Nutrition 32 (2016) 1138–1143

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

The insulin-signaling pathway of the pancreatic islet is impaired in adult mice offspring of mothers fed a high-fat diet

Isabele Bringhenti R.D., Ph.D., Fernanda Ornellas R.D., Ph.D., Carlos Alberto Mandarim-de-Lacerda M.D., Ph.D., Marcia Barbosa Aguila R.D., Ph.D. *

Laboratory of Morphometry, Metabolism, and Cardiovascular Diseases, Biomedical Center, State University of Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 29 November 2015
Accepted 1 March 2016

Keywords:
Fetal programming
Insulin signaling
Pancreatic islet
Insulin resistance
Mice

ABSTRACT

Objective: Mothers fed a high-fat (HF) diet can cause different adverse alterations in their offspring. The study aimed to verify the pancreatic islet structure and insulin-signaling pathway in adulthood of offspring of mothers fed a HF diet during the pregnancy.
Methods: Female mice (mothers) were randomly assigned to receive either standard chow (Mo-SC) or a HF diet (Mo-HF) ad libitum. After 2 mo on the experimental diets, 3-mo-old female mice were mated with male C57 BL/6 mice that were fed a SC diet. The male offspring was evaluated at 6 mo old.
Results: At 6 mo of age, Mo-HF offspring had an increment in body mass and adiposity, hypercholesterolemia, and hypertriacylglycerolemia, higher levels of insulin, and leptin with a concomitant decrease in adiponectin levels. In the islet, we observed an alteration in the structure characterized by the migration of some alpha cells from the edge to the core of the islet in association with an increase in the masses of the islet, beta cell, and alpha cell, featuring a pancreatic islet remodeling. Additionally, the Mo-HF offspring demonstrated a decrease in IRS1, PI3 k p-Akt, Pd-1, and Glut2 protein expressions compared to Mo-SC offspring. However, an increase was observed in FOXO1 and insulin protein expressions in Mo-HF offspring compared to Mo-SC offspring.
Conclusion: The present study demonstrated that a maternal HF diet is responsible for remodeling the islet structure coupled with an adverse carbohydrate metabolism and impairment of the insulin-signaling pathway in adult male mice offspring.

Introduction

The animal model supports the concept that a high-fat (HF) diet is associated with an adverse metabolic outcome featuring by dyslipidemia, overweight/obesity, and insulin resistance (IR) [1]. Moreover, the effects of a HF diet during critical periods of development have a direct action on the failure of the pancreatic beta cells, resulting in the appearance of IR and type 2 diabetes mellitus (DM2) [2,3].

The IR development is followed by hyperglycemia, inflammatory cytokines, and free fatty acids circulating, which impair glucose-stimulated-insulin secretion [4–6].

Several studies have found the impact of maternal programming of insulin signaling in target organs in the offspring. It has been demonstrated impairment of hepatic insulin signaling pathway [7], as well skeletal muscle IR in mice offspring, associated with oxidative stress in this tissue [8]. Studies relating maternal low-protein diet and pancreatic alterations in offspring indicate defects of insulin secretion and glucose homeostasis [9,10], but the insulin signaling pathway in the pancreas of offspring is still a scarce theme in literature.

The authors would like to thank Ms. Aline Penna, Ms. Thatiany Marinho, Ms. Michele Soares, and Ms. Gezileia Lau for their technical assistance. This research was supported by the Brazilian agencies CNPq (Brazilian Council of Science and Technology, www.cnpq.br, grant #302.154/2011-6 to CAML, and #306.077/2013-2 to MBA), FAPERJ (Rio de Janeiro Foundation for Research, www.faperj.br, grant #102.944/2011 to CAML, and #103.062/2011 to MBA), and CAPES (Coordination for Perfectionnement of Superior Personal, www.capes.br), scholarship to IB. The authors disclose no other conflicts of interest in this research.

* Corresponding author. Tel.: 55 21 2868 8316; fax: 55 21 2868 8033.
E-mail address: Marciaguila@gmail.com (M. B. Aguila).
URL: http://www.lmmc.uerj.br

http://dx.doi.org/10.1016/j.nut.2016.03.001
0899-9007/

It is well described that the phosphoinositide 3-kinase (PI3 K) has a critical role in the metabolic action of insulin [11]. Previous data have shown that a disruption in insulin-PI3 K signaling may induce a development of beta cell dysfunction, leading to the onset of IR and consequently DM2 [6,12].

The initiation of IR is characterized by a decrease in receptor concentration and kinase activity. In addition, the concentration and phosphorylation of IRS-1 and -2, PI3 K activity, glucose transporter translocation, and the activity of intracellular enzymes also decrease in the IR and DM2 framework [13].

In this context, the aim of the present study was to verify if the maternal HF diet programming induces beta cell failure couple with insulin signaling is impaired as mice mature in later life.

Material and methods

Animals and diets

Female C57 BL/6 mice were taken immediately after weaning and kept under controlled temperature (21 ± 2°C) and humidity (60 ± 10%) with free access to food and water on a 12/12-h light/dark cycle. The experimental protocol was approved by the Ethics Committee of the State University of Rio de Janeiro (protocol CEUA 070/2012) and followed the rules established in the current guideline for experimentation with animals (NIH Publication No. 85-23, revised 1996).

The animals were randomly assigned to receive either standard chow (control group SC, n = 10) or an HF diet (experimental group HF, n = 10). The standard chow had 64% of energy from carbohydrates, 19% of energy from protein, and 17% of energy from lipids (70 g soybean oil/kg diet, total energy: 16.5 kJ/kg diet). The HF diet had 32% of energy from carbohydrates, 19% of energy from protein, and 49% of energy from lipids (200 g of animal lard and 70 g of soybean oil/kg diet, with total energy: 20.7 kJ/kg diet). The diets were manufactured by PragSolucoes (Jau, SP, Brazil) with purified ingredients. Both diets had identical content of vitamins and minerals according to the standards recommended by the American Institute of Nutrition for rodent diets to support growth during pregnancy, lactation and postweaning periods of life (AIN-93 G) [14]; see details in Table 1.

After 2 mo on the diets, 3 mo old females were mated with mature male C57 BL/6 mice, outside of the experiment, that were fed standard chow for rodents all the time. Females were accompanied during the pregnancy until the birthday of the offspring. Day 0 of gestation was determined by observing the formation of a vaginal plug, and the pregnant dams were maintained on their respective diets throughout pregnancy and lactation.

After birth, the litter size in each cage was randomly adjusted to seven pups (four males, when possible) to ensure adequate and standardized nutrition until the pups were weaned. Offspring sex was assessed by analyzing the anogenital distance [15]. Afterward, one male from each litter was randomly assigned to the experimental groups according to the mother's diet (Mo), meaning Mo-SC, derived from mothers fed standard chow, and Mo-HF derived from mothers fed the HF diet. After weaning, the pups were fed standard chow until 6 mo of age, when they were humanely killed.

Table 1
Diet compositions

Nutrients (g/kg)	SC	HF
Casein	190.00	230.00
Corn starch	539.50	299.50
Sucrose	100.00	100.00
Soybean oil	70.00	70.00
Lard	–	200.00
Fiber	50.00	50.00
Vitamin Mixture (mg)	10.00	10.00
Mineral Mixture (mg)	35.00	35.00
Cysteine	3.00	3.00
Choline	2.50	2.50
Antioxidants	0.01	0.01
Total mass (g)	1.00	1.00
Energy (kcal/kg)	3.95	4.95
Carbohydrates (%)	64.00	32.00
Protein (%)	19.00	19.00
Lipids (%)	17.00	49.00

SC, standard chow; HF, high fat

Fresh chow was provided daily, and any remaining chow from the previous day was discarded. Both the food intake and energy intake of the offspring were determined, and the body mass was measured until the end of the experiment.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) was performed after 6 h of fasting in offspring at 6 mo of age. A known amount of hypertonic glucose (1 g/kg body weight) was administered via orogastric gavage to induce an overload of glucose. Blood was collected from the tail at 0, 15, 30, 60, and 120 min following glucose administration, and blood glucose was measured (glucometer Accu-Chek Performa, Roche, Mannheim, Germany). The area under the curve (AUC) was calculated from zero to 120 min (GraphPad Prism version 6.05 for Windows; GraphPad Software, La Jolla, CA, USA).

Euthanasia

The offspring was humanely killed at 6 mo of age. The animals were humanely killed and discarded in accordance with standards established by the local ethics committee. The animals were fasted overnight (food-deprived from 01:00–07:00 h) and then deeply anesthetized (sodium pentobarbital, 150 mg/kg intraperitoneal). Blood samples were rapidly obtained by cardiac puncture, and blood centrifugation separated the plasma (120 g for 15 min) that was stored at −20°C.

Dissection of pancreas and fat pads

The pancreas was dissected (n = 5), or the islets were isolated by collagenase digestion (n = 5). When dissected (the dissections were made by one of the authors, I.B.), the pancreas was accurately weighed and then rapidly fixed in a freshly prepared fixative solution (4% [w/v] formaldehyde and 0.1 M phosphate buffer, pH 7.2) for analysis by light microscopy. Afterward, the pancreas was embedded in Paraplas plus (Sigma-Aldrich Corporation, St. Louis, MO, USA) and exhaustively sectioned at a nominal thickness of five micrometers, and sections were stained with hematoxylin and eosin, or alternatively were used in immunofluorescence (at least five nonconsecutive sections per animal).

Both inguinal and abdominal fat pads were dissected and weighed. The inguinal fat pad as kept as the subcutaneous fat occupying the place between the lower part of the rib cage and the midthigh. The abdominal fat pad was considered as the sum of the retroperitoneal fat (attached to the posterior abdominal wall near the kidneys) and the genital fat (located in the lower part of the abdomen that is connected to the epididymis in males). The adiposity index was determined as the ratio of the sum of the fat masses divided by the total BM [16].

Plasma analyses

Total cholesterol and triacylglycerol were measured with an automatic spectrophotometer using a commercial kit (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil). Insulin, adiponectin, and leptin concentrations were analyzed in duplicate using commercially available enzyme-linked immunosorbent assay kits (Rat/Mouse Insulin ELISA kit cat. #EZRMI-13 K; Rat/Mouse Adiponectin ELISA kit Cat. #EZMADP-60 K; Rat/Mouse Leptin ELISA kit Cat. #EZML-82 K, Millipore, Missouri, USA), using Fluostar Omega equipment (BMG Labtech GmbH, Ortenberg, Germany).

Pancreas

Immunofluorescence and confocal laser scanning microscopy

Antigen retrieval was accomplished using citrate buffer, pH 6.0, at 60°C for 20 min and blocked with ammonium chloride, 2% glycine, and phosphate-buffered saline, pH 7.4 (PBS). Sections were incubated with the double labeling of insulin and glucagon expression in islets to observe the distribution of alpha and beta cells. Primary antibodies guinea pig antiinsulin (A0564, Dako, Glostrup, Denmark) and rabbit antiglucagon (Ab1846, Abcam, Cambridge, MA, USA) were diluted 1:50 in blocking buffer (PBS/1% bovine serum albumin, BSA), and incubations were conducted overnight at 4°C. The samples were then incubated for 1 h at room temperature with a fluorochrome-conjugated secondary antibody, goat antiguinea pig IgG-Alexa 546, and Alexa 488 (Invitrogen, Molecular Probes, Carlsbad, CA, USA), diluted 1:50 in PBS/1% BSA. After rinsing in PBS, the slides were mounted with DAPI Nucleic Acid Stain and SlowFade Antifade (Invitrogen, Molecular Probes, Carlsbad, CA, USA). Indirect immunofluorescence images were observed in a Nikon confocal microscope model C2 (Nikon Corporation, Tokyo, Japan).

Islet

The islet volume density (Vv[islet]) was estimated by point-counting as the ratio between the points hitting the islet (Pp) and the test-points in the grid: Vv