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Basic nutritional investigation

Pregestational maternal obesity impairs endocrine pancreas in male F1 and F2 progeny

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of maternal obesity on pancreas structure and carbohydrate metabolism in early adult life, focusing on the F1 and F2 generations after F0 maternal pregestational, gestation, and lactation high-fat diet (HF).
Methods: C57 BL/6 female mice (F0) were fed standard chow (SC) or an HF diet for 8 wk before mating and during the gestation and lactation periods to provide the F1 generation (F1-SC and F1-HF). At 3 mo old, F1 females were mated to produce the F2 generation (F2-SC and F2-HF). The male offspring from all groups were evaluated at 3 mo old.
Results: F0-HF and F1-HF dams were overweight before gestation and had a higher body mass gain and energy intake during gestation, although only F0-HF dams presented pregestational hyperglycemia. The F1-HF offspring had higher body mass, energy intake, fasting glucose levels, and were glucose intolerant compared with F1-SC offspring. These parameters were not significantly altered in F2-HF offspring. Both F1-HF and F2-HF offspring showed hyperinsulinemia, hyperleptinemia, decreased adiponectin levels, increased pancreatic mass, and islet volume density with elevated α- and β-cell mass, hypertrophied islet characterized by an altered distribution of α- and β-cells and weak pancreatic-duodenal homeobox (Pdx)1 immunoreactivity.
Conclusions: Maternal HF diet consumed during the preconception period and throughout the gestation and lactation periods in mice promotes metabolism and pancreatic programming in F1 and F2 male offspring, implying intergenerational effects.

Introduction

Increased availability of energy-dense foods during the last century and sedentarism, more common in the lifestyles of Western populations, are directly linked to the current obesity epidemic [1]. It has been well established that obesity and its comorbidities can stem from nutritional inadequacies in early life [2]. In this context, maternal high-fat (HF) feeding emerged as a risk factor for metabolic disorders involving abnormal glucose homeostasis and reduced whole-body insulin sensitivity, which worsen the structure of the pancreas, causing islet hypertrophy and insulin resistance (IR) [3,4].

Currently, offspring from pregestational obese dams have demonstrated more pronounced metabolic changes such as hyperinsulinemia, dyslipidemia, and hyperleptinemia at early adult age, despite being fed with control diet after weaning [5,6]. The fact that developmental programming effects could persist even if excessive lipid intake is interrupted suggests that metabolic alterations could be intergenerational. In other words, when inadequate diet is applied to a mother during pregnancy, it will directly influence the offspring developing in utero (F1) and their later-life health, which can affect future generations (F2 and beyond) [7].

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The notion that type 2 diabetes mellitus (T2DM) can develop due to excessive saturated fat in maternal diet calls the attention of the scientific community to metabolic programming of pancreas [8]. Previously, pregestational maternal obesity yielded hyperglycemia coupled with rapid catch-up growth of pancreatic islets in the first generation offspring at 10 d old [9]. This suggests that when dams are obese before becoming pregnant, the effects of a plentiful intrauterine milieu on offspring might be more deleterious. Also, it shows the intergenerational effects of pregestational maternal obesity on insulin sensitivity in mice [10]; although the possible effect of the obesogenic intrauterine environment on islet morphophysiology of the second-generation offspring has not been addressed thus far.

This study aimed to evaluate the effects of maternal obesity on the endocrine pancreas structure and carbohydrate metabolism in early adult life of two consecutive mice generations. This study focused on the F1 and F2 generations after F0 maternal pregestational, gestation, and lactation HF diet feeding.

Methods and materials

The Animal Ethics Committee of the State University of Rio de Janeiro (Protocol no. CEUA/024/2012) approved animal protocols, and all procedures were conducted in accordance with the guidelines for experimentation with animals (NIH Publication no. 85-23, revised 1996). Animals were housed at controlled temperature (21°C ± 1°C) and humidity (60% ± 10%), with a 12-h light/dark cycle and free access to food and water.

Animals and diet

Four-wk-old female C57 BL/6 mice (F0) were randomly assigned to receive standard chow (SC) or HF diet (n = 10 per group) during the 8 wk before mating. F0-SC dams (F0-SCD) were fed a diet with 64% of the energy derived from carbohydrates, 19% from protein, and 17% from lipids (70 g soybean oil/kg food). F0-HF dams (F0-HFD) were fed a diet containing 32% of the energy from carbohydrates, and 19% from protein, 49% from fat (200 g lard/kg food and 70 g soybean oil/kg food). Vitamin and mineral contents of both diets were identical and followed the recommendations of the American Institute of Nutrition for rodents to support growth (AIN-93 G) [11]. Both SC and HF experimental diets were manufactured by PragSolucoes (Jau, São Paulo, Brazil). Females (F0) were mated with breeding males; the day of fecundation was set as the day on which vaginal plugs were noted. Diets were maintained until the end of lactation and dams were single housed in pathogen-free cages.

At birth, litter was randomly reduced to six offspring (with a 1:1 sex ratio) to ensure a standard plane of nutrition. F1 offspring were weaned onto the SC diet at postnatal day 21. The groups were formed by randomly picking up one male per litter (n = 5). Animals were group housed in pathogen-free cages. At 3 mo old, one female offspring of each litter (F1 lineage) was randomly selected and mated with a male from dams not subjected to dietary manipulations to produce F2 offspring. Females (F1) were fed the SC diet throughout gestation and lactation and the same procedures used with F1 generation were applied after F2 animals were born. F2 offspring were also weaned onto the SC diet. It is noteworthy that only F0-HFD were fed the HF diet and, therefore, all effects observed in F1-HF and F2-HF generations are due to the effects of their mother's and grandmother's diets, respectively, on organs physiology. The following male offspring were studied: F1-SC, F1-HF; F2-SC; F2-HF, all fed with SC diet from weaning to 3 mo old.

Dams

Body mass, food intake, and fasting glucose

Body mass (BM) from F0 and F1 dams was measured weekly until 3 mo and 3 wk of age, when they were mated. Weight gain was assessed as the difference between BM at the last week of gestation and BM at the week before mating.

Daily food consumption was measured as the difference between the amount of food provided and the remaining food after 24 h. The energy intake was estimated as the product of food consumption and the energy content of the diet. Two days before mating, glycemia was measured after 6-h fasting, using a glucometer (Accu-Check, Roche Diagnostics, Germany).

Offspring

Body mass and food intake

BM was measured weekly until 3 mo old. Food intake and energy intake from F1 and F2 offspring have been evaluated as described before for dams.

Animals were food deprived for 6 h and sacrificed (150 mg/kg of sodium pentobarbital, intraperitoneally) for blood collection by cardiac puncture. Fasting glucose was measured in a semiautomatic spectrophotometer (Bioclin, Belo Horizonte, MG, Brazil).

Metabolic profile

Glucose metabolism

Oral glucose tolerance test (OGTT) was performed after 6 h of food deprivation (01:00 h–07:00 h). Glucose (1 g/kg) was administered by orogastric gavage, blood samples were collected from the caudal vein before and at 15, 30, 60, and 120 min after glucose overload. Blood glucose concentrations were measured using a handheld glucometer (Accu-Chek, Roche Diagnostics, Germany). The area under the curve (AUC) was calculated for OGTT from 0 to 120 min using the trapezoid rule (GraphPad Prism version 6.02 for Windows, La Jolla, CA, USA) to assess glucose intolerance. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as fasting glucose (mmol/L) multiplied by the fasting insulin level (μIU/L), divided by 22.5 [12].

Plasma analysis

Plasma was obtained from offspring to determine insulin, leptin, and adiponectin concentrations, which were analyzed in duplicate using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Rat/Mouse Insulin ELISA kit Cat. no. EZRMI-13 K, Rat/Mouse Adiponectin ELISA kit Cat. no. EZMADP-60 K and Rat/Mouse Leptin ELISA kit Cat. no. EZML-82 K, Millipore, St. Charles, MO, USA), using Fluostar Omega equipment (BMG LABTECH GmbH, Germany).

Pancreas

Pancreas stereology

The pancreas was carefully removed, weighed, and fixed in freshly prepared formalin for 48 h. It was embedded in Paraplast Plus (Sigma-Aldrich Co., St. Louis, MO, USA), serially sectioned with a nominal thickness of 5 μm, and stained with hematoxylin and eosin. The average islet diameter (considering the smallest and largest diameters) was calculated in at least 150 islets per group (Image-Pro Plus version 7.0, Media Cybernetics, Silver Spring, MD, USA).

Islet volume density (Vv[islet]) and islet mass (M[islet]). Vv[islet] was estimated by point-counting as the ratio between the number of points that hit the pancreatic islet (Pp) and the total number of test points in a test system made up of 36 test points (PT): Vv[islet] = Pp[islet]/PT (%). Subsequently, M[islet] was obtained by multiplying the Vv[islet] by pancreatic mass [13].

α-Cell volume density (Vv[α-cell]) and alpha cell mass (M[α-cell]). A threshold tool from the software Image-Pro plus version 7.0 was used to perform image segmentation. Afterward, (Vv[α-cell]) was estimated using the glucagon-positive areas of the islets after immunohistochemistry, which was expressed as a percentage of the islet (Image-Pro Plus version 7.0). Then, M[α-cell] was estimated as the product of Vv[[α-cell] and M[islet] [14].

β-Cell volume density (Vv[β-cell]) and beta cell mass (M[β-cell]). Vv[β-cell] was estimated by image analysis using the density threshold selection tool applied to islets with insulin-positive areas after immunohistochemistry. Vv[β-cell] was expressed as a percentage of the islet (Image-Pro Plus version 7.0). Thus, M[β-cell] was estimated as the product of Vv[β-cell] and M[islet] [14].

Immunofluorescence and immunohistochemistry

For immunofluorescence, antigen retrieval was accomplished using citrate buffer, pH 6.0, 60°C for 20 min and blocked with ammonium chloride, glycine 2%, and phosphate buffer saline (PBS), pH 7.4. Pancreatic sections were simultaneously incubated with rabbit antiglucagon (ab1846, Abcam) and guinea pig anti-insulin (ab7842, Abcam). Primary antibodies were diluted 1:50 in blocking buffer (PBS/bovine serum assay [BSA] 1%) and incubated overnight at 4°C. Furthermore, the samples were incubated for 1 h at room temperature with fluorochrome-conjugated secondary antibodies: Donkey antirabbit immunoglobulin (Ig) G-Alexa 488 for glucagon and goat anti-guinea pig IgG-Alexa 546 for insulin (Invitrogen, Molecular Probes, Carlsbad, CA, USA), both diluted at 1:50 in PBS/BSA 1%. After rinsing in PBS, the slides were mounted with DAPI Nucleic Acid Stain and SlowFade Antifade (Invitrogen, Molecular Probes, Carlsbad, CA, USA). Double indirect immunofluorescence images were captured using confocal microscopy (System Microscope Confocal Laser Scanning Nikon brand, model C2, Tokyo, Japan).

For immunohistochemistry, sections were incubated with rabbit anti-Pdx1 (AB3503; Chemicon), guinea pig anti-insulin (ab7842, Abcam) and rabbit anti-glucagon (ab1846, Abcam), all of them diluted in 1:100. The reactions were amplified with a biotin–streptavidin complex followed by incubation with biotinylated secondary antibodies and streptavidin peroxidase conjugates (HistostainPlus Kit, Invitrogen, Carlsbad, CA, USA). Sections were washed in PBS,