrates, 20% and 30%, respectively, are stored in the form of hepatic and muscular glycogen^{6,7}.

Glycogenesis and glycogenolysis alterations can contribute to increase postprandial hyperglycemia found in glycemic metabolic changes, as diabetes. In uncontrolled type 1 Diabetes mellitus (DM1) individuals, deregulation in endogenous glucose production occurs after food intake, which is not properly supplied. Hence, hepatic glycogen accumulation (glycogenesis) is reduced in these individuals^{8,9}.

In addition to the interference by these pathologies, environmental factors may alter liver glycogen storage. Cigarette smoking decreases fasting insulin levels and leads a transient increase in blood glucose levels after an oral glucose challenge^{10,11}.

Many experimental studies have been performed in order to reproduce the diabetic state, to evaluate diabetes outcomes and to enhance the understanding of the physiopathological mechanisms involved in this syndrome. Then, beta-cytotoxic drugs, such as streptozotocin (STZ), are used in laboratory animals. In general, animals with STZ-induced diabetes present reduced hepatic capacity to synthesize glycogen during acute phase of diabetes¹². Since the liver controls glucose homeostasis and there are no studies relating diabetes and cigarette smoke exposure. By this way, in this study, control and diabetic rats were exposed to cigarette smoke with the purpose to examine the interaction of these two conditions during pregnancy in glucose and liver glycogen profile, and in the maternal and litter weight evaluation.

Methods

Six-week-old female (n=60) and nine-week-old male Wistar rats (n=15), weighing approximately 180g and 220g respectively, were kept in collective cages in controlled conditions of temperature of (22 ± 3° C), light (12h light/dark cycle) and relative humidity (60 ± 5%). The animals were fed with laboratory chow (Purina®) 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.

A total of 60 female rats were randomly distributed into six groups (n=10/group); non-diabetic exposed to filtered air (G1); non-diabetic exposed to cigarette smoke before pregnancy (G2);

non-diabetic exposed to cigarette smoke before and during pregnancy (G3); diabetic exposed to filtered air (G4); diabetic exposed to cigarette smoke before pregnancy (G5), and diabetic exposed to cigarette smoke before and during pregnancy (G6). The composition of the cigarette used consisted of 10 mg of tar, 0.80 mg of nicotine and 10 mg of carbon monoxide. For exposure to tobacco cigarette smoke, approximately 47-day-old rats were placed in hermetically sealed chambers before pregnancy (pre pregnancy) period while others were exposed before and during pregnancy period. The respective control groups were exposed to filtered air during similar periods of time. In the first week of exposure, non-pregnant rats were submitted to an adaptation period and exposed to smoke using 5 cigarettes for 30 minutes/day during seven days. After adaptation, rats were exposed to smoke from 10 cigarettes for 30 minutes on a daily basis with 15-minute resting intervals for release of all cigarette smoke contained in the chamber. Following the experimental procedure, the same animals were exposed to smoke from another 10 cigarettes for 30 more minutes; this proceeding method was used for approximately two months. Carbon monoxide concentration in the chamber was 193.50 particles per million (ppm); temperature was maintained at 22-25°C and relative humidity was approximately 40%¹³.

Diabetes was induced by streptozotocin (STZ - SIGMA Chemical Company, St. Louis, MO, USA) 7 days before the mating period as previously described method¹⁴. A dose of 40 mg/kg body weight was used to produce a permanent severe diabetic state (glycemia >300 mg/dL). Blood glucose levels were measured on days 0 and 21 at approximately 9:00 a.m. using glucose oxidase strips (One-Touch Ultra Johnson & Johnson®, Milpitas, CA, USA) by glucose oxidase method¹⁵. Criteria inclusion for this study was rats with glycemia higher than 300 mg/dL were used in diabetic groups.

All female rats were mated overnight to non-diabetic male rats unexposed to cigarette smoke. The morning when sperm was found in the vaginal smear was designated gestational day 0. At day 21 of pregnancy, fed rats were weighed (maternal weight gain) and anesthetized with sodium pentobarbital (Hypnol® a 3%). 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 accomplished with the 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 balance. Rapidly, the stomach was moved to the right to expose the larger hepatic lobe. By scissors, about 50% of this portion was dried up, the sample was weighed in analytical balance to obtain 500 mg of the tissue. These liver samples were stored in KOH (30%) at -80°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 nm¹⁶.

The results were reported as mean ± 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 significant¹⁷.

Results

Glycemia

In non-diabetic rats, normoglycemia was confirmed with mean glucose values below 100 mg/dL (day 0 and 21 of pregnancy); while in diabetic rats hyperglycemia consisted of a diabetic state with mean glucose levels above 460 mg/dL (day 0 and 21 of pregnancy). Cigarette smoke exposure did not alter glycemia of groups G2 and G3 (early and late pregnancy) compared to group G1. At day 21 of pregnancy, in presence of cigarette smoke, diabetic rats of groups G5 and G6 presented increased glycemia related with group G4 ($p < 0.05$) (Table 1).

Liver glycogen levels

There was decreased liver glycogen levels in G4 group in relation to those of G1 group ($p < 0.05$). G2 and G3 group also showed a decrease in liver glycogen storage as compared to G1 group ($p < 0.05$). In G5 and G6 groups, there was no statistically significant difference in liver glycogen levels as compared to those of group G4 ($p > 0.05$). G2, G3, G4, G5 and G6 groups showed reduction in liver glycogen levels related with to G1 group ($p < 0.05$). Rats exposed to association of severe diabetes and cigarette smoke before and during pregnancy (G6) showed decreased glycogen levels compared to G3 group ($p < 0.05$) (Table 1).

TABLE 1 - Glycemic levels on days 0 and 21 of pregnancy and liver glycogen concentration obtained at term pregnancy of rats from different experimental groups

	Non-diabetic			Diabetic		
	G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=10)	G6 (n=10)
Glycemia day 0 (mg/dL)	81.31±5.42a	87.85±2.79a	87.57±2.45a	460.62±22.35b	502.75±11.44b	484.64±14.82b
Glycemia day 21 (mg/dL)	96.69±4.85a	90.85±3.68a	92.21±3.20a	471.83±21.93b	525.14±25.70c	562.07±19.91c
Liver glycogen (mg/100mg)	4.20 ± 0.18a	2.43 ± 0.43bc	3.20 ± 0.49b	2.62 ± 0.34bc	2.65 ± 0.27bc	1.94 ± 0.35c

Data are reported as mean ± standard error mean. Non-diabetic exposed to filtered air (G1), non-diabetic exposed to cigarette smoke before pregnancy (G2), non-diabetic exposed to cigarette smoke before and during pregnancy (G3); diabetic exposed to filtered air (G4), diabetic exposed to cigarette smoke before pregnancy (G5), and diabetic exposed to cigarette smoke before and during pregnancy (G6).

$p < 0.05$ – different letters represent significant difference among groups (Student Newman Keuls Test).

Maternal parameters

It was observed decreased maternal weight gain and litter weight in diabetic groups exposed to cigarette smoke (G5 and G6) compared to other groups (G1, G2, G3 and G4). Non-diabetic rats exposed to cigarette smoke (G2 and G3) and diabetic rats exposed to filtered air (G4) did not present alteration in maternal weight gain and litter weight compared to G1 group (Figure 1).

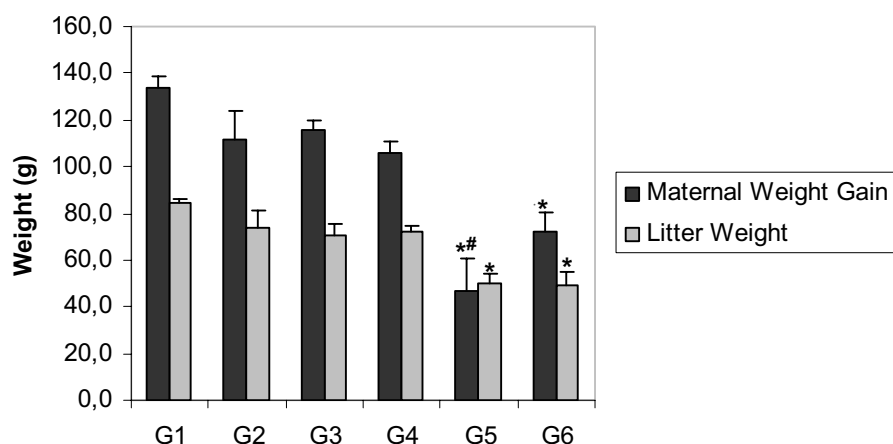


FIGURE 1 - Maternal weight gain and litter weight on day 21 of pregnancy of rats from different experimental groups. Data are reported as mean \pm standard error mean. Non-diabetic exposed to filtered air (G1), non-diabetic exposed to cigarette smoke before pregnancy (G2), non-diabetic exposed to cigarette smoke before and during pregnancy (G3); diabetic exposed to filtered air (G4), diabetic exposed to cigarette smoke before pregnancy (G5), and diabetic exposed to cigarette smoke before and during pregnancy (G6)

* $p < 0.05$ – significant difference among groups (Student Newman Keuls Test).

$p < 0.05$ – significant difference compared to G6 group (Student Newman Keuls Test).

Discussion

The aim of the present paper was to study the correlation between cigarette smoke exposure and diabetes during pregnancy and not was designed to trying to explain the pathophysiology these two conditions. In the present study, non-diabetic rats exposed to cigarette smoke presented decreased litter weights but no change in the maternal weight gain. These results disagree with Bertolini *et al.*¹⁸, which demonstrated that smoke-exposed rats during pregnancy presented no alterations on the litter weight and reduced maternal weight gain. Studies with women pregnant smokers showed contradictory results about maternal weight gain^{19,20,21}. The association of diabetes and cigarette smoke impaired maternal weight gain and litter gain, fetal weight and number of fetus (data not shown).

In this study, the highest glicemic levels was observed in diabetic rats exposed to cigarette smoke, regardless moment, specially on late pregnancy, suggesting that exposure to cigarette smoke contributed even more to increase the hyperglycemia. Cigarette smoking generally increases insulin resistance by altering the distribution of body fat or by exerting a direct toxic influence on pancreatic tissue²². Chemical components of cigarettes may directly alter intracellular glucose transport or may indirectly alter it through changes in serum chemistry or diminished vascular blood flow²³. As cigarettes contain about 3,500 different compounds in the particulate phase and 500 gaseous compounds in the volatile phase, precisely elucidating such mechanisms may be a formidable task indeed²⁴. Theoretically, insulin resistance could be caused by direct effects of nicotine, carbon monoxide or other agents in tobacco smoke²⁵.

Diabetic rats, regardless cigarette smoke exposure, presented decreased glycogen levels. This finding can be explained because streptozotocin inhibits insulin synthesis and secretion, causing hyperglycemia, which reproduces clinical signals found in patients with uncontrolled type 1 Diabetes mellitus. Insulin

triggers a remarkable set of biological responses and is the main hormone responsible for controlling the uptake, use and storage of cellular nutrients. In the absence of insulin, glucose uptake by the liver is interrupted, leading to a reduction in the additional synthesis of liver glycogen²⁶, which justifies the low glycogen levels in diabetic rats exposed to filtered air or cigarette smoke found in the present study.

However, the glycogen storage of rats exposed to smoke only prior to pregnancy (G2 and G5 groups) did not differ from those of rats that had always been exposed (G3 and G6 groups), respectively. This result corroborates with Will *et al.*²⁷, who observed that the risk factor for women smokers for developing diabetes only ceased to exist in those who had stopped smoking at least five years before. This fact shows the importance of women smokers stopping smoking long before planning a pregnancy. Besides, G6 rats had reduced glycogen levels compared to G3 group, therefore, the association of severe diabetes and cigarette smoke exposure impaired the liver glycogen metabolism and storage in maternal organism.

Several plausible biological mechanisms have been advanced to explain an association between cigarette smoking and the incidence of diabetes, but much more research is needed in this area.

Thus, the isolated exposure to cigarette smoke and isolated severe diabetes caused decreased glycogen storage in rats. Cigarette smoke exposure and severe diabetes association impaired glycogen levels in pregnant rats, but it did not exacerbate a reduction of glycogen determination related to severe diabetes. This fact might be explained because highest glycemic level and exposure period masked cigarette smoke effect in this parameter. These results suggest more studies are required using experimental mild diabetes to better understand the insulin resistance and/or glucose tolerance state in the presence of this association. Hence, in view of the obtained results, cessation of smoking only after conception did not promote sufficient improvement in glycemia and liver glycogen metabolism. Considering that smoking and pregnancy lead insulin resistance and that the latter is directly or indirectly involved in the pathogenesis of hyperglycemia and other manifestations, women smokers should count on medical follow-up before becoming pregnant, since intrauterine exposure to smoking during pregnancy may increase the risk of fetal complications and cause both diabetes and obesity in the offspring, resulting in lifelong metabolic dysfunction, possibly due to fetal malnutrition or toxicity. Besides, due to the fact that liver glycogen storages were considered to be determinant for glucose tolerance, it is relevant to point out a rigid clinical glycemic control and to stop smoking so earlier in pregnancy programming.

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