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ORIGINAL CONTRIBUTION

Maternal high-fat diet is associated with altered pancreatic remodelling in mice offspring

Bianca Martins Gregorio · Vanessa Souza-Mello · Carlos Alberto Mandarim-de-Lacerda · Marcia Barbosa Aguila

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Abstract
Purpose To investigate whether a maternal high-fat diet (HF) during pregnancy and/or suckling periods predisposes adult C57BL/6 mice offspring to morphological pancreatic modifications.
Methods Male pups were divided into 5 groups: SC (standard chow)—from dams fed SC during gestation and lactation, maintaining an SC diet from postweaning to adulthood; G—from dams fed HF diets during gestation; L—from dams fed HF diets during lactation; GL—from dams fed HF diets during gestation and lactation; and GL/HF—from dams fed HF diets during gestation and lactation, maintaining an HF diet from postweaning to adulthood. We analysed body mass (BM), plasma insulin, pancreas and adipose tissue structures.
Results During the entire experiment, the SC group had the lowest BM. However, GL/HF offspring were heavier than the other groups. This weight gain was also accompanied by adipocyte hypertrophy. At 3 months, G offspring showed an increased insulin levels and impairment in carbohydrates metabolism. Furthermore, pancreatic islets were hypertrophied in G, GL and GL/HF offspring in comparison with SC offspring.
Conclusion HF diet administration during the gestation period is more harmful than during the lactation period, exerting deleterious effects on pancreatic morphology in addition to larger fat deposits in adult mice offspring.

B. M. Gregorio · V. Souza-Mello · C. A. Mandarim-de-Lacerda · M. B. Aguila (✉)
Laboratório de Morfometria, Metabolismo e Doença Cardiovascular, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Av. 28 de Setembro 87 (fds), 20551-030 Rio de Janeiro, RJ, Brazil
e-mail: mbaguila@uerj.br
URL: www.lmmc.uerj.br

Keywords Pancreas remodelling · Maternal high-fat diet · Beta-cell mass · Foetal programming

Background

Obesity and the related metabolic disorders are prevalent in modern society [1]. Insulin resistance has emerged as the central feature of metabolic syndrome (MetS) development [2]. Lately, increasing MetS prevalence has been attributed to environmental factors such as high availability of fat-rich foods and sedentary behaviour [3]. However, recent evidence suggests that maternal nutrition is of paramount importance in regard to insulin resistance determinants [4].

A high-fat diet during gestation and/or lactation has been related to alterations in metabolic pathways and growth rate in pups. Foetal macrosomia has been a frequent observation after feeding mothers with excessive fatty acids [5]. The resulting metabolic changes are accompanied by structural alterations in key metabolic organs, such as the liver, adipose tissue (mainly epididymal fat pad that is the deposit of fat equivalent to visceral fat in humans) and pancreas [6–8]. Non-alcoholic fatty liver disease (NAFLD) has been found after administration of maternal high-fat diets, and the intensity of hepatic lesions is dependent on the period during which the maternal high-fat diet was administered. In a previous study, we observed that the administration of HF diet during the pregnancy and lactation periods, and over the perinatal and postnatal periods caused in offspring hepatic steatosis accompanied by ultrastructural changes in the hepatocytes as well as altered carbohydrate metabolism as hyperglycaemia and hyperinsulinemia, both of which denote insulin resistance [9].

Cross talk between the liver and pancreas occurs as NAFLD amplifies insulin resistance. Insulin resistance plays

a pivotal role in structural and functional pancreatic alterations, as evidenced by islet hypertrophy and hypersecretion [8]. There is compelling evidence that non-alcoholic fatty pancreatic disease (NAFPD) occurs due to lipotoxicity. Diet-induced obesity models develop NAFLD concurrently with NAFPD [9], confirming the importance of excessive free fatty acids (FFA) in the determination of this disease. When caloric intake chronically exceeds energy expenditure, there is a progressive increase in triglyceride (TG) accumulation throughout the body. This is augmented by the fact that the hyperinsulinemia associated with the excess caloric intake upregulates enzymes involved in lipogenesis, whereas intermediates in the lipogenic pathway inhibit FFA oxidation, increasing the amount of FFAs available for storage as TGs [10, 11]. Thus, the lipid content of pancreas, liver, heart, and skeletal muscle could be increased [12]. NAFPD determines long-term beta-cell loss and overt diabetes due to gluco-lipotoxicity, considering that hyperinsulinemia and hyperglycaemia are associated findings. Little is known about the influence of maternal nutrition upon pancreatic islet size, beta-cell mass and islet mass [13].

The aim of the present study was to evaluate the effects of maternal high-fat intake on offspring fat mass, pancreatic alterations and carbohydrate metabolism at different stages of the perinatal period, followed by maintenance or change of the diet after weaning.

Materials and methods

Experimental protocol

This study was approved by the local ethics committee (Protocol number 009/2009), according to conventional guidelines for experimentation with animals (NIH Publication No. 85-23, revised 1996). The animals have been studied previously with regard to NAFLD evaluating in conjunction the body mass (BM) and feed efficiency [14]. Here, we studied the pancreatic and adipose tissue alterations in this foetal programming model.

Animals were maintained under controlled conditions of temperature and humidity, and 12:12 h dark–light cycle, with free access to water and food. C57BL/6 mature virgin female mice were caged with males overnight, and mating was confirmed by observation of vaginal plugs. Twenty-five 3-month-old females, weighing approximately 22 g before breeding, were then assigned to be fed either standard chow (SC: 17 % fat, 19 % protein and 64 % carbohydrate) or high-fat chow, rich in cholesterol-saturated fatty acid (HF: 49 % fat, 19 % protein and 32 % carbohydrate). Both experimental diets included a micronutrient mineral mix, following the American Institute of Nutrition recommendations (AIN-93G) [15] (Table 1).

Table 1 Nutritional composition of experimental diets

Nutrients (g/kg)	Diets	
	HF	SC
Casein	230.00	190.00
Corn starch	299.50	539.50
Sucrose	100.00	100.00
Fat (soybean oil)	70.00	70.00
Fat (lard)	200.00	–
Fibre (cellulose)	50.00	50.00
Vitamin mix (AIN-93G)	10.00	10.00
Mineral mix (AIN-93G)	35.00	35.00
Cystine	3.00	3.00
Choline	2.50	2.50
Antioxidant	0.01	0.01
kJ/g	20.691	16.511
Carbohydrates (%)	32.00	64.00
Protein (%)	19.00	19.00
Fat (%)	49.00	17.00

HF high-fat diet, *SC* standard chow diet. Vitamins and minerals mix formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodents

HF feeding was assigned to specific periods in gestation and/or lactation: (a) G (HF diet was only offered during gestation); (b) L (HF diet was only offered during lactation); (c) GL (HF diet was administered in both gestation and lactation), and d) GL/HF (HF diet was administered in both gestation and lactation, and continued from post-weaning until 3 months of age). Food intake and maternal BM were recorded daily. As soon as delivery occurred, litters were adjusted to six animals in order to ensure adequate and standardised nutrition until weaning [16]. Within 24 h of delivery, pups' birth weights were obtained. At weaning, one male pup per litter was randomly assigned to form the study groups (five animals per group) [17]. BM was monitored weekly until the offspring reached 3 months of age. Feed efficiency (FE) was calculated as grams of BM gain/kilojoules of food consumed per animal (100×).

Glucose levels, euthanasia, plasma insulin and carbohydrate metabolism

At 3 months (1 week before euthanasia), blood was collected from the tail vein by a small incision at the tip of the tail. Blood glucose was measured immediately using a glucometer (Accu-Chek, Roche, SP, Brazil). On the day before euthanasia, animals were deprived of food for 6 h (1 a.m.–7 a.m.), and they were anaesthetised (intraperitoneal sodium pentobarbital). Blood was collected by cardiac puncture and immediately centrifuged and stored at −20 °C for plasma insulin analysis. Genital fat pads and

pancreas were then excised, weighed and fixed (1.27 mol/L formaldehyde in 0.1 M phosphate buffer, pH 7.2) for 48 h at room temperature. Fasting insulin concentration was measured by radioimmunoassay using a mouse insulin RIA kit (Cat. RI-13K, Linco Research, St. Charles, MO, USA Cat. RI-13K). All samples were analysed in duplicate, with an intra-assay coefficient of variation of 1.4 %. The oral glucose tolerance test (OGTT) was performed, and 25 % glucose in sterile saline (0.9 % NaCl) (1 g/kg body mass [BM]) was administered by orogastric gavage after 6 h of a fasting period. Blood was collected from the tail vein at 0, 15, 30, 60 and 120 min after the glucose load, and blood glucose was measured using a glucometer (Accu-Chek; Roche, Sao Paulo, Brazil). The analysis considered the area under the curve (in arbitrary units, a.u.) to assess glucose intolerance (Prism version 5.03 for Windows; GraphPad Software, SanDiego, CA).

Immunofluorescence

Antigen retrieval was performed with citrate buffer (pH 6.0), and Triton X-100 (0.5 %) was used to facilitate localisation of intracellular proteins. Blocking with ammonium chlorate and glycine (2 %) was then performed. Non-specific binding of polyclonal antibodies was blocked by incubation with 5 % bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Subsequently, sections (5/group) were incubated with anti-insulin (anti-guinea pig, A0564, Dako, Glostrup, Denmark) and anti-glucagon (anti-rabbit, A0565, Dako, Glostrup, Denmark) antibodies. Secondary antibodies were conjugated with Alexa fluor conjugates. Slides were mounted with slow-fade (Invitrogen, Molecular Probes, Carlsbad, CA, USA) to maintain fluorescence. Control sections were obtained after omission of the primary antibodies. A laser confocal microscope (Zeiss Confocal Laser Scanning Microscopy—LSM 510 Meta, Germany) with an EC-Plan-Neofluar (20x) objective lens was used to assess slices.

Immunohistochemistry

Sections obtained through the entire pancreas (5 sections/group) were deparaffinised, and antigen retrieval was performed with citrate buffer (pH 6.0) for 30 min at 60° C. Then, endogenous peroxidase blockade was prepared with hydrogen peroxidase (3–0.3 % H_2O_2 in PBS) and finally was inhibited with phosphate-buffered saline/bovine serum albumin (5 %). Sections were incubated with anti-insulin antibody (dilution 1:100, A0564, Dako, Glostrup, Denmark) and were treated with a biotinylated secondary antibody (K0679; Universal DakoCytomation LSAB + Kit, Peroxidase, Glostrup, Denmark); the immunoreaction was amplified with a biotin–streptavidin system (K0679; Universal DakoCytomation LSAB + Kit, Peroxidase, Glostrup, Denmark). Immunostaining was visualised after incubating the sections with 3,3 diaminobenzidine tetrachloride (K3466, DakoCytomation, Glostrup, Denmark) and counterstaining with Mayer haematoxylin.

Image analysis

Digital images from pancreatic islets (20 pictures/animal/group) were obtained and studied by image analysis. A selection tool from Image-Pro Plus software (version 7.0, Media Cybernetics, Silver Spring, MD, USA) was used to identify islets areas with positive immunoreactions. This selection was segmented into a black/white image, where white showed immunostained areas (insulin). Islet boundaries were delimited using an irregular AOI (area of interest) tool, and the islet area filled in with white was quantified using the image histogram tool. The beta-cell area was expressed as density stain/islet (%) [18].

Stereology

Fragments of the pancreas (5/group) were rapidly fixed (freshly prepared fixatives, 1.27 mol/L formaldehyde in 0.1 M phosphate buffer, pH 7.2) for 48 h at room temperature. The material was embedded in Paraplast plus (Sigma-Aldrich Co., St. Louis, MO, USA), sectioned into 5-μm-thick slices and stained with haematoxylin and eosin. Digital images from pancreatic tissue (15 pictures/animal/group) were obtained using an Olympus BX51 light microscope (Olympus America Inc., Miami, FL, USA) and an LC evolution camera (Media Cybernetics, Silver Spring, MD, USA), and the pancreatic islets were quantified. The smallest and largest diameters of each islet were measured with a ruler to calculate the mean islet diameter. At least 20 islets per mouse were analysed.

For stereological analysis, the islet volume density (*Vv[islet]*) was estimated by counting points, using 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 (P_T):*Vv[islet]* = Pp*[islet]*/*PT (%)*. The islet mass (*M[islet]*) was then obtained by multiplying the *Vv[islet]* by the mass of the pancreas [19]. The beta-cell volume density (*Vv[beta-cell]*) was estimated by image analysis using the density threshold selection tool inside the islet insulin-positive area, which was expressed as a percentage of the islet (Image-Pro Plus version 7.0, Media Cybernetics, Silver Spring, MD, USA) [18]. Thus, beta-cell mass was estimated as the product of *Vv[beta cell]* and *M[islet]* in at least 30 islets per group.

Adipocyte morphometry

Genital adipose tissue is the equivalent to visceral fat in humans [6]. Genital adipose tissue was removed, fixed (formaldehyde 0.1 M phosphate buffer, pH 7.2), embedded in paraffin, sectioned and stained with haematoxylin and eosin. The cross-sectional area of adipocytes was then estimated. Digital images (TIFF format) were obtained from histological sections, and at least 350 adipocytes per group were randomly analysed using an Olympus BX51 light microscope (Olympus America Inc., Miami, FL, USA) and an LC evolution camera. Cell diameters were measured with ImagePro Plus (Media Cybernetics).

Statistical analysis

Data were reported as the mean ± standard error of the mean. Data between groups were analysed using one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test. P value ≤ 0.05 was considered statistically significant (GraphPad Prism v. 5.04 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Body mass (BM) and Feed efficiency (FE)

The maternal HF diet yielded high birth weights in HF offspring compared with SC offspring. At birth, SC and G mice weighed 1.27 ± 0.02 g and 1.35 ± 0.02 g, respectively (+6 %, $P = 0.03$). Throughout the experiment, the SC group was the lightest. At 3 months of age, the BM of GL/HF offspring (32.2 ± 1.9 g) was 45 % higher than SC offspring (22.2 ± 0.5 g, $P < 0.001$), 32 % higher than G offspring (24.4 ± 0.41 g, $P < 0.0001$), 26 % higher than L offspring (25.61 ± 0.69 g, $P < 0.0001$) and 35 % higher than GL offspring (23.79 ± 0.44 g, $P < 0.0001$). In addition, the BM of L offspring was 15 % higher than SC offspring ($P < 0.05$).

FE was only different between SC and GL/HF offspring (5.6 ± 0.3 g/Kj versus 7.2 ± 0.4 g/Kj, respectively, $P = 0.007$), which exhibited FE nearly 30 % greater than SC offspring. The remaining groups had intermediary values of FE, between those of SC and GL/HF offspring, but without significant differences.

Genital fat pad

Animals subjected to postnatal HF diets had greater epididymal fat mass (1.41 ± 0.23 g) than SC (0.45 ± 0.02 g, $P = 0.002$), G (0.48 ± 0.02 g, $P = 0.0004$), L (0.48 ± 0.02 g, $P = 0.0006$) and GL (0.50 ± 0.01 g, $P = 0.003$) offspring, corresponding to an increase of 209, 191, 193 and 182 % ($P < 0.002$, one-way ANOVA) in this variable, respectively.

Insulin levels and oral glucose tolerance test

No difference was detected in glucose levels among the groups at 3 months (Table 2). However, insulin levels were significantly higher in the G offspring in comparison with SC (+147 %, $P < 0.03$) and L offspring (+216 %, $P < 0.03$) (Table 2), suggesting negative effects of high-fat intake during the gestation period. Similarly, Table 2 shows greater OGTT values in G offspring (23.48 ± 1.38 a. u.) than in SC offspring (17.27 ± 1.08 a. u.; $P < 0.05$).

Pancreas morphology

The mass of the pancreas was similar in all offspring (Table 2). Figure 1 shows the morphology of islets from the different groups. SC (329.00 ± 16.47 μm) and L offspring (347.90 ± 16.88 μm) showed the smallest diameters, being normal. Hypertrophy approached 49 % in G offspring (488.60 ± 21.19 μm, $P < 0.0001$), 39 % in GL offspring (457.90 ± 16.52 μm, $P < 0.0001$) and surpassed 77 % in GL/HF offspring (582.90 ± 20.77 μm, $P < 0.0001$, one-way ANOVA), compared with SC offspring. These findings agree with the findings on insulin immunodensity, where all groups whose mothers received high-fat diets, apart from the period of administration, presented higher immunodensity for insulin in comparison with SC offspring ($P < 0.0001$, Table 2).

Vv[islet], beta-cell mass and islet mass are shown in Table 2 and yielded similar results. G offspring showed increased *Vv[islet]* (106 %, $P < 0.005$), which is reflected in greater islet mass (+99 %, $P = 0.002$) compared with SC offspring. Increase in beta-cell mass was also significant in G offspring compared with SC (+113 %, $P < 0.001$) and L (+135 %, $P < 0.001$) offspring, which agrees with the increased area labelled for insulin. Taken together, these confirm islet hypertrophy in G offspring.

Immunofluorescence

There were positive immunoreactions for glucagon (alpha cells) and insulin (beta cells) in pancreatic islets from all groups. Photomicrographs illustrate that GL offspring showed an abnormal distribution of alpha cells, which were arranged not only in the periphery but also distributed within the pancreatic islets (Fig. 2). In contrast, L offspring showed pancreatic islet morphology that was not significantly different from that found in SC offspring. In addition, we also infer that the marked area for insulin was proportional to islet size in all groups.

Table 2 Metabolic and pancreatic parameters of experimental groups

Data	Group SC		Group G		Group L		Group GL		Group GL/HF		ANOVA
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P* value
Feed intake (g/animal/week)	17.70	0.35	17.78	0.29	18.94	0.73	17.20	0.39	17.89	0.38	*0.11*
Caloric intake (Kcal/animal/week)	419.15	35.98	320.73[a]	10.88	450.43[b]	16.52	416.86[b]	9.37	804.18[a,b,c,d]	25.54	*0.0001*
FE (g/KJ) ($\times 10^{-2}$)	5.60	0.30	6.70	0.30	6.40	0.20	6.20	0.30	7.20[a]	0.40	*0.02*
Glucose (mmol/L)	7.17	0.84	8.26	0.61	6.44	1.04	8.74	0.08	7.36	0.23	*0.13*
Insulin (µl U/mL)	5.39	1.12	13.29[a]	2.83	4.20[b]	0.34	9.66	1.41	7.74	0.42	*0.01*
OGTT (AUC, a.u.)	17.27	1.08	23.48[a]	1.38	18.43[b]	1.14	20.86	0.68	20.03	0.95	*0.01*
Pancreas mass (mg)	290.00	0.01	290.00	0.02	250.00	0.02	250.00	0.01	270.00	0.01	*0.13*
Islet diameter (µm)	328.96	16.47	488.58[a]	21.19	347.89[b]	16.88	457.93[a,c]	16.52	582.94[a,b,c,d]	20.77	*0.0001*
Vv [islet] (%)	11.19	0.66	23.05[a]	3.67	9.29[b]	0.83	16.76	2.03	15.34	2.57	*0.01*
Islet mass (mg)	33.00	1.43	65.60[a]	10.62	27.75[b]	3.52	40.62	7.29	39.40	8.32	*0.01*
Islet insulin immunodensity (%)	74.06	0.53	79.19[a]	0.49	79.80[a]	0.71	74.33[b,c]	0.60	78.08[a,d]	0.86	*0.01*
β-cell mass (mg)	24.44	1.09	52.08[a]	8.54	22.13[b]	1.33	30.25	5.56	30.90	6.78	*0.01*
Adipocyte diameter (µm)	52.36	1.03	61.29[a]	1.16	59.65[b]	1.05	56.34[a]	1.16	59.65[a,b,c,d]	1.05	*0.0001*

Groups: SC, maternal and postweaning standard chow diet; G, maternal high-fat diet during gestation; L, maternal high-fat diet during suckling period; GL, maternal high-fat diet during gestation and lactation periods; GL/HF, maternal high-fat diet during gestation and lactation periods plus high-fat diet postweaning until euthanasia. One-way ANOVA and post hoc test of Tukey: in signalled cases, when compared, $P < 0.05$; if: [a] when compared with SC, [b] with G, [c] with L, [d] with GL

The data were expressed as mean ± SEM ($n = 5$ animals each group)

Abbreviations: *FE* feed efficiency, *I/G* insulin/glucose, *OGTT* oral glucose tolerance test, *Vv[islet]* volume density of pancreatic islet, *a.u.* arbitrary units, *AUC* area under the curve

Adipocyte morphometry

Greater adipocyte hypertrophy was observed in animals from G (+17 %, $P < 0.0001$), GL (+14 %, $P < 0.0001$) and GL/HF (+41 %, $P < 0.0001$) groups as compared with SC offspring. Moreover, GL/HF offspring had larger adipocyte sizes than G (+21 %, $P < 0.0001$), L (+31 %, $P < 0.0001$) and GL (+24 %, $P < 0.0001$) groups. For this parameter, HF intake during gestation was worse than during the lactation period. The G group showed an increase of 8 % in adipocyte size as compared with L offspring ($P = 0.003$). Figure 3 shows the microscopic appearance of the adipocytes, confirming smaller adipocytes in SC and L offspring, larger adipocytes in GL/HF offspring and medium-sized adipocytes in G and GL offspring.

Discussion

The current study has highlighted the importance of unbalanced maternal nutrition for inadequate beta-cell function in young offspring. We administered a HF diet (rich in saturated fatty acids) not only during pregnancy and/or lactation but also to offspring from postweaning up to 3 months. We opted for saturated fatty acids because this sort of fatty acid is predominantly present in industrialised food, fast foods and fried foods, which are highly consumed worldwide. A major finding of this study was hyperinsulinemia and impaired carbohydrates metabolism, in agreement with previous report [20], and an elevation in beta-cell mass in offspring from dams kept on an HF diet exclusively during pregnancy. The animals from HF dams were born heavier, and those who continued to receive HF diet after weaning have shown increased body weight until the end of the experiment (at the 3 months old). This result was corroborated with the increased intake and FE.

Critical windows of development involve the intrauterine environment and the period of lactation. The administration of HF diet during these periods can alter metabolic and physiological permanently and irreversibly in adulthood [21, 22] programming the pups to the development of obesity and glucose intolerance [20]. Similarly, this diet can also lead to hypertriglyceridaemia and hepatomegaly, which mimics the phenotype of the MetS in humans [23]. Our data are according to these findings, mainly the group that received the HF diet during the period of gestation (G group).

In this context, the present findings confirm previous reports that critical events during sensitive periods of development may programme the long-term structure or function of the body [24, 25]. In particular, both the quantity and the quality of nutrition during early development have lifetime consequences, supporting the concept

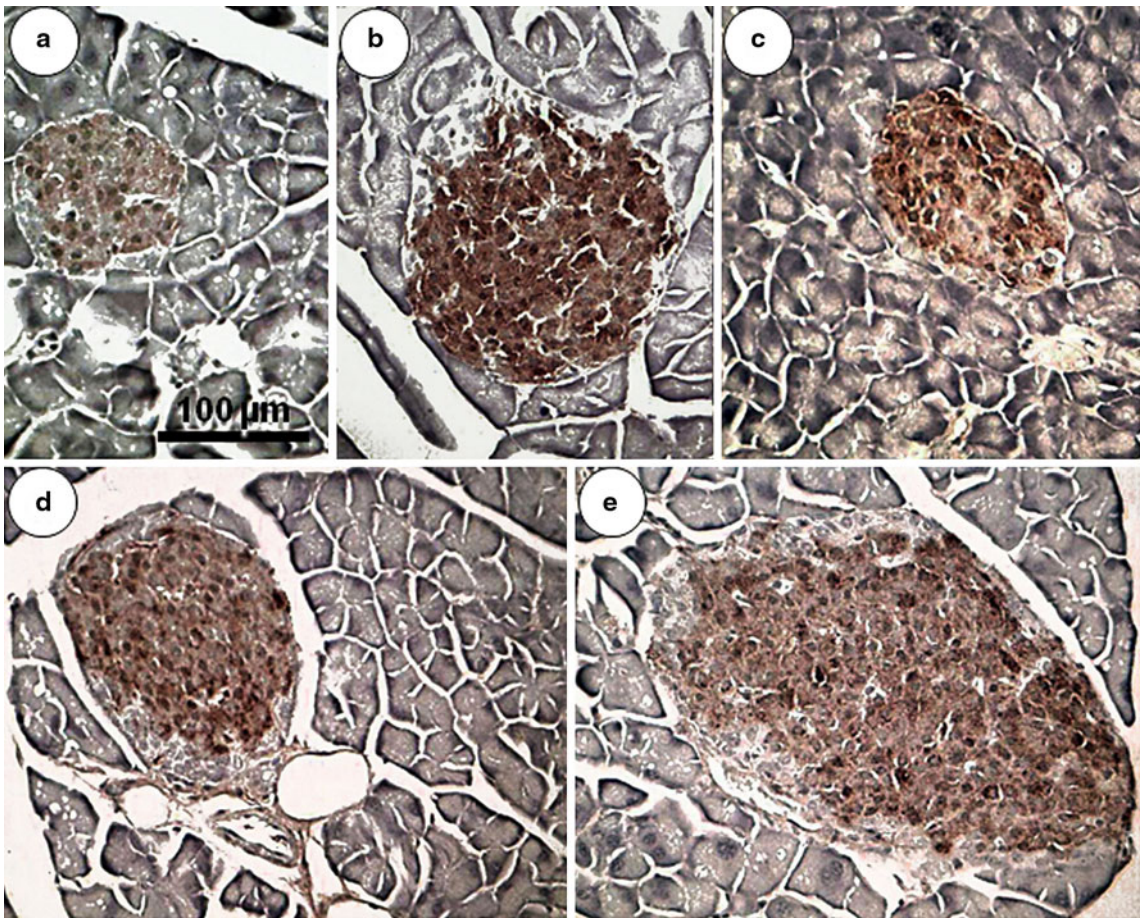

Fig. 1 Immunostaining for insulin in three-month-old offspring. Photomicrographs show pancreatic islets immunostained with 3,3 diaminobenzidine tetrachloride (same magnification for all pictures): **a** SC, maternal and postweaning standard chow diet; **b** G, maternal high-fat diet during gestation; **c** L, maternal high-fat diet during suckling period; **d** GL, maternal high-fat diet during gestation and lactation periods; **e** GL/HF, maternal high-fat diet during gestation and lactation periods plus high-fat diet postweaning until euthanasia

of metabolic programming of early adaptive responses into adulthood [26, 27]. Recent research provides compelling evidences that healthy conditions in adult life are determined by conditions under which an organism develops in the womb.

HF diet, characterised by a saturated fatty acid component, promotes obesity and insulin resistance [6, 28]. To provide HF diet to the dams, the content of carbohydrates was reduced accordingly on the basis that protein should be kept within the recommendations. Otherwise, a mildly deficiency of protein would compromise result interpretations once it represents another type of foetal programming. The reduction in carbohydrate was related to complex carbohydrate, which does not pose any risk of foetal programming as long as sucrose is offered in accordance with dietary recommendations [15]. Here, we found that administration of diets rich in saturated fatty acids, especially during pregnancy, perinatal and postnatal periods, affected BM, adipocyte size and carbohydrate metabolism in young offspring. Our group has previously published evidence that excessive food provision during perinatal life (focusing on excessive maternal fat intake) is capable of inducing hepatic steatosis with hepatic resistance to insulin, stemming from hepatic mitochondrial abnormalities and defective lipid oxidation pathways. Of note, it was previously showed that offspring from mothers subjected to HF diets exclusively during gestation presented insulin resistance through increased HOMA-IR and a bigger area under the curve for oral glucose tolerance tests, which led us to examine pancreatic alterations more closely [14].

Insulin resistance has been described as the central causative component in the development of MetS [29]. In rats, HF diet consumption programmes the male offspring for glucose intolerance and increased BM in adulthood [30]. Some of the observed consequences include reduced whole-body insulin sensitivity, impaired or normal insulin secretion and changes in the structure of pancreas, as was

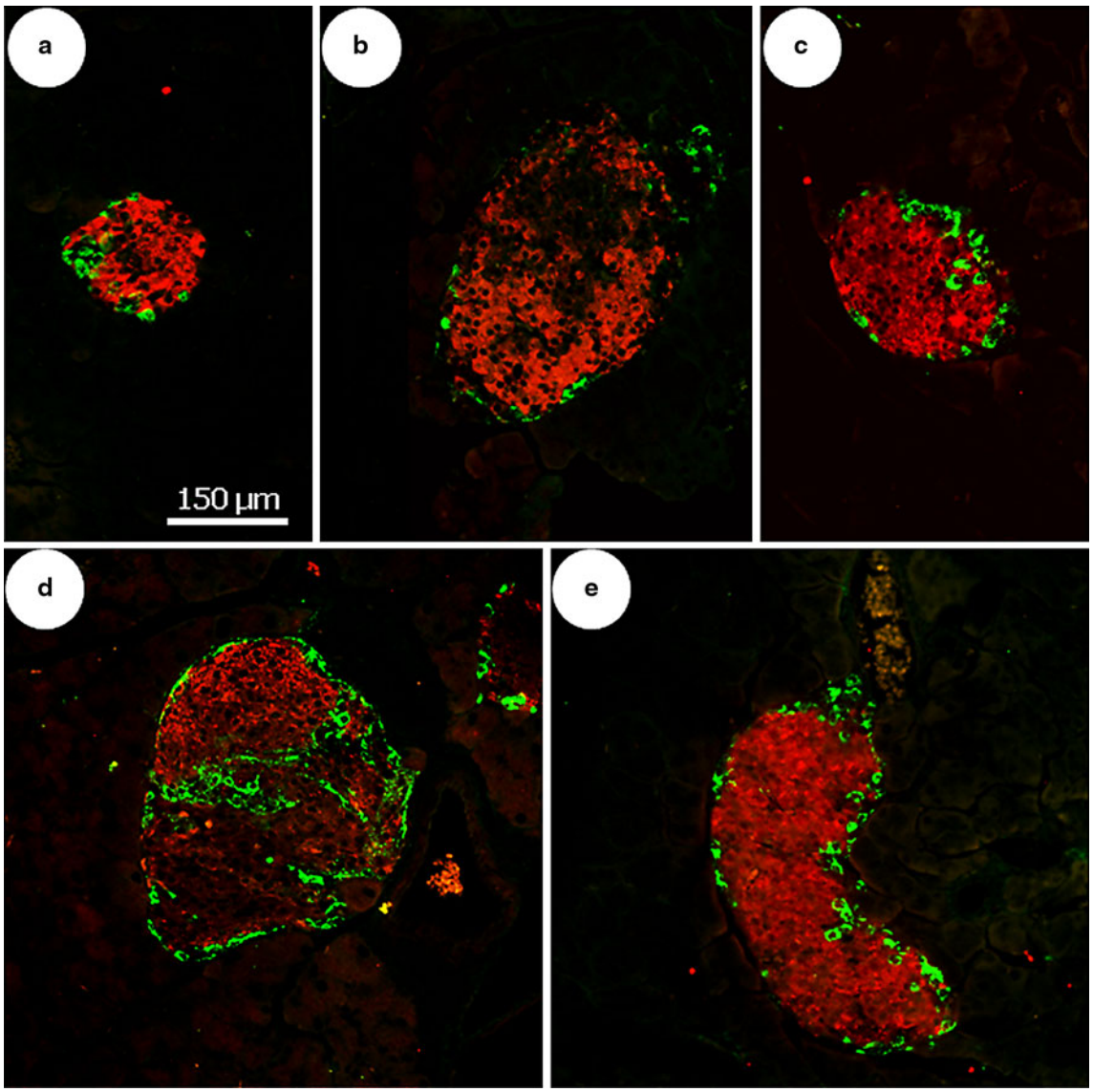

Fig. 2 Immunofluorescence for insulin (*red*) and glucagon (*green*) in 3-month-old offspring (same magnification for all pictures): **a** SC, maternal and postweaning standard chow diet; **b** G, maternal high-fat diet during gestation; **c** L, maternal high-fat diet during suckling period; **d** GL, maternal high-fat diet during gestation and lactation periods; **e** GL/HF, maternal high-fat diet during gestation and lactation periods plus high-fat diet postweaning until euthanasia

observed in G group [31]. Maintenance of beta-cell mass is critical for the regulation of glucose homoeostasis and prevention of type 2 diabetes [32]. However, previous reports show that an HF diet promotes an increase in beta-cell mass, which undergoes hypertrophy and hypersecretion as an adaptive reaction [33, 34], before the onset of overt hyperglycaemia and progression towards type 2 diabetes [35].

The observation that insulin resistance in offspring was consistently programmed by maternal dietary imbalance was of paramount importance. Offspring from mothers that received HF diets during pregnancy (G group) had an increase in beta-cell mass. In this group, as in the others whose mothers were fed HF diet during critical periods of development (lactation as an exception), we also detected an increase in islet diameter, which was accompanied by an increase in beta-cell mass. The increase in islet size might be explained by increased feed consumption given that excessive calorie intake represents a challenge to islet secretion machinery [6, 36]. Consequently, islets increase their volume in order to cover higher demand for insulin in insulin resistant state, as animals from some groups were (mainly G and GL/HF). This adverse islet remodelling may be related not only to compensating hypertrophy to maintain adequate glycemic levels but also may be explained by alterations in mitochondrial pathways within the islet,

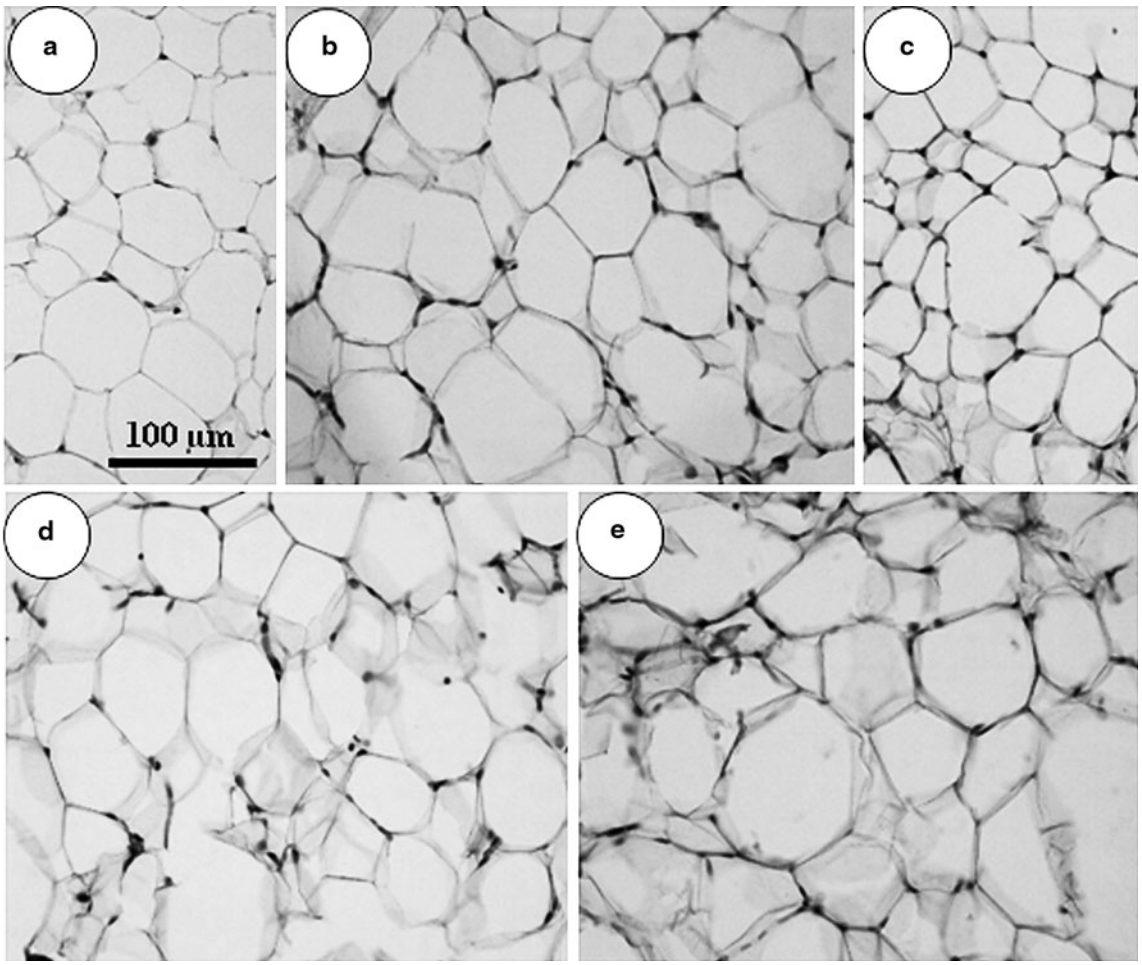

Fig. 3 Adipocyte diameter in 3-month-old offspring. Photomicrographs show adipose tissue stained with haematoxylin and eosin (same magnification for all pictures): **a** SC, maternal and postweaning standard chow diet; **b** G, maternal high-fat diet during gestation; **c** L, maternal high-fat diet during suckling period; **d** GL, maternal high-fat diet during gestation and lactation periods; **e** GL/HF, maternal high-fat diet during gestation and lactation periods plus high-fat diet postweaning until euthanasia

characterising insulin resistance as an inheritable alteration in this model [37]. These observations highlight the fact that type 2 diabetes phenotype can be programmed by an unsuitable intake of saturated fatty acids during gestation. Then, it would be important to encourage clinicians to focus on the category of lipid ingested during pregnancy in order to prevent of obesity and type 2 diabetes mellitus among children and teenagers worldwide [38, 39].

The literature reports that offspring exposed to HF diet for the entire pregnancy were hyperglycaemic with reduced beta-cell volume and number [40–42], but that was not the case in the present results. The decrease in the number and the volume of the beta cell might indicate the installation of type 2 diabetes [4, 43], which was not found in the present study. Here, we can suggest that the animals that received the HF diet in pregnancy showed hyperglycaemia and hyperinsulinemia, configuring insulin resistance, which precedes the installation of type 2 diabetes mellitus. Furthermore, we do not know the nature of the fats used by other studies, that is, whether they were saturated, unsaturated or a mixture. Thus, it is hard to compare those results with our findings. Studies showing the effects of excess administration of monounsaturated and polyunsaturated fatty acids during the perinatal period are still scarce in the literature. Our group has shown that diets rich in monounsaturated fatty acid (olive oil), polyunsaturated fatty acid (sunflower and canola oil) and saturated fatty acid (lard) alter the carbohydrate metabolism in mice. These modifications seem to be more influenced by the contents of lipid than the absolute amount of lipid [44]. In order to evaluate the effects promoted by this diet (rich in saturated fatty acid), the present study included not only a group of suckling mice fed HF diet but also a group that received this diet in perinatal and in the postweaning periods.

Immunofluorescence revealed evidence to support previously described islet abnormalities due to maternal HF diet [25]. Marked differences were depicted concerning the

composition and architecture of pancreatic islets in mice. The primary findings were that pups from GL group showed an abnormal distribution of alpha cells, arranged not only in the periphery but also distributed within the islets. This expansion of the mass of alpha cells may be indicative of a compensated state of the organ due to the increased metabolic demand experienced by beta cells in cases of altered metabolism of carbohydrates [45]. In contrast, the L group showed a distribution pattern of alpha cells not significantly different from that observed in SC group, without significant differences in islet cytoarchitecture.

Our findings suggest that exposure to neonatal HF diets (rich in saturated fatty acids), as well as changes in the type of postnatal diet, alter the glycaemic control of offspring in adulthood. When administered exclusively during gestation, the HF diet yielded islet hypertrophy, hyperinsulinemia, changed in glycaemic control after glucose overload, increased beta-cell mass and islet mass, which made gestation the most critical period. Maternal HF intake during lactation failed to programme pancreatic alterations as the present findings suggested or hepatic alterations [14]. GL offspring showed the most significant alterations concerning cell type distribution of islets, even though the animals were not resistant to insulin and did not present other features suggestive of type 2 diabetes. These alterations in islet architecture together with increased islet diameter imply that type 2 diabetes will develop in the long run, as the animals suffer from the change of diet at weaning.

In contrast, the GL/HF group did not present the majority of metabolic alterations addressed here, which could be explained by the hypothesis of "predictive adaptive response", suggesting that the maintenance of maternal diet confers a kind of protection during the early postweaning phase. Foetal programming encompasses physiological, morphological and metabolic adaptation that aims to guarantee foetal survival even with adverse intrauterine conditions. In this context, adaptations are efficient if the foetus faces during early life the same conditions of the womb. Modification of postnatal pre-existing conditions (oversupply or lack of nutrients) causes a metabolic disorder, predisposing the foetus/offspring to the onset of future diseases [46, 47]. However, although GL/HF offspring have not presented hyperglycaemia, hyperinsulinemia and increased mass of pancreatic beta-cell mass, the presence of islet hypertrophy and increased immunodensity for insulin may be indicative of further insulin resistance. This observation raises the possibility that the predictive adaptive response may be restricted to early life and thus should be addressed in ageing animals to clarify whether it is a temporary protective mechanism.

The damage to pancreatic architecture characterised in this study emphasises the need to improve the quality of food intake and health conditions. We can conclude from the present data set that maintenance or changing of diet after weaning and especially HF diets during pregnancy represent great harm to offspring, causing significant overweight, adipocyte hypertrophy, structural changes in beta cells and impairment of carbohydrate metabolism. Although GL/HF offspring did not present hyperglycaemia, hyperinsulinemia or increased mass of beta-cell mass, this group showed the largest adipocytes of all groups, and these findings may indicate future insulin resistance. Consideration should be given to the effects of different sources of fat intake during the perinatal period, and therefore, further studies are relevant. It is not known whether other types of fat-rich diets (i.e., those rich in monounsaturated or polyunsaturated fatty acids) could also cause adverse pancreatic and adipose tissue remodelling. In the present study, the notion that excessive energetic intake during gestation is more dangerous than during lactation create new perspectives aiming at unravelling the precise mechanisms that underlie pancreatic remodelling in these offspring. The relevance of these discoveries is the opportunity to tackle diabetes mellitus, which affects around 285 million adults worldwide and might develop since intrauterine life due to excessive maternal intake.

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Conflicts of interest None.

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