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Neonatal streptozotocin-induced diabetes in mothers promotes metabolic programming of adipose tissue in male rat offspring

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ABSTRACT

Aims: Maternal hyperglycemia during pregnancy can lead to fetal changes, like macrosomia or obesity in adult life. Experimental models of diabetes have been studied to evaluate the consequences of offspring lipid metabolism. This study aimed to investigate the metabolic changes in adipose tissue of offspring of streptozotocin-induced diabetic mothers during neonatal period.
Main methods: Diabetes was induced in female rats by streptozotocin administration on 5th day of life. In adulthood, female rats were bred with control male rats. Male puppies were sacrificed on 12th week of life and epididymal (EP) and subcutaneous (SC) adipose fat pads were excised and weighted. Adipocytes were isolated and evaluated for basal and insulin-stimulated 2-deoxyglucose uptake, oxidation of glucose into CO_2, and incorporation of glucose into lipids and lipolytic capacity.
Key findings: Body weight, EP fat pad weight and diameter of adipocytes from offspring of diabetic mothers were increased in comparison to offspring of control mothers. EP adipocytes from offspring of diabetic mothers presented increased basal and insulin stimulated glucose uptake in comparison to control ones. Similar pattern was observed for glucose oxidation into CO_2 and incorporation into lipids. However, significant difference in lipolytic capacity in vitro was not observed. Protein content of GLUT4, insulin receptor and acetyl-CoA carboxylase was significantly increased in EP fat pad of offspring of diabetic mothers in relation to control group.
Significance: Metabolic programming occurred in the adipose tissue of offspring of diabetic mothers, increasing its capacity to store lipids with no changes in lipolytic capacity.

1. Introduction

Gestational diabetes causes hyperglycemia and hyperinsulinemia in fetal internal milieu during intrauterine life which affects the whole animal, promoting changes in fetal metabolism and compromising many organs and tissue functions. Exposure to this diabetic intrauterine environment also causes changes in fetal growth, which predispose to overweight and obesity in adulthood, even in the absence of macrosomia at birth [1,2].

Several important aspects of the pathophysiology of gestational diabetes can be studied using appropriate animal models. The induction of diabetes during adulthood produces severe diabetes that often needs insulin administration to ensure survival for longer periods. On the other hand, the induction of diabetes by streptozotocin (STZ) during the neonatal period (5 days old) disrupts the metabolic control causing hyperglycemia, hypoinsulinemia, insulin resistance, polyuria, polydipsia, polyphagia, with a lower incidence of ketosis and mortality [3–5].

The diabetes induced in neonatal life displays some characteristics similar to naturally occurring type 1 diabetes in humans, with asymptomatic beginning and partial destruction of pancreatic β cells, while the remaining cells differentiate to compensate for insulin secretion, in order to maintain normoglycemia [6]. Starting at the pubertal period until adulthood, a symptomatic process takes place, in which pancreatic β cells lose its functionality, resulting in hypoinsulinemia and hyperglycemia [4–6]. It is already known that in rats this model of diabetes induces metabolic disturbances and functional inability in white adipose tissue in adulthood [4].

It is indicated that the intensity of fetal exposure to mother's hyperglycemia is similar in offspring of type 1 and type 2 diabetic mothers. However, the offspring's metabolic phenotype is not identical, since

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the ones from type 1 diabetic mothers can develop secondary insulin deficiency, overweight and obesity in adulthood [8,9]. Although some research has described metabolic changes in offspring of diabetic mothers, some aspects, especially those concerned to investigate adipose tissue metabolism have been neglected. Therefore, the present study sought to assess this issue in offspring of neonatally streptozotocin-induced diabetic rat mothers.

2. Materials and methods

2.1. Animals

Five-day-old female Wistar rats were separated from their mothers for 8 h. They were divided into two groups: 1) diabetic mothers, which received an intraperitoneal (i.p.) injection of STZ (120 mg/kg b.w.) freshly diluted in a citrate buffer (10 mM, Na citrate, pH 4.5), and 2) control mothers, which received only the vehicle (i.p.) in an equivalent volume (C group, $n = 6$). Glycemia was determined by tail puncture after weaning (21 days) in STZ-treated animals, and those with 150 mg/dL of glucose levels or above were selected for the study. After the 12th week of age, these females (diabetic and non-diabetic mothers) were placed to mating.

Six pregnant females (three non-diabetic and three diabetic) were transferred to individual cages and stayed with their pups until weaning period (21 days after birth), when the male pups were divided into two experimental groups: control group (C) — offspring of non-diabetic mothers; and offspring of diabetic mothers group (ODM). On the birth day, puppies were weighted and only two male puppies were kept with each mother during lactation in both groups. It was established due to diabetic mothers that give birth to only 4 to 5 animals. After weaning, mothers from both groups were weighted, sacrificed, and the blood was collected for plasma glucose and insulin determinations (Table 2).

Rats from the two groups were housed (one per cage) under a 12-h light–dark cycle (lights on at 0600 h), and maintained at 23 °C, with full access to food (Nuvilab balanced chow pellets, Nuvital SA, Colombo, Brazil). The composition of chow (Nuvilab CR-1) according to the manufacturers was: carbohydrate (56% vegetable starch), protein (30% vegetable protein), lipid (14% unsaturaded oils); and caloric content (2.9 kcal/g).

Body weight and food intake were measured weekly, from weaning to sacrifice (12 weeks old).

2.2. Glucose tolerance test

Oral glucose tolerance test (oGTT) was performed in 10-week-old animals. After 12 h fasting, 750 mg glucose/kg b.w. was given by gavage in a bolus, and blood samples (from a cut of the tail) were collected at 0, 5, 15, 20, 30, 60 and 90 min after glucose administration for glycemia analysis.

2.3. Euthanasia

On the 12th week, all animals (12 h fasted) were decapitated under anesthesia with sodium thiopental (20 mg/kg, b.w.) and trunk blood was collected. The serum was used for glucose and insulin determinations. After laparotomy, the subcutaneous (from inguinal region) (SC) and epididymal (EP) fat pads were excised, weighed and processed for adipocyte isolation. All procedures were approved by the Institute of Biomedical Sciences Ethical Committee for Animal Research (CEEA) and were in accordance with Ethical Principles in Animal Research and the UFAW Handbook on the Care Management of Laboratory Animals.

2.4. Adipocyte isolation

Adipocytes from SC and EP fat pads were isolated by tissue collagenase digestion as previously described [11]. The procedures for diameter determination were performed as Chimin et al. [10]. 100 adipocytes from all visual field were considered, and from these, arithmetic media were calculated.

2.5. Insulin measurements

Insulin was quantified using a specific rat radioimmunoassay kit (Millipore Corporation, St Charles, MO, USA), and was performed following the manufacturer's instructions.

2.6. Insulin-stimulated 2-deoxy-D-glucose uptake

Experiments with 2-deoxy-D-glucose (2DG) were performed as described elsewhere [11] with some modifications. Briefly, isolated adipocytes (40 μL in a 30% cell suspension), from SC and EP fat pads, were incubated with or without insulin (10 nmol/L) diluted in EHB (Earle's salts, Hepes 10 mM, BSA 1%) buffer (pH 7.4), for 15 min at 37 °C. At the end of this period, a 10 μL-aliquot of 2-deoxy-D-[^{3}H]-glucose (^{3}H-2DG, 0.4 mmol/L final concentration and 1850 Bq/tube) was added and after 3 min the reaction was stopped by adding 250 μL of ice-cold phloretin (0.3 mmol/L in EHB and DMSO 0.05%). From this final mixture, a 200 μL-aliquot was transferred to microfuge tubes, and then 200 μL of silicone oil (density = 0.963 mg/mL) was added and the tubes were centrifuged (Microfuge E, Beckman Instruments, Palo Alto, CA) for 10 s at 11,000 *g*. The cell pellet on top of the oil layer was removed and transferred to vials containing 2.5 mL of scintillation cocktail (EcoLume, ICN Pharmaceuticals, Costa Mesa, CA, USA) for radioactivity counting (1450 LSC, Counter MicroBeta, Trilux, PerkinElmer). Unspecific radioactivity was determined using 250 μL of ice-cold phloretin to stop transport reaction before adding the tracer, and the value was used in the final calculation. Values are expressed as picomoles per square centimeter of cell surface area (pmol/cm^2). The within-run variation was <8%.

2.7. Incorporation of D-[U–^{14}C]-glucose into lipids and its conversion into $^{14}CO_2$

Isolated adipocytes excised from SC and EP fat pads were suspended in Krebs/Ringer/phosphate buffer (pH 7.4) containing 1% BSA and 2 mM glucose (to a final concentration of 1×10^6 cells/mL), and saturated with a gas mixture of 95% O_2 and 5% CO_2. 450 μL of cell suspension was incubated with [U–^{14}C]-D-glucose (1850 Bq/tube), in the presence or absence of 10 nM insulin for 1 h at 37 °C (with a 95% O_2 and 5% CO_2 atmosphere). After this period, 200 μL of 8 N H_2SO_4 was added, and the $^{14}CO_2$ released from the mixture was trapped in filter paper moistened with 200 mL of ethanolamine for an additional 30 min. The radioactivity trapped on the filter paper was counted in β-counter (1450 LSC, Counter MicroBeta, Trilux, PerkinElmer). For lipid extraction, dole's reagent (isopropanol:n-heptane:H_2SO_4, 4:1:0.25 vol./vol./vol.) (2.5 mL) n-heptane (1.5 mL) and distilled water (1.5 mL) were added to the remaining reaction mixture, vortexed, and decanted for 5 min. Five hundred microliters of the upper phase were collected for the determination of the radioactivity trapped into lipids. The results were expressed as nanomoles of $^{14}CO_2$ released and glucose incorporated into lipids per 10^6 cells × hour. The within-run variation was <8%.

2.8. Measurement of lipolysis

Lipolytic activity was performed in isolated adipocytes, from SC and EP fat pads, as described elsewhere [12]. The glycerol content of incubated medium was measured following the manufacturer's protocol.