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Basic nutritional investigation

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

Francielle Graus-Nunes M.Sc., Eliete Dalla Corte Frantz Ph.D., Wilian Rodrigues Lannes M.Sc., Mariel Caroline da Silva Menezes R.D., Carlos Alberto Mandarim-de-Lacerda M.D., Ph.D., Vanessa Souza-Mello R.D., Ph.D. *

Laboratory of Morphometry, Metabolism and Cardiovascular Disease, Biomedical Center, Institute of Biology, State University of Rio de Janeiro, Brazil

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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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* Corresponding author. Tel.: +55 212 868 8689; fax: +55 212 868 8033.
E-mail address: souzamello.uerj@gmail.com (V. Souza-Mello).

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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,

Table 1
Metabolic data from dams*

Data	F0-SCD	F0-HFD	F1-SCD	F1-HFD
Pregestational BM (g)	27.99 ± 0.26	32.36 ± 0.64†	27.59 ± 0.31	30.18 ± 0.23†,‡
Fasting glucose (mmol/L)	5.66 ± 0.14	7.26 ± 0.12†	5.63 ± 0.16	5.96 ± 0.54

ANOVA, analysis of variance; BM, body mass; F0-HFD, high-fat fed dams; F0-SCD, standard-chow fed dams; F1-HFD, dams born to high-fat fed dams; F1-SCD, dams born to standard-chow fed dams

* Values represent means ± SEM, n = 10.

† $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with the SC dam.

‡ $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with their F0 counterpart.

revealed with liquid diaminobenzidine (HistostainPlus Kit, Invitrogen), and counterstained with hematoxylin.

Statistical analysis

Data are expressed as mean and the associated SEM. Differences among the groups were analyzed using one-way analysis of variance followed by the post hoc test of Holm-Sidak. A *P*-value <0.05 was considered statistically significant.

Results

Dams

Pregestation

F0-HFD and F1-HFD were overweight compared with FO-SCD (+16%, $P < 0.0001$) and F1-SCD (+9%, $P = 0.0012$) before pregnancy. Additionally, higher energy intake was found during this period compared with their counterparts (+65% for F0-HF, $P < 0.0001$ and +28% for F1-HF, $P = 0.0013$). Moreover, F0-HFD, directly exposed to HF diet, showed hyperglycemia prepregnancy when compared with F0-SCD (+25%, $P = 0.024$) (Table 1, Fig. 1)

Gestation

Although F1-HFD did not show pregestational hyperglycemia, pregestational excessive BM coupled with higher energy intake was accompanied by a higher weight gain during gestation in both F0-HFD and F1-HFD compared with their counterparts (+39%, $P < 0.0001$ and +25%, $P = 0.0001$, respectively). Both F0-HFD and F1-HFD showed higher energy intake during gestation compared with their counterparts (+39%, $P < 0.0001$ and +20%, $P = 0.0046$), albeit with the consumption of the SC diet by F1-HFD (Figs. 1 and 2).

Offspring

Body mass and energy intake

Final BM of F1-HF was significantly higher (+21%, $P < 0.0001$) than F1-SC. As for the second generation, no differences were observed between F2-SC and F2-HF. However, F2-HF was significantly lighter than F1-HF (−11%, $P < 0.0001$). BM gains (from weaning to 3 mo old) resembled BM results (Table 2, Figs. 1 and 2).

In agreement with BM, F1-HF showed significantly higher energy intake (+18%, $P < 0.0001$) than F1-SC. In F2-HF, no differences were observed regarding energy intake compared with F2-SC. Moreover, there was a decrease in energy intake in F2-HF in relation to F1-HF (−16%, $P < 0.0001$).

Glucose metabolism

Regarding glycemia, F1-HF had higher levels than F1-SC (+52%, $P < 0.0001$). In the second generation, there was no difference between HF and SC, however, when comparing different generations, F2-HF had reduced glycemia compared with F1-HF (−26%, $P < 0.0001$) (Table 2, Fig. 3).

The analysis of the AUC of OGTT revealed that F1-HF had glucose intolerance, showing greater AUC than F1-SC (+26%, $P = 0.0006$). Despite showing significant later peak of blood glucose after glucose overload (at 30 min, +29%, $P = 0.0084$), F2-HF were not intolerant to glucose compared with F2-SC. It is also noteworthy that there was no difference regarding AUC between F1-HF and F2-HF.

HOMA-IR was elevated in F1-HF compared with F1-SC (+124%, $P < 0.0001$). Likewise, F2-HF showed significantly higher HOMA-IR index compared with F2-SC (+63%, $P = 0.025$).

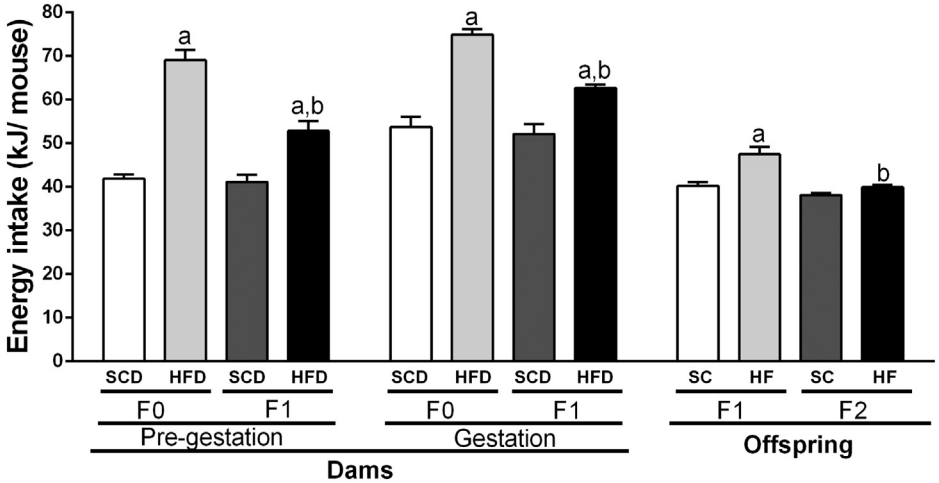

Fig. 1. Energy intake of dams (pregestation and during gestation, n = 10) and offspring (from weaning to 3 mo old, n = 5), mean ± SEM. In signaled cases, $P < 0.05$ when compared with SC, same generation [a] and with previous generation [b] (one-way ANOVA and Holm-Sidak posthoc test). ANOVA, analysis of variance; F0-HFD, high-fat fed dams; F0-SCD, standard-chow fed dams; F1-HF, first generation from pregestational high-fat fed dams; F1-HFD, dams born to high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F1-SCD, dams born to standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from standard-chow fed dams.

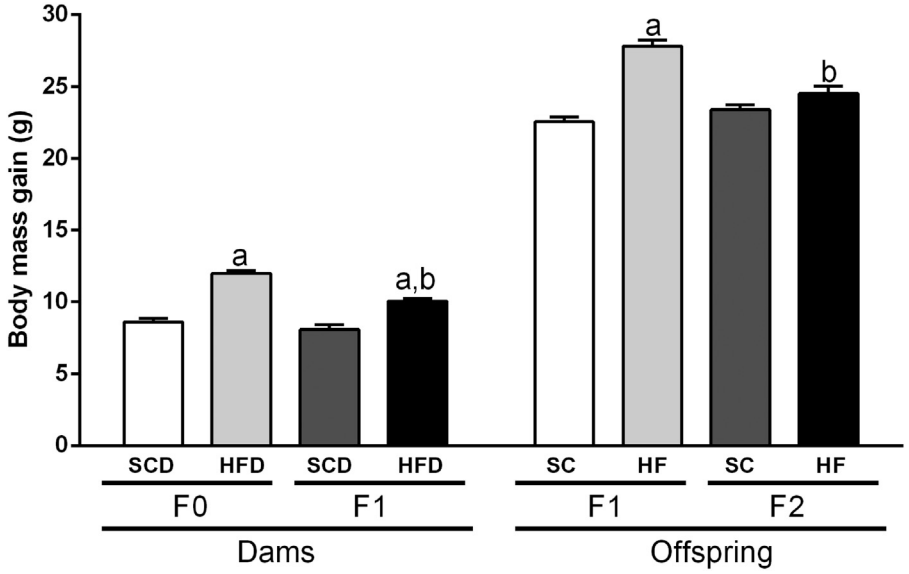

Fig. 2. Body mass gain of dams (during gestation, n = 10) and offspring (from weaning to 3 mo old, n = 5), mean ± SEM. In signaled cases, $P < 0.05$ when compared with SC, same generation [a] and with previous generation [b] (one-way ANOVA and Holm-Sidak post hoc test). ANOVA, analysis of variance; F0-HFD, high-fat fed dams; F0-SCD, standard-chow fed dams; F1-HF, first generation from pregestational high-fat fed dams; F1-HFD, dams born to high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F1-SCD, dams born to standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from standard-chow fed dams.

When comparing different generations, F2-HF showed reduction of this index compared with F1-HF (–40%, $P = 0.0003$).

Plasma analysis

Insulin levels were higher in F1-HF than in F1-SC (+48%, $P = 0.0169$). Furthermore, F2-HF had hyperinsulinemia compared with F2-SC (+50%, $P = 0.0412$). Likewise, F1-HF had hyperleptinemia compared with F1-SC (+34%, $P = 0.0177$). In the second generation, leptin levels were also higher in F2-HF than in F2-SC (+28%, $P = 0.0311$) (Table 2).

Conversely, plasma adiponectin levels were reduced in F1-HF compared with F1-SC (–30%, $P = 0.0082$). Likewise, F2-HF showed hypoadiponectinemia compared with F2-SC (39%, $P = 0.0015$).

Endocrine pancreas remodeling

Concerning the first generation, F1-HF had heavier pancreatic mass than F1-SC (+43%, $P < 0.0001$). As for endocrine pancreas, the islet volume density in the F1-HF was significantly higher than in F1-SC (+83%, $P < 0.0001$), which was accompanied by larger islet mean diameter (+95%, $P < 0.0001$) and greater islet mass (+160%, $P < 0.0001$) (Table 3, Figs. 4 and 5).

F1-HF also demonstrated higher volume density of glucagon immunostaining (+73%, $P < 0.0001$), which, coupled with enhanced islet mass was directly linked to increased α-cell mass (+321%, $P < 0.0001$). Likewise, increased insulin immunoreactive-positive β-cells were found in F1-HF (+30%, $P < 0.0001$) but not in F1-SC, which, in turn, was accompanied by an augment of +234% in β-cell mass compared with F1-SC ($P < 0.0001$). Moreover, F1-HF showed weaker expression of Pdx1 when compared with its counterpart.

Taking into account the second generation, F2-HF had heavier pancreatic mass than F2-SC (+43%, $P < 0.0001$). Concerning endocrine pancreas, islet volume density in F2-HF was significantly higher than in F2-SC (+65%, $P < 0.0001$), which was followed by an increase in islet mean diameter (+72%, $P < 0.0001$) and islet mass (+137%, $P < 0.0001$).

Furthermore, F2-HF showed higher volume density of glucagon immunostaining (+46%, $P = 0.003$) and insulin immunoreactive-positive β-cells (+21%, $P = 0.0026$) compared with F2-SC (Table 2). Consequently, both α-cell mass and β-cell mass increased in F2-HF compared with F2-SC (+239% and +188%, respectively, $P < 0.0001$). Moreover, F2-HF showed weaker expression of Pdx1 than F2-SC.

Photomicrographs illustrate the positive double immunofluorescence reaction to insulin (β-cells) and glucagon (a-cells) in pancreatic islet of all studied offspring. Complying with larger α-cell mass, F1-HF and F2-HF showed disarranged distribution of α-cells, which were arranged not only in the periphery but also distributed within the islet core. Also, islet hypertrophy and higher β-cell mass can be observed, corroborating with the previous results.

Table 2
Metabolic data from F1 and F2 generations*

Data	F1-SC	F1-HF	F2-SC	F2-HF
Final BM (g)	24.15 ± 0.35	29.13 ± 0.37†	24.65 ± 0.40	26.01 ± 0.47‡
Fasting glucose (mmol/L)	6.33 ± 0.17	9.60 ± 0.15†	6.46 ± 0.26	7.20 ± 0.2‡
Insulin (μUI/L)	5.54 ± 0.73	8.21 ± 0.58†	4.47 ± 0.56	6.71 ± 0.13†
HOMA-IR	1.56 ± 0.21	3.50 ± 0.25†	1.29 ± 0.19	2.15 ± 0.07†,‡
Leptin (pg/mL)	639.2 ± 28.8	856.1 ± 67.5†	646.9 ± 25.5	827.5 ± 40.6†
Adiponectin (10^6 pg/mL)	7.1 ± 0.6	5.0 ± 0.1†	6.9 ± 0.5	4.3 ± 0.3†

BM, body mass; F1-HF, first generation from pregestational high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from pregestational standard-chow fed dams; HOMA-IR, homeostasis model assessment of insulin resistance index

* Values represent means ± SEM, n = 5.

† $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with the SC offspring.

‡ $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with their F1 counterpart.

Discussion

This study described the effects of a maternal HF diet during the pregestation, gestation, and lactation periods on the endocrine

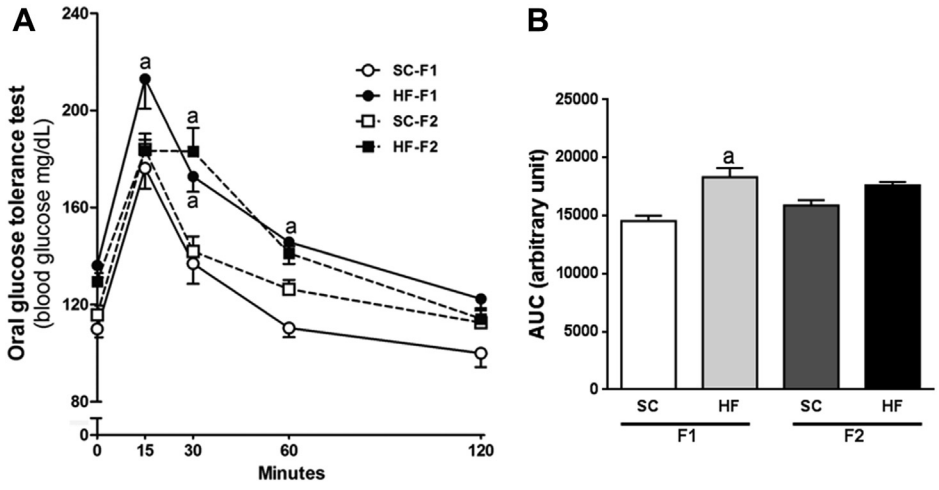

Fig. 3. Oral glucose tolerance test in offspring (A) and area under the curve for oral glucose tolerance test (B) (mean ± SEM, n = 5 per group). In signaled cases, $P < 0.05$ when compared with SC [a] and with F1 [b] (one-way ANOVA and Holm-Sidak posthoc test). F1-HF, first generation from pregestational high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from standard-chow fed dams.

pancreas structure and carbohydrate metabolism in F1 and F2 mice offspring. Excessive saturated fat in maternal diet led to morphologic, hormone, and functional changes in the pancreases of two subsequent offspring generations despite the fact that all the offspring consumed a standard diet after weaning.

Increased BM gain during gestation in F0-HFD, which exhibited pregestational overweight due to the HF diet exposure during the 8 wk before mating, has been described previously [9,15,16]. Of note, increased saturated fat intake seems to be more important than increased energy intake as a whole because dietary fat triggers hyperphagia due to higher energy density coupled with higher palatability and lower satiety properties [17,18]. In the present study, maternal diet had 49% of energy as lipids, which was compensated by a reduction in carbohydrate content because protein content should be equal to the amount of SC as any manipulation in protein content could add bias to the study as it would configure another trait of fetal programming [19]. Considering that higher protein intake is linked to greater satiety effect than excessive fat intake [20], it can be argued that hyperphagia exhibited by F0-HFD is a result of excessive fat in the diet.

Likewise, F0-HFD daughters (F1-HFD) showed increased BM gain during gestation coupled with pregestational overweight, albeit with the intake of SC diet during the whole experiment. This fact might reside in the biggest energy intake when compared with F1-SCD. In this case, in utero exposure to excessive saturated fat played a key role in the development of hypothalamic circuits [21], explaining the hyperphagia found in F1-HFD. Moreover, morphologic, physiological, and metabolic adaptations due to HF programming were developed in utero to prepare the fetus for a similar postnatal environment. However, F1-HFD faced a change of the diet at weaning, which also contributes to extensive adaptations [22].

It is noteworthy that the time of exposure to HF diet directly influenced offspring BM. Recently, it was shown that exposure to the HF diet during the first week of gestation produced reduced birth weight, resembling the maternal protein restriction model due to in utero growth retardation at middle and late gestation [23]. Conversely, animal models of diet-induced maternal obesity through administration of HF diet during gestation and/or lactation have demonstrated a link between BM gain in early life and metabolic complications later, even if the descendants received a balanced diet at weaning [24,25]. More recently, early increased BM was found in F1 from pregestational obese dams at postnatal day 14, being males heavier than females at 1 mo old [5]. Our results confirm this, as F1-HF offspring were heavier than F1-SC and F2-HF offspring.

The differences in BM gain between F1 and F2 offspring could be explained by energy intake. The energetic intake of F1-HF was significantly higher than F2-HF, an expected behavior due to possible hypothalamic changes following maternal consumption of a HF diet, which can lead to obesity in adulthood [6,16]. Conversely, energetic intake of F2-HF did not differ from F2-SC, which may explain the lower BM gain in this group compared with F1-HF. Previously, it has been shown that obesity phenotype did not reach the second generation from HF fed dams as F2 offspring are not hyperphagic, regardless of the postnatal diet [10]. We hypothesize that although F2 offspring were exposed to excessive energy intake in utero, it was not derived from excessive dietary saturated fatty acid, which seems to play an important role in determining offspring BM and food behavior.

Table 3
Pancreatic stereology, α- and β-cell masses*

Data	F1-SC	F1-HF	F2-SC	F2-HF
Pancreas				
Mass (mg)	139.4 ± 6.6	200.0 ± 7.1†	135.0 ± 7.8	191.0 ± 3.1†
Islet				
Volume density (%)	4.0 ± 0.22	7.3 ± 0.34†	4.1 ± 0.42	6.8 ± 0.07†
Mass (mg)	5.6 ± 0.4	14.4 ± 0.4†	5.5 ± 0.4	13 ± 0.3†,‡
Diameter (μm)	74.0 ± 3.1	144.0 ± 1.6†	77.0 ± 3.2	133.0 ± 4.6†
α-cell mass				
Glucagon (%)	13.3 ± 0.5	23.0 ± 1.7†	11.6 ± 0.6	16.9 ± 0.3†,‡
Mass (mg)	0.7 ± 0.06	3.0 ± 0.29†	0.6 ± 0.08	2.2 ± 0.06 †,‡
β-cell mass				
Insulin (%)	51.52 ± 1.98	66.75 ± 1.29†	50.50 ± 2.51	60.91 ± 0.56†
Mass (mg)	2.89 ± 0.28	9.64 ± 0.24†	2.73 ± 0.16	7.86 ± 0.20†,‡

F1-HF, first generation from pregestational high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from pregestational standard-chow fed dams

* Values represent means ± SEM, n = 5 per offspring.

† $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with the SC offspring.

‡ $P < 0.05$ using one-way ANOVA and post hoc Holm-Sidak test when compared with their F1 counterpart.

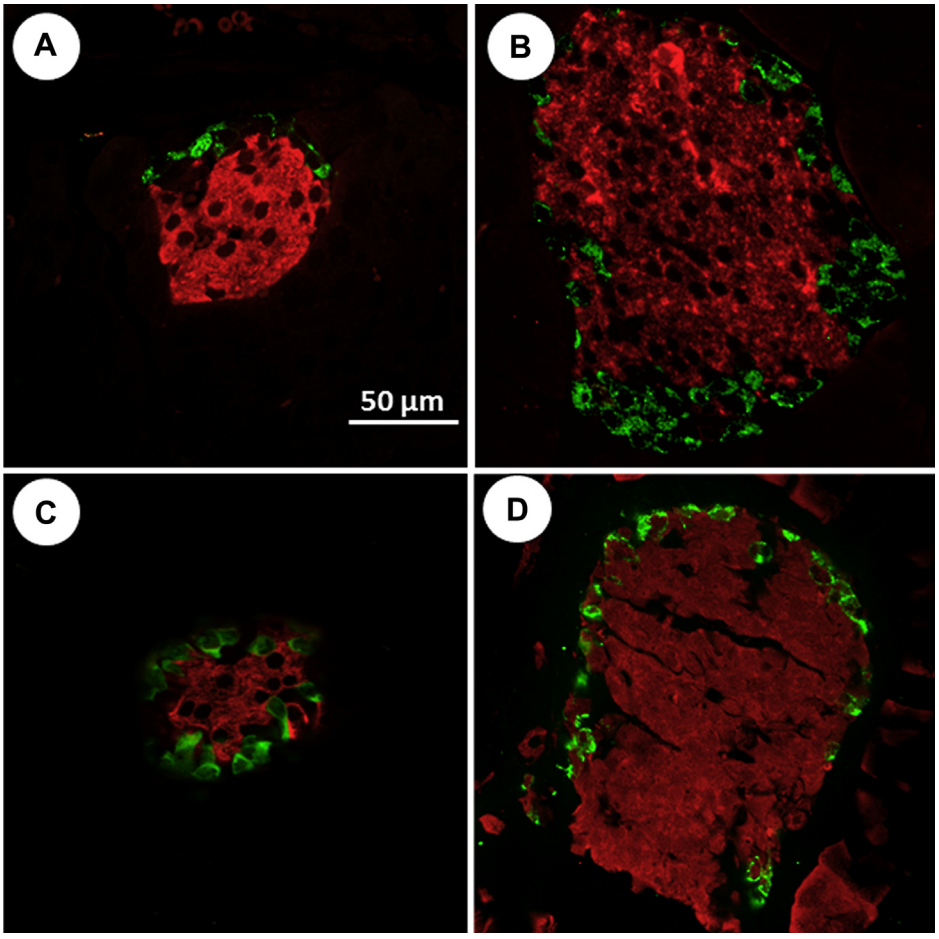

Fig. 4. Pancreatic islet cell immunofluorescence. Cells were double labeled for insulin (red) and glucagon (green) and show normal distribution of α-cell/β-cell in F1-SC (A) offspring, but altered α-cell/β-cell distribution in F1-HF (B). F2-SC (C) offspring show normal distribution of α-cell/β-cell, but altered α-cell/β-cell distribution in F2-HF (D), characterized by the infiltration of α-cells (green, stained with glucagon) into the islet core. F1-HF, first generation from pregestational high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from standard-chow fed dams. (The color version of this figure is available online at www.nutritionjrnl.com.)

Adiponectin levels correlate inversely with IR and metabolic syndrome [26]. In agreement with the literature, we observed hypoadiponectinemia in F1-HF offspring, in addition to the hyperleptinemia, which is correlated with larger fat pads [27]. Surprisingly, F2-HF offspring showed hypoadiponectinemia and hyperleptinemia, albeit with normal BM. It is widely described that, in the long run, these conditions lead to leptin resistance, by which leptin loses its capacity to reduce insulin secretion at postprandial stage, favoring IR establishment [28,29]. In this way, both F1-HF and F2-HF showed altered adipokine profile compared with their counterparts, despite the normal BM of F2-HF. It is possible that altered glucose metabolism underlie these effects.

Maternal obesity can affect offspring phenotype and predisposition to obesity and metabolic diseases. Previous studies demonstrated that dams that consumed HF diet had a higher adiposity index and body weight than SC dams, which is correlated with higher levels of insulin, blood pressure, and impaired fasting glucose levels [4,5]. These conditions can affect pancreas structure and insulin homeostasis of their offspring once insulin is able to transpose the placenta, causing greater stimulation of fetal pancreatic β-cells [8,9].

In the present study, lipotoxicity due to excessive maternal saturated fat intake induced adverse structural pancreatic remodeling coupled with altered insulin sensitivity in first-generation mice. It has been shown that carbohydrate metabolism is more sensitive to the quality of dietary lipid than to the amount of lipid in the diet [30], which implies the overriding role of qualitative dietary fat composition on fetal programming. Although F1-HFD did not have access to an HF diet, they were directly exposed to excessive fat intake when they were in utero, which, coupled with their higher energy intake while pregnant produced similar structural pancreatic alterations in their F2 offspring. So, it can be argued that these effects on glucose metabolism and pancreas are intergenerational. Confirming the important role of parental fat intake on offspring metabolism, intergenerational transmission of impaired glucose metabolism has been recently verified in the female progeny of fathers that consumed HF diet before mating [31].

As for endocrine pancreas, β-cell proliferation occurs at a high rate near the end of embryogenesis, which leads to a massive increase in β-cell mass [9]. The increase in β-cell mass slows down considerably in adult animals, although variations in insulin demands due to physiological and pathologic states such as pregnancy and obesity can lead to adaptive changes in the β-cells, such as hyperplasia, hypertrophy, and increased insulin synthesis and secretion [32].

Early hyperglycemia (at 10 and 21 d old) has been described previously in the first generation of mice offspring born to HF fed

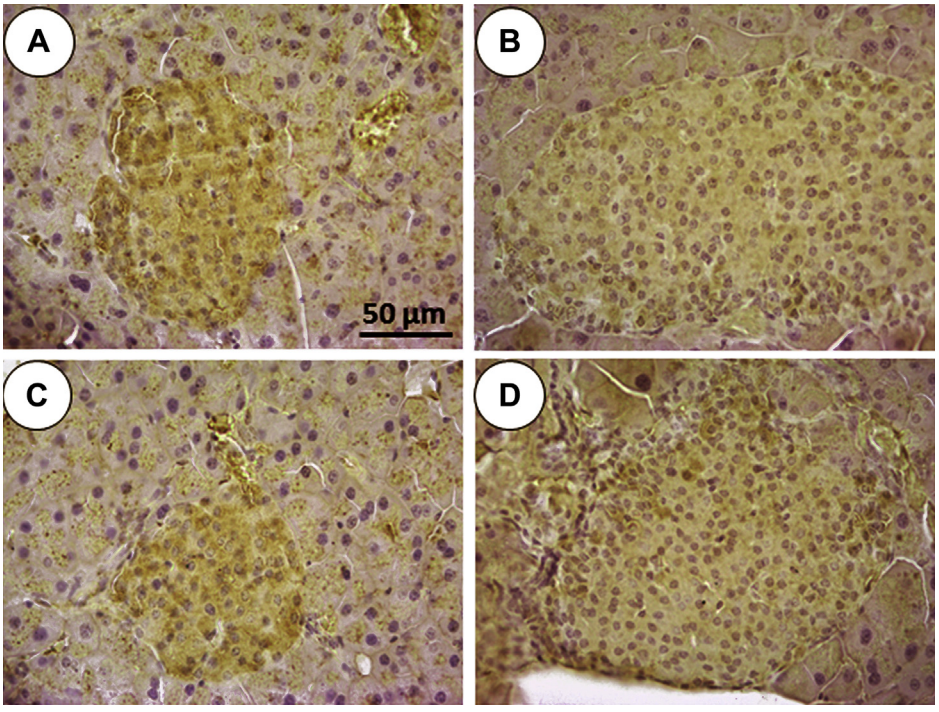

Fig. 5. Offspring islet Pdx1 immunostainning, same magnification in all pictures. In F1-SC (A) offspring smaller islet with strong immunostaining was observed in comparison with F1-HF (B). The same was observed for the second generation, in which, F2-SC (C) offspring smaller islet with strong immunostaining, was observed in comparison with F2-HF (D). F1-HF, first generation from pregestational high-fat fed dams; F1-SC, first generation from standard-chow fed dams; F2-HF, second generation from pregestational high-fat fed dams; F2-SC, second generation from standard-chow fed dams.

dams, which was followed by adverse pancreatic remodeling and altered function [9,33]. In our data, altered glucose metabolism was demonstrated in both generations of pregestational obese dams through islet hypertrophy, by which pancreatic β-cells undergo a compensatory response involving the expansion of β-cell mass, enhanced insulin biosynthesis, insulin secretion, and increased responsiveness of nutrient to secretion coupling, manifested by hyperinsulinemia in F1-HF and F2-HF [34,35].

Increased β-cell mass and the resulting hyperinsulinemia were not enough to maintain euglycemia in F1-HF, which presented hyperglycemia coupled with abnormal OGTT, configuring glucose intolerance, a condition that precedes T2DM. Conversely, islet hypertrophy and hypersecretion prevented F2-HF from hyperglycemia and these animals were tolerant to glucose at 3 mo old, despite presenting higher HOMA-IR. It can be postulated that glucose intolerance was temporarily blunted by high insulin levels in F2-HF and that these animals will probably present glucose intolerance later in life, once their pancreatic architecture bore resemblance to F1-HF.

Impaired glucose metabolism also could be depicted by some histologic findings. The disarrangement in pancreatic islet cell distribution has been reported through the observation of augmented α-cell mass and β-cell mass, implicating it as an important adaptive process typically observed in IR animal models [36,37]. The expression of glucagon was so intense in F1-HF and F2-HF that positive, α-cells were not restricted to the islet periphery, but also infiltrated the entire structure, including islet core. Increased α-cell mass is commonplace in obesity and becomes necessary due to the hypertrophy and hypersecretion of beta cells in insulin resistant states [4,38]. These observations put forward the progression to early T2DM onset for two consecutive generations (sons and grandsons) from obese dams.

It is worth mentioning that our results are opposite to the outcomes from the maternal protein restriction model, albeit with similar effects to offspring glucose homeostasis later in life [39,40]. Maternal protein restriction yields reduced β-cell mass in the early stages of life in three subsequent generations of mice offspring [41]. This alteration is followed by decreased insulin secretion, but increased insulin sensitivity through the enhanced expression of insulin receptor [40,41]. When the postnatal environment is plentiful, adaptations that guaranteed fetal survival under an intrauterine-restricted environment turn into a faster deterioration of glucose homeostasis due to higher demand for nutrient coupling under a limited endowment of islets [42].

Concerning a possible progression to T2DM, both F1-HF and F2-HF showed reduced Pdx1 immunostaining in pancreatic islets. Pdx1 has antiapoptotic action and gene transcription activation that promote glucose-stimulated insulin secretion and supports the maintenance of β-cell mass [43]. Moreover, Pdx1 improves mitochondrial metabolism and studies demonstrated increased DNA methylation and decreased expression of Pdx1 in the development of T2DM [44]. Our results demonstrated reduced Pdx1 expression in the islets from F1-HF and F2-HF, suggesting indirectly worsening of β-cell function and insulin secretion, which, coupled with progressive hypertrophy and hypersecretion of β-cells, can configure in a predisposition to β-cell failure in the long run.

Overall, our results showed that hyperinsulinemia, islet hypertrophy, enlarged α- and β-cell masses parallel to decreased Pdx1 expression were exhibited by the first generation and were transferred to the second-generation offspring from pregestational obese dams. Considering that animal studies on fetal programming target to a translational perspective, the present findings are relevant and alarming. As plentiful resources lead to higher rates of

obese pregnant women nowadays, the present observations allow us to infer that morphophysiological endocrine pancreas impairments and the resulting early T2DM onset could not be avoided in the next two generations even if the offspring exhibits normal BM and adopts adequate nutritional patterns.

Conclusions

Maternal HF diet during pregestation, gestation, and lactation promotes pancreatic programming in F2-HF mice offspring, despite the maintenance of a balanced diet of F1 dams. Interestingly, pancreas from F2 remains with increased islet mass and islet volume, albeit with normal BM and adequate nutrition, implying that these pancreatic changes are intergenerational.

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