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Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny

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Abstract Nutrition during fetal life is a critical factor contributing to diabetes development in adulthood. The aim of our study was to verify: *1*) whether a high-fat (HF) diet in young adult mice induces alterations in β-cell mass, proliferation, neogenesis, and apoptosis, as well as insulin sensitivity and secretion; *2*) whether these alterations may be reversible after HF diet suspension; *3*) the effects in a first (F1) and second generation (F2) of mice without direct exposure to a HF diet after birth. Type 2 diabetes developed in adult mice on a HF diet, in F1 mice that were HF diet-exposed during fetal or neonatal life, and in F2 mice whose mothers were HF diet-exposed during their fetal life. β-cell mass, replication, and neogenesis were high in HF diet-exposed mice and decreased after diet suspension. β-cell mass and replication remained high in F1 mice and decreased in F2 mice whose mothers were exposed to a HF diet. β-cell neogenesis was present in adult mice on a HF diet and in F1 mice that were HF diet-exposed during fetal and/or neonatal life. We conclude that a HF diet during fetal life, particularly if combined with the same insult during the suckling period, can induce the type 2 diabetes phenotype, which can be directly transmitted to the progeny even in the absence of additional dietary insults.—Gniuli, D., A. Calcagno, M. E. Caristo, A. Mancuso, V. Macchi, G. Mingrone, and R. Vettor. **Effects of high-fat diet exposure during fetal life on type 2 diabetes development in the progeny. *J. Lipid Res.* 2008.** 49: **1936–1945.**

Supplementary key words beta cell • PDX-1 • KI67

Parallel with significant improvements in socioeconomic conditions in Westernized societies, the incidence of type 2 diabetes has increased dramatically, gaining the nature of an epidemic (1). In light of such a rapid increase in type 2 diabetes prevalence worldwide, it is unrealistic to derive a single measure of heritability for this condition. In the past few years, genes responsible for monogenic and syndromic forms of diabetes have been identified. However, the vast majority of diabetes types do not recognize a specific genetic pattern (2).

Because the genetic pool has remained stable, the explanation for the explosion of the diabetes epidemic must involve changes in eating habits and/or increasingly sedentary lifestyles (3–8). Epidemiologic and migration studies indicate that a Western lifestyle is associated with a higher prevalence of type 2 diabetes (9). Dietary factors are important predictors for diabetes risk, and they have often been considered as major elements in several diabetes prevention trials (10, 11). Moreover, it has been shown that a drastic reduction of metabolizable lipid intake, as observed after malabsorptive bariatric surgical procedures, is able to cure diabetes mellitus not only in formerly obese patients (12–14), but also in normal-weight subjects (15, 16).

Epidemiological studies have investigated the importance of adequate nutrition during fetal development in humans (17) and in animals (18, 19) as one of the critical factors contributing to the etiology of obesity and diabetes in adult life (20), probably imputable to an organism's potential to modulate "biological switches" when encountering an altered nutritional environment during the early stages of life. Once these biological switches are imprinted, the physiology of the organism is permanently modified, abnormally responding to various stimuli later in life (21).

Consistent evidence in the literature indicates that maternal undernutrition during gestation leads to hyperinsulinemia and insulin resistance, often associated with obesity in adult offspring (22, 23). More interestingly, the insulin resistance trait is transmitted to a well-nourished second generation of rats (24).

It has been demonstrated that neonatal female rat pups raised artificially on a high-carbohydrate milk formula dur-

This work was supported by MIUR (Ministero Italiano dell'Istruzione e Ricerca scientifica, Italian Ministry of Education and scientific research) Grants RBNE01KCX4_008 MIUR-FIRB and 2003061834_006 MIUR-COFIN (R.V.).

Manuscript received 18 January 2008 and in revised form 19 March 2008 and in re-revised form 12 May 2008.

Published, JLR Papers in Press, May 20, 2008.
DOI 10.1194/jlr.M800033-JLR200

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ing the suckling period maintained chronic hyperinsulinemia in the postweaning period on a laboratory diet and developed obesity in adulthood without any further dietary intervention (21).

Studies concerning maternal fat intake during pregnancy have mainly addressed cardiovascular outcomes in the offspring (25–27). At least to our knowledge, there is only one study (28) reporting the effects of exposure to a high-fat (HF) diet during different periods of gestation. This study shows a compromised β-cell development, altered fasting glycemia, and low birth weight in neonates. However, dynamic measures of glucose disposal after a glucose challenge were not performed.

We therefore hypothesized that HF diet-induced alteration in pancreatic β-cell formation might be transmissible to further generations. We aimed at verifying whether exposure to a milieu rich in saturated fat during either fetal life or lactation in experimental animals might induce type 2 diabetes mellitus and whether this acquired diabetes could be inherited by the progeny. In particular, we investigated: *1*) the effect of a HF diet in young adult mice in terms of β-cell mass, proliferation, neogenesis, and apoptosis, as well as the effects on insulin secretion and sensitivity; *2*) whether the metabolic and histological effects of a HF diet might be reversible after ceasing the diet; and *3*) the effect of a HF diet in terms of β-cell mass, proliferation, neogenesis, and apoptosis, as well as on insulin secretion and sensitivity in first- (F1) and second-generation (F2) mice.

RESEARCH DESIGN AND METHODS

Experimental animals

Randomization of mice receiving different types of diet is described in **Fig. 1** and in **Table 1**. Briefly, 50 female Swiss mice obtained from an in-house breeding colony were randomized at ~2 months of age into two groups. One group received a standard chow (C) diet (Allied Feeds; Rhodes, NSW, Australia) ad libitum and the other a HF diet (Allied Feeds) ad libitum. The HF diet supplied 60% of calories as fat and 20% of calories as carbohydrates versus 10% of calories as fat and 60% of calories as carbohydrates in the C diet. The carbohydrates present were corn starch (45%), maltodextrin (50%), and sucrose (5%) in both diets. Fatty acid composition was as follows. C diet: 25.1% saturated fatty acids (mainly stearic acid and palmitic acid), 34.7% monounsaturated fatty acids (mainly oleic acid), and 40.2% polyunsaturated fatty acid (linoleic acid). HF diet: 42.0% saturated fatty acids (palmitic and stearic acid), 43% monounsaturated fatty acids (oleic acid), and 15.0% polyunsaturated fatty acid (linoleic acid and linolenic acid). The C diet and HF diet contained 18 mg and 300 mg cholesterol/kg and 0.3 and 4.2 g of arachidonic acid/4,057 kcal, respectively. Vitamins, minerals, and protein content were matched in order to be identical.

The diets were administered for 60 days before breeding. Half of the HF diet mice were then shifted to a C diet for 2 more

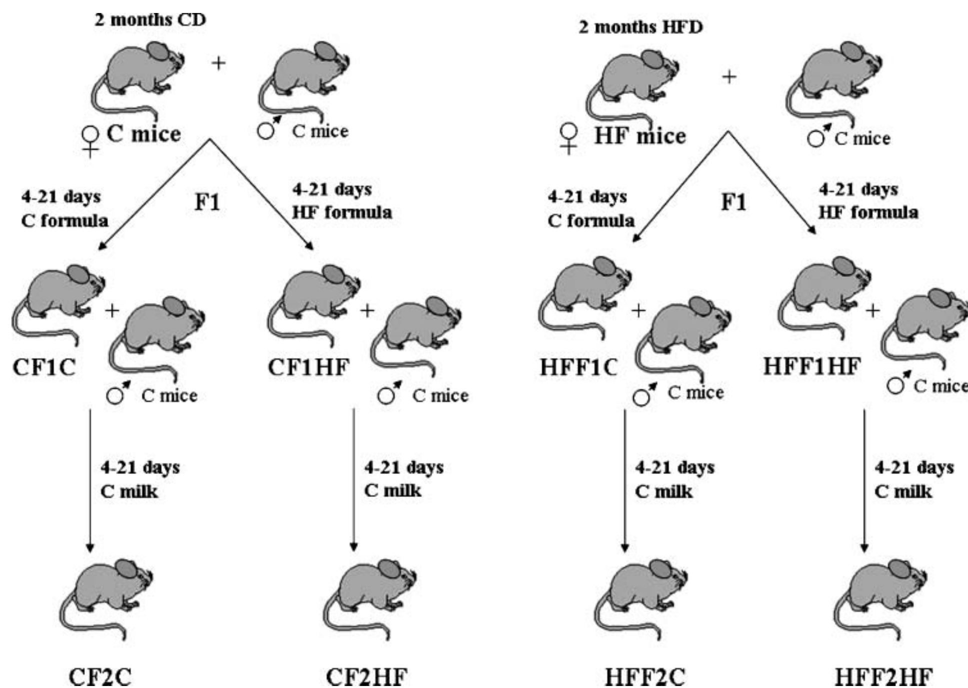

Fig. 1. First (F1) and second generation (F2) of mice production. F1 mice: CF1C, mice receiving a chow (C) diet in utero and during the suckling period; HFF1C, mice receiving a high-fat (HF) diet in utero and a C formula during the suckling period; CF1HF, mice receiving a C diet in utero and a HF formula during the suckling period; HFF1HF, mice receiving a HF diet both in utero and during the suckling period. F2 mice: all mice were fed on C milk by foster mothers and fed a C diet after 21 days of age. Mice were named from their progenitor mother: from CF1C (CF2C), from CF1HF (CF2HF), from HFF1C (HFF2C), and from HFF1HF (HFF2HF).

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TABLE 1. Mice production from young adult mice to F1 and F2 and their relative weight at the end of the dietary treatment

Mice Groups	Mother's Diet in Utero	Suckling Period Diet	Mother's Background	Diet During F1 Pregnancy	F2 Suckling Period Diet	Weight
						g
Foster Mothers						
C	C	C	C			30.1 ± 9.1
HF	C	C	C			27.3 ± 7.4
HFC	C	C	C			29.3 ± 8.2
F1						
CF1C	C	C	C			28.3 ± 6.1
CF1HF	C	HF	C			27.2 ± 7.1
HFF1C	HF	C	C			29.5 ± 5.3
HFF1HF	HF	HF	C			26.8 ± 8.1
F2						
CF2C			CC	C	C	28.3 ± 8.2
CF2HF			HFC	C	C	29.3 ± 6.8
HFF2C			HFC	C	C	23.2 ± 5.1[a]
HFF2HF			HFHF	C	C	22.1 ± 4.4[a]

[a] HFF2C and HFF2HF mice were significantly smaller than the other F2 mice ($P < 0.05$).

months, so forming a third group (HFC). Six animals from the HF group, six from the C group, and six from the HFC group underwent an intraperitoneal glucose tolerance test (IPGTT) 2 months after the beginning of either a HF or a C diet. Twenty C and twenty HF females were bred with C males in order to obtain the F1 mice. On postnatal day 4, each F1 mouse was fed by an intragastic cannula introduced under mild anesthesia and received the same HF formula or C formula as the mothers for 20 min every 2 h at a rate of 0.40 kcal/g_{bw}/day, so forming four groups of mice (CF1C, mothers on C diet and suckling F1 pups on C diet; HFF1C, mothers on HF diet and suckling F1 pups on C diet; CF1HF, mothers on C diet and suckling F1 pups on HF diet; and HFF1HF, mothers on HF diet and suckling F1 pups on HF diet). The complexity of the procedure, the length of the treatment, and cannula failure resulted in a 60% success rate in all groups. The surviving mice were healthy and, when not euthanized for the experimental sessions, reached adulthood. On postnatal day 24, all four groups were fed on normal adult laboratory chow diet. On the 60th day, 40 female F1 mice (10 mice from each group) underwent an IPGTT, and then after euthanization, the pancreas was removed for morphometrical and immunohistochemical evaluation. The remaining F1 females were bred with normal male mice in order to obtain the F2 mice, named HFF2HF, HFF2C, CF2HF, and CF2C, according to their respective group of provenience. Foster mothers under C diet nursed the resulting progenies until day 24, when they were weaned on laboratory C diet ad libitum. On day 60, all F2 mice (48 mice) underwent an IPGTT, and then the pancreas was removed for morphometrical and immunohistochemical evaluation.

Animals had free access to water and were subjected to controlled temperature (22 ± 1°C) and lighting (lights on 0600–1800). Experimental procedures were performed under mild anesthesia (75 mg/kg ketamin + 1 mg/kg domitor), as approved by the Catholic University Animal Experimentation Ethics Committee and in accordance with national guidelines for the use of animals in research.

IPGTT

The IPGTT was performed according to previously described methods (29). Mice were fasted overnight for 12 h and then injected intraperitoneally with glucose (2 g/kg body weight). Venous blood was obtained from the tail vein at 0, 15, 30, 60, and 120 min after the injection. Blood glucose was measured with an automated glucometer. Insulin levels were measured by radio immuno assay using a rat insulin standard (Linco; St. Charles, MO).

Immunohistochemical and morphometric analyses

Tissue fixation and immunohistochemistry. Although the experiments were carried out in Rome, the immunohistochemestry was performed in Padua by a blinded observer. Pancreas was removed from all groups of mice, weighed, and fixed overnight in 10% formalin in PBS. Tissues were then processed routinely for paraffin embedding, and 4 μm-thick sections were prepared for histology and immunohistochemistry. For each pancreas, from three to five sections were cut along the length. Hematoxylin and eosin-stained sections were used for direct microscopic examination. Negative staining controls were utilized in order to evaluate and subtract background.

β-cell mass. Slides were immunostained for β-cells using mouse monoclonal anti-insulin antibody (NCL-INSULIN; Castra, Newcastle upon Tyne, UK). The antibody was at a dilution of 1:100 in PBS. Slides were then exposed to a secondary biotinylated IgG (ScyTek Laboratories, Inc.; Logan, UT) and visualized by incubation with a peroxidase substrate solution containing 3,3′-diaminobenzidine (DAB) tetrahydrochloride. Slides were then washed and counterstained with hematoxylin. β-cell mass was determined from the insulin antibody-stained sections using an image analysis system (30).

The proportion of islet β-cell surface area versus surface area of the whole pancreas was determined planimetrically by digitally imaging 14–20 (young mice) or 20–40 (adult mice) non-overlapping, about 4 mm^2 fields per pancreas on a Zeiss Universal microscope coupled to a 3CCD color video camera (KY-F55BE; JVC, Japan) and to a Koehler's illuminations setting. Eight to ten non-overlapping random 20× objective frames were captured by a frame grabber (Kontron; Eching, Germany), analyzed with an image analysis program (CIRES, Zeiss; Jena, Germany) operating on line with the camera and hosted in a personal computer. Image files were tabulated in pixel value and entered into Microsoft Excel for statistical analyses. This planimetric method also allowed us to estimate relative islet numbers and compare islet sizes. The possibility of measuring large islets twice was minimized due to the wide sampling interval between sections. β-cell mass was estimated for each animal by deter-

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mining the average β-cell surface area per animal multiplied by their pancreatic weight and expressed in milligrams (31).

Morphometric analysis. Morphometric analysis was done on three sections per animal. Sections were hematoxylin and eosin stained and were viewed through a 20× objective of a conventional light microscope (Leica DMLB; Glostrup, Denmark). In each section, the total islet size was measured using image analysis software (see above). The average of three measurements per section was determined, then islet area was multiplied by pancreatic weight and the results expressed in milligrams, in order to compare islet size with β-cell mass.

GPR-40. For detection of pancreatic GPR-40 (32, 33), immunohistochemical staining was performed using a rabbit polyclonal antibody to GPCR GPR-40 peptide (ab12569; concentration: 1.00 mg/ml; Abcam, Cambridge, UK). Antigen retrieval was carried out using protease-14 for 30 min at 25°C. Rabbit anti-GPR-40 immunoglobulins were applied at a diluition of 1:100 in PBS for 50 min. The secondary biotinylated antibody was by ScyTek Laboratories, and the cellular positivity was visualized by incubation with the chromogen DAB, added for 5 min at room temperature. The sections were counterstained with hematoxylin. An image analysis system was used as reported above. Results were expressed as percentage of positive cells to GPR-40 immunostaining on total pancreatic area.

β-cell replication. Cellular proliferation was evaluated using immunohistochemistry with the cell cycle antigen Ki67 (NCL-Ki 67p; Castra). The antibody was at a dilution of 1:100 in PBS. The secondary biotinylated antibody was by ScyTek Laboratories, and the nuclear positivity was visualized by incubation with a peroxidase substrate solution containing 3,3′-DAB tetrahydrochloride. Mouse anti-insulin antibody was used for double staining with anti-Ki67 and anti-insulin Abs. Quantification of β-cell replication was performed by counting Ki67-positive cells in five sections spaced more than 40 μm apart in each pancreas. The average value of Ki67-positive cells was multiplied by β-cell mass as calculated above to obtain an arbitrary Ki67 labelling index (31).

Pancreatic duodenal homeobox-1-positive cells and β-cell neogenesis. Pancreatic duodenal homeobox-1 (PDX-1) is one of the transcriptional factors needed by pancreatic stem cells in order to develop a mature pancreas and to regulate the transcription of endocrine pancreas-specific genes in young adult mice (34–37). For detection of PDX-1, the mounted sections were microwaved in citrate buffer (pH 6.0), for antigen retrieval before being preincubated with blocking serum, 1:10 in PBS for 15 min. They were then incubated in PDX-1 anti-serum at a dilution of 1:100 in PBS for 50 min.

An affinity-purified goat polyclonal antibody was used, and it was raised against a peptide mapping within an internal region of PDX-1 of human origin (sc-14664 P; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The secondary antibody was an anti-goat IgG rinsed in rabbit, diluted 1:200 in PBS, for 30 min. The immunohistochemical visualization was performed by peroxidase anti-peroxidase complex (1:50 in PBS). The chromogen DAB was added for 5 min. All passages were at room temperature. Control studies were performed using the above methods with deletion of individual primary or secondary antisera. Finally, slides were stained with hematoxylin, shed in water and in alcohol, and mounted in synthetic resin.

For PDX-1-positive cell detection purposes, the percentage of cells immunoreacting to PDX-1 antiserum on the total cells of an islet was calculated. Four to six non-overlapping random 40× objective frames for slides were considered. A conventional light microscope (Leica DMLB) was used. For β-cell neogenesis evaluation, the number of exocrine duct cells immunoreactive for PDX-1 was divided by the number of ductal cells.

Apoptosis. Islet cell apoptosis was investigated by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining (38) using an in situ apoptosis detection kit (DeadEnd Colorimetric Tunel System; Promega Corporation) according to the manufacturer's protocol. Duel staining for apoptosis and insulin was performed. Five to ten sections spaced more than 40 μm apart for each pancreas were analyzed. An average of eight to ten were examined per section, and the mean number of positive cells per islet calculated for each section. Because the relative β-cell mass differed among groups, the frequency of β-cell apoptosis was divided by the relative β-cell mass.

Statistical analysis

Results are expressed as mean ± SEM. Differences between means were evaluated using Bonferroni-corrected Mann-Whitney U tests. Differences were considered significant at $P < 0.05$. The area under the curve (AUC) of glucose and insulin plasma levels after IPGTT was calculated by the trapezoidal method. Bivariate correlations and multiple regression analysis were calculated using the SPSS 12.0 system for Windows.

RESULTS

Experimental animals

The dietary treatment of the mothers did not affect litter size and birth weight of F1 and F2 mice. Young adult mice, F1 mice, CF2HF, and CF2C mice did not differ significantly in weight during the entire period of treatment, whereas HFF2C and HFF2HF mice were significantly smaller than the other young adult F2 mice ($P < 0.05$), due to decompensated diabetes with subsequent polyuria (Table 1).

Metabolic studies

Fig. 2 reports the glucose levels after IPGTT and the glucose AUC in the three groups of mice (young adult, F1, and F2 mice). **Fig. 3** reports insulin levels after IPGTT and insulin AUC in the three groups of mice.

Young adult mice. Glucose levels were significantly higher at any IPGTT time in female mice fed on the HF diet compared with control mice ($P < 0.001$), and blood glucose levels returned to normal 2 months after HF diet suspension (C vs. HFC, P = n.s.; HF vs. HFC, $P < 0.01$ at any time) (Fig. 2A). Insulin levels were significantly higher at any IPGTT time in mice fed a HF diet compared with control mice ($P < 0.001$). Two months after the suspension of the diet, the insulin levels were significantly reduced during the whole time course ($P < 0.001$), but still higher than those observed in the control group ($P < 0.01$) (Fig. 3A).

F1 mice. All F1 mice showed normal fasting glucose levels (P = n.s. among groups), whereas blood glucose

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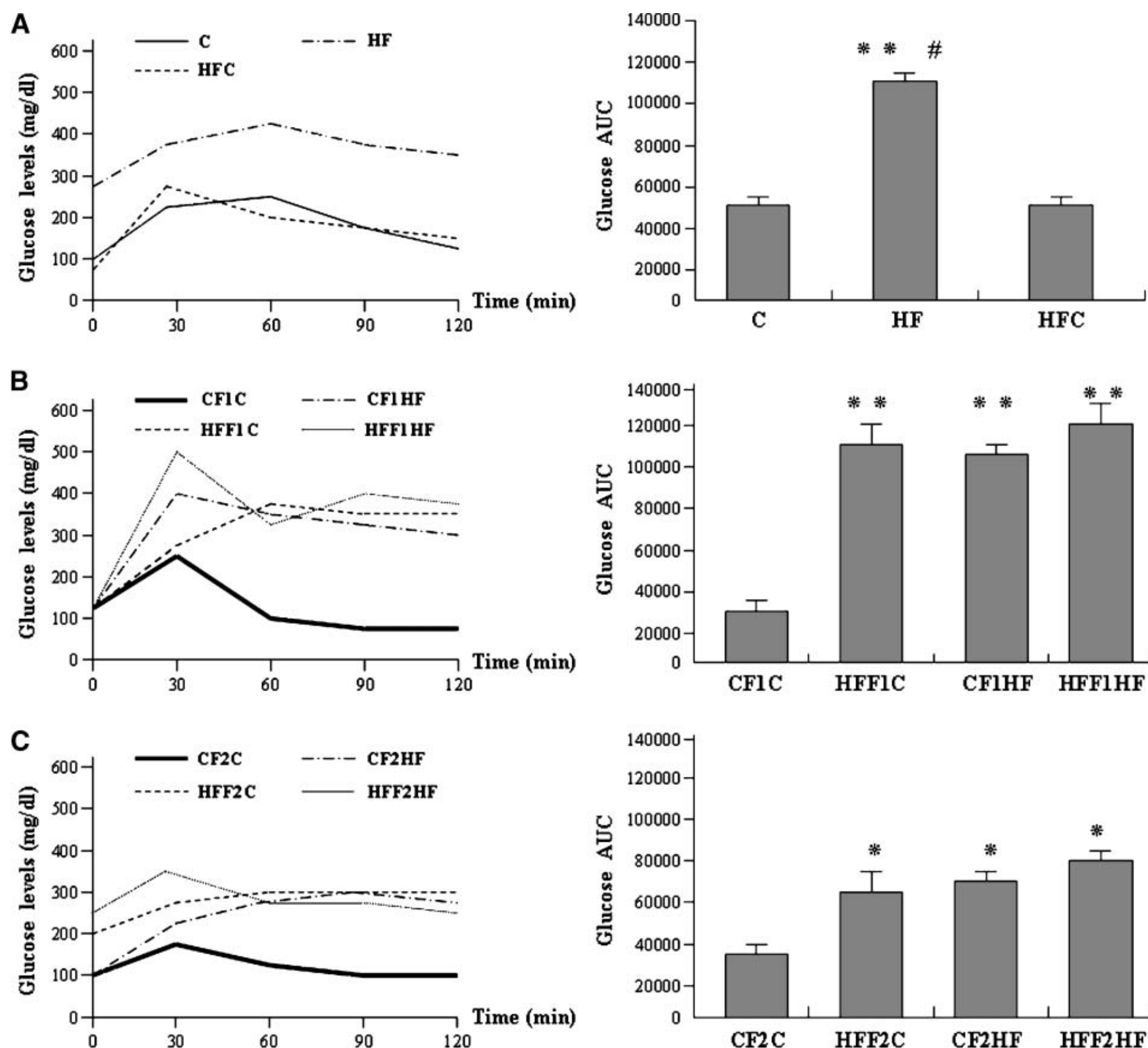

Fig. 2. Glucose time course and area under the curve (AUC) after an intraperitoneal glucose tolerance test (IPGTT) (2 g glucose per kg of body weight). A: Young adult female mice. ** Glucose AUC: HF vs. C mice ($P = 0.0003$); # HF vs. HFC ($P = 0.002$). HFC, HF diet mice shifted to a C diet. B: F1. ** Glucose AUC: HFF1HF, CF1HF, and HFF1C vs. CF1C mice ($P = 0.009$). C: F2. * Glucose AUC: CF2HF, HFF2HF, and HFF2C vs. CF2C mice ($P = 0.03$). Error bars indicate ± SD.

concentration was significantly higher after the glucose load in HFF1HF and CF1HF mice compared with the CF1C group (Fig. 2B). Fasting insulin levels were similar in CF1C and HFF1C mice, whereas they were significantly higher in CF1HF and HFF1HF mice ($P < 0.01$ in both groups vs. CF1C mice). The insulin time course in both HFF1C and CF1HF mice showed significantly higher insulin levels than in CF1C mice, but a similar shape. In contrast, HFF1HF mice showed a 4-fold insulin increase at 30 and 60 min after the IPGTT, and a slight decrease over the following hour (Fig. 3B).

F2 mice. Fasting blood glucose levels were significantly higher only in HFF2HF and HFF2C mice compared with CF2C (both $P < 0.01$). CF2HF mice glycemia 30 min after IPGTT was higher than that in CF2C mice ($P < 0.05$) and similar to that in HFF2C mice (P = n.s.). After the glucose load, blood glucose levels were significantly higher in HFF2HF, HFF2C, and CF2HF mice than in CF2C mice at any single time (Fig. 2C). Fasting insulin levels, as well as the entire insulin time-course, was significantly lower in HFF2HF ($P < 0.05$) and HFF2C mice ($P < 0.05$) than in CF2C mice (Fig. 3C). In contrast, fasting insulin levels were significantly higher in CF2HF mice ($P < 0.01$), but the insulin time-course was similar to that in CF1HF mice (Fig. 3B, C).

Immunohistochemical and morphometric analysis

β-cell mass.

Young adult mice. β-cell mass (**Fig. 4A**) was higher in mice fed a HF diet than in C mice ($P < 0.001$); after HF diet suspension, β-cell mass greatly diminished, but remained higher than in C mice ($P < 0.05$ C vs. HFC and HF vs. HFC).

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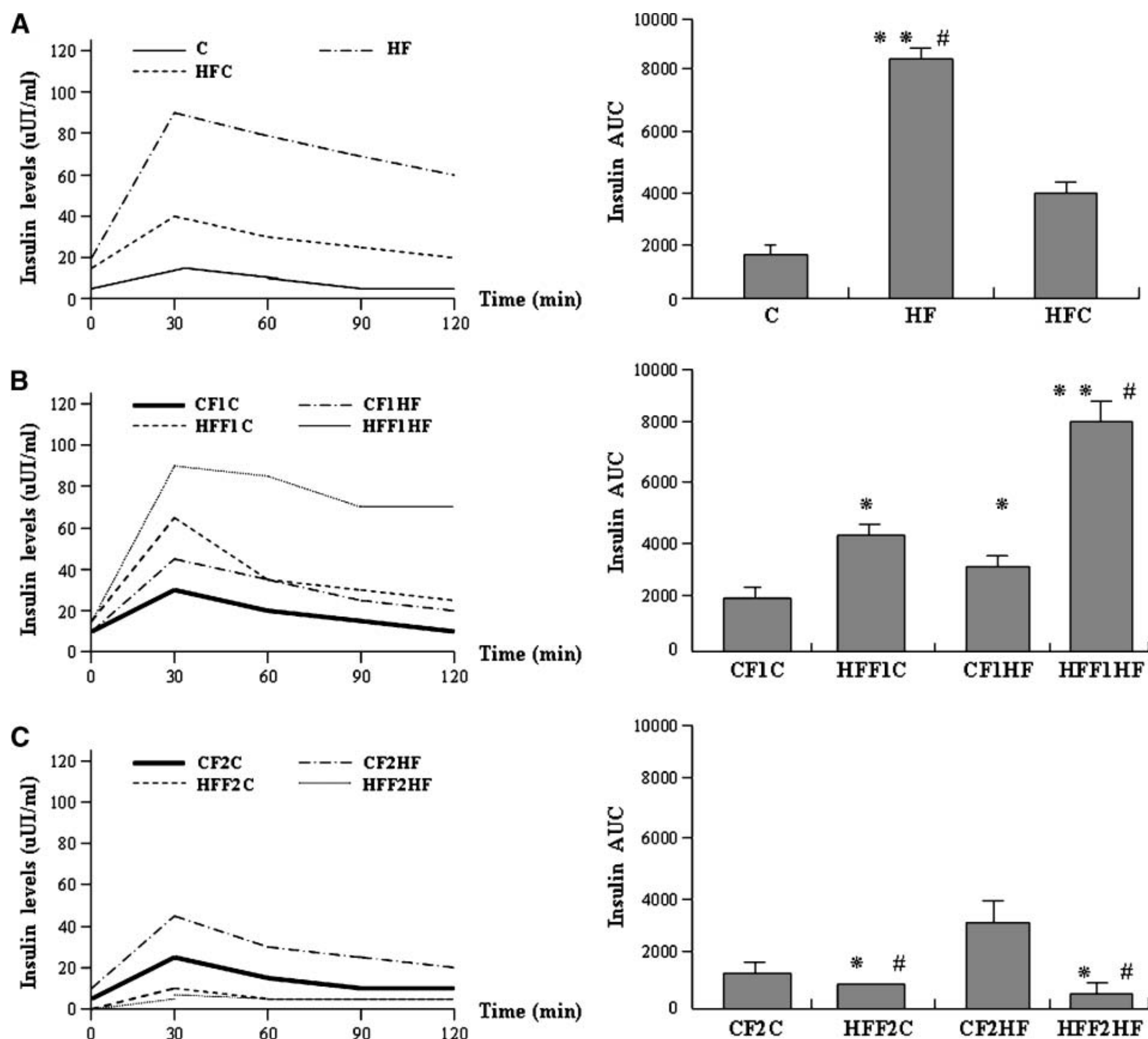

Fig. 3. Insulin time course and AUC after an IPGTT (2 g glucose per kg of body weight). A: Young adult mice. ** Insulin AUC: HF vs. HFC mice ($P = 0.0003$); # HF vs. C mice ($P = 0.007$). B: F1. * $P < 0.05$. ** $P < 0.001$; insulin AUC: CF1HF and HFF1HF vs. CF1C mice ($P = 0.03$); # HFF1HF vs. HFF1C and CF1HF mice ($P = 0.0003$). C: F2. * Insulin AUC: HFF2HF and HFF2C vs. CF2C mice ($P = 0.04$); # CF2HF vs. HFF2HF, HFF2C, and CF2C mice ($P = 0.007$). Error bars indicate ± SD.

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F1 mice. HFF1HF mice had a higher β-cell mass than CF1C mice ($P < 0.05$). No significant difference was found between the CF1C group and the other groups (P = n.s.).

F2 mice. HFF2HF mice had lower β-cell mass than CF2C mice ($P < 0.05$). No significant difference was found between the CF2C group and the other groups (P = n.s.).

Pancreatic islet size. Mean value of islet size is reported in Fig. 4B. These results are consistent with the increase in β-cell mass in the same groups.

GPR-40. GPR-40-positive cells in the pancreas islets were counted, and the percentage of FFAs sensing GPR-40 protein are shown in Fig. 4C. Co-staining with insulin showed that only β-cells were positive for anti-GPR-40 antibodies.

Young adult mice. Percent GPR-40-positive cells was higher in HF mice than in C mice ($P < 0.05$).

F1 mice. Percent GPR-40-positive cells was higher in F1 mice on a HF diet during their fetal life or lactation than in CF1C mice ($P < 0.05$).

F2 mice. Percent GPR-40-positive cells did not differ in any of the F2 groups (P = n.s.).

β-cell replication. Results of β-cell replication are reported in **Fig. 5A**.

Young adult mice. β-cell replication was almost three times larger in HF mice than in C mice ($P < 0.01$); after suspension of the HF diet, β-cell replication greatly di-

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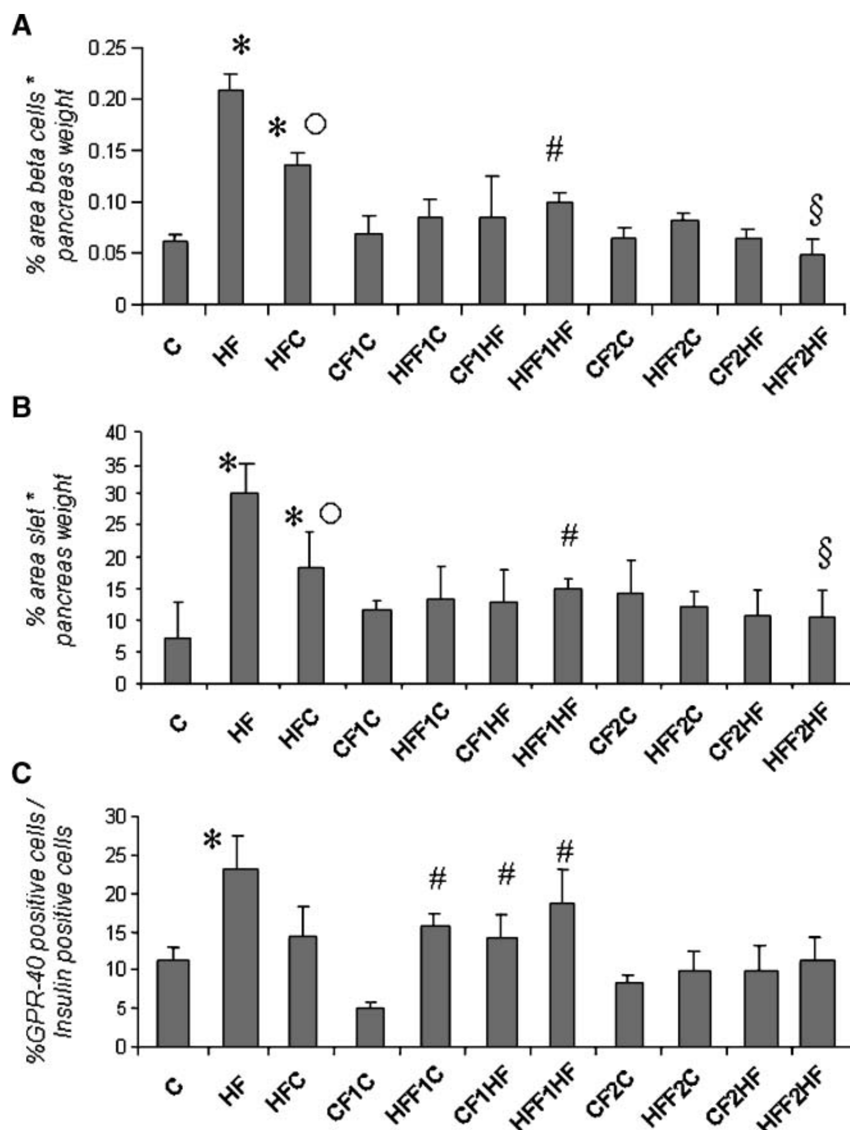

Fig. 4. β-cell mass (A), islet size (B) and percentage of cells positive to the FFA-sensing GPR-40 protein in all groups of mice (C). A: β-cell mass. Young adult mice: * HF vs. C mice ($P = 0.003$); ○ C vs. HFC mice ($P = 0.03$); HF vs. HFC mice ($P = 0.04$). F1: # HFF1HF vs. CF1C mice ($P = 0.03$). F2: § HFF2HF vs. CF2C mice ($P = 0.045$). B: Islet size. Foster mothers: * HF vs. C mice ($P = 0.003$); ○ C vs. HFC mice ($P = 0.03$); HF vs. HFC mice ($P = 0.04$). F1: # HFF1HF vs. CF1C mice ($P = 0.03$). F2: § HFF2HF vs. CF2C mice ($P = 0.045$). C: GPR-40. Foster mothers: * HF vs. C mice ($P = 0.045$). F1: # HFF1C vs. CF1C mice ($P = 0.045$); CF1HF and HFF1HF vs. CF1C mice ($P < 0.03$). F2 mice that were not directly exposed to a HF diet did not show a rise in GPR-40. Error bars indicate ± SD.

minished, remaining, however, twice as large as the replication level in C mice ($P < 0.05$ C vs. HFC and HF vs. HFC).

F1 mice. CF1HF and HFF1HF mice had significantly larger values (twice) of β-cell replication than CF1C ($P < 0.01$ in both cases); HFF1HF had values of β-cell replication three times larger than CF1C mice ($P < 0.0001$). No significant differences were found among the other groups (P = n.s.).

F2 mice. β-cell replication did not differ in any of the F2 groups of mice (P = n.s.), and was comparable to that in C young adult mice (P = n.s.).

PDX-1-positive cells and β-cell neogenesis. PDX-1-positive nuclei were found mainly in β-cells. The percentage of PDX-1-positive cells in the islets of all groups studied is reported in Fig. 5B. In C and HFC (young adult mice), CF1C and CF1HF (F1 mice), and in all groups of F2 mice, nuclear immunoreactivity for PDX-1 was found only in acinar cells. A nuclear PDX-1 immunoreactivity in ductal cells was observed only in HF (young adult mice), HFF1C, and HFF1HF mice (F1 mice), suggesting a β-cell neogenesis in those groups of mice (Fig. 5C).

Apoptosis. Because no significant difference in apoptosis absolute values was found among groups, the frequency of

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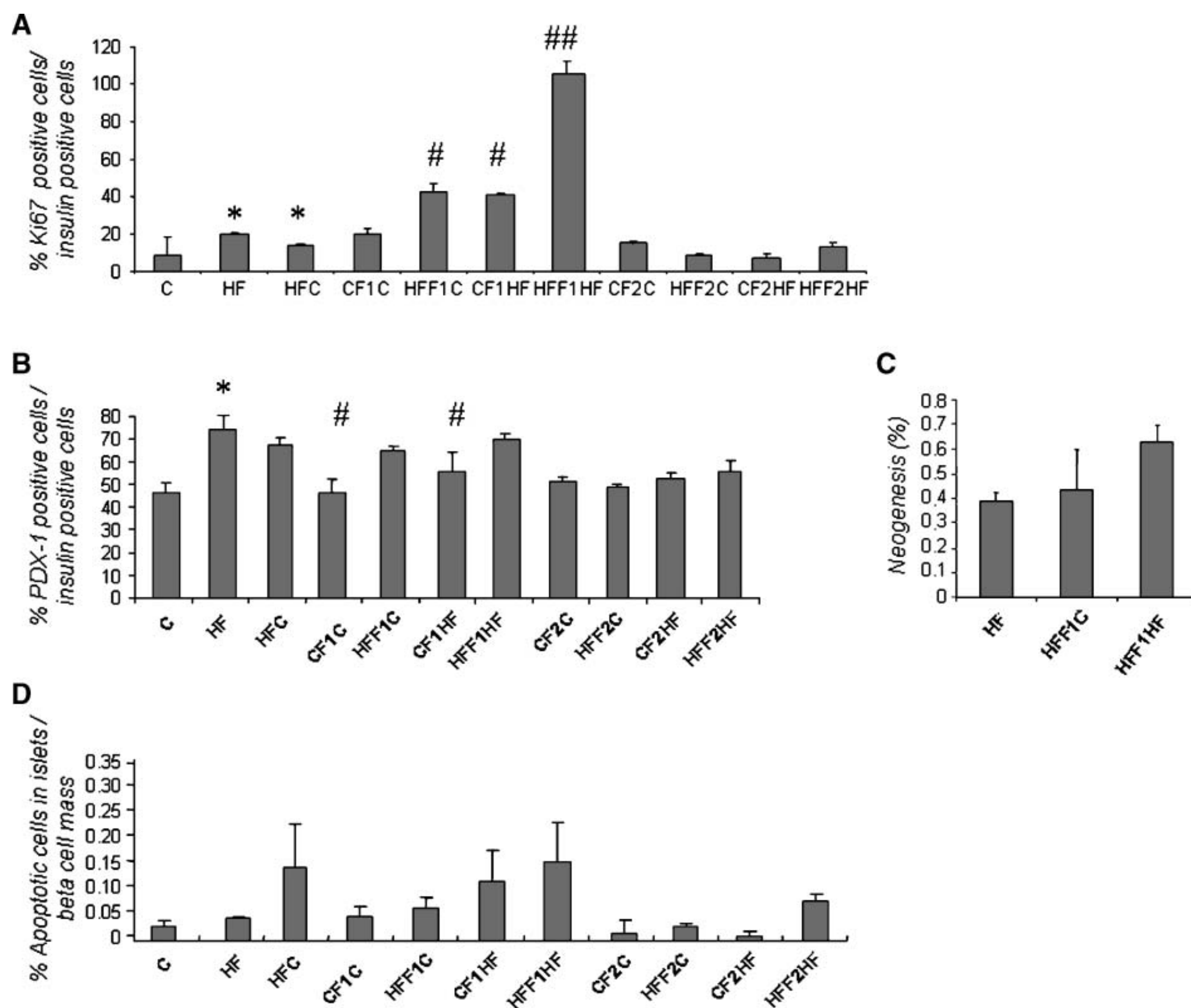

Fig. 5. Percentage of β-cell replication (A), percentage of PDX-1-positive cells (B), percentage of β-cell neogenesis expressed as PDX-1-positive cells within the ductal cells (C), and percentage of β-cell apoptosis (D). A: β-cell replication. Young adult mice: * HF vs. C mice ($P = 0.003$); and ° C vs. HFC ($P = 0.03$). F1: # CF1HF and HFF1HF vs. CF1C mice ($P = 0.009$). ## HFF1HF vs. CF1C mice ($P = 0.0001$). B: PDX-1-positive cells. Young adult mice: * HF vs. C mice ($P = 0.003$). F1: # CF1HF, HFF1HF, and HFF1C vs. CF1C mice ($P = 0.04$). C: β-cell neogenesis. A nuclear immunoreactivity for PDX-1 in ductal cells was observed in HF (young adult mice), HFF1C, and HFF1HF mice (F1 mice). D: β-cell apoptosis. Young adult mice: A trend of increased apoptosis was observed in HFC mice ($P = 0.06$). F1: CF1HF and HFF1HF vs. CF1C mice ($P = 0.6$). Error bars indicate ± SD.

β-cell apoptosis was normalized by the β-cell mass (Fig. 5D). Although a clear trend of increased apoptosis was observed in some groups of young adult mice (HF, HFC) and F1 mice on a HF diet (CF1HF and HFF1HF), this trend did not reach statistical significance.

DISCUSSION

The mechanism leading from obesity to type 2 diabetes is currently unknown, but it has two hallmark features: *i*) insulin resistance and *ii*) compromised function of pancreatic β-cells (39). In the majority of the prediabetic population, insulin resistance precedes the development of glucose intolerance. In the prediabetic phase, β-cells increase insulin secretion in order to compensate for insulin resistance until they become unable to meet the rising demand for insulin, with subsequent development of frank diabetes.

It is generally accepted that dietary fat affects insulin resistance and β-cell function in both obesity and type 2 diabetes (40, 41). FFAs have generally been proposed to regulate pancreatic insulin release by an intracellular mechanism involving inhibition of CPT-1. In fact, GPR-40, a member of a subfamily of homologous G protein-coupled receptors, is specifically expressed in insulin-producing β-cells, and it functions as a receptor for long-chain FFAs to amplify glucose-stimulated insulin secretion in experimental animal islets (42, 43). In our series, Swiss mice on a diet rich in saturated fat developed frank type 2 diabetes, typically represented by hyperinsulinemia associated with hyperglycemia. These features were associated with increased FFA-specific sensing of GPR-40 in β-cells. In contrast, F2 mice, which were not directly exposed to a HF diet, did not show

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any rise in GPR-40. It has been shown, in rodents and in humans, that β-cells possess great capacity of expansion in response to changes in peripheral insulin requirements (29, 44–46). Studies in mice identified a key role for PDX-1 (47, 48) in regulating β-cell mass in response to progressive insulin resistance. The relative contribution to pancreatic islet expansion of β-cell hyperplasia/hypertrophy, or neogenesis from pancreatic epithelial precursors, or of β-cell apoptosis is not clear. Recently, the importance of β-cell proliferation was put forward as a pivotal mechanism of β-cell growth and mass maintenance (48–50).

Our data provide several insights that contribute to a better knowledge of the "programming" of type 2 diabetes and insulin resistance in mice. First, it is noteworthy that the same HF diet that in adult Swiss mice is able to induce a reversible form of type 2 diabetes, when administered during a formative period of life, such as the fetal stage or the combination of both fetal and neonatal stages, induces diabetes transmissible to the progeny. In the adult HF group, a HF diet delivers a direct insult to β-cells, which respond by increasing both their neogenesis and replication, resulting in an increased β-cell mass and frank hyperinsulinemia. When the insult is removed during the adult life, a compensatory β-cell apoptosis restores a normal β-cell mass, thus normalizing the glucose tolerance (44, 45). In the offspring, where a HF diet was administered only during fetal life or in combination, during both fetal and neonatal life, the β-cell insult was severe, as shown by the sustained hyperglycemia during adulthood, and irreversible, in spite of the phenomena of replication and neogenesis observed.

Second, it is also noteworthy that a dietary insult can induce permanent alterations in β-cell function, altering, in addition, the physiological mechanisms of damage compensation, only if occurring during the fetal stage. Therefore, a dietary insult occurring during early infancy can be considered as an additional stimulus, which worsens an already-compromised β-cell function. When a HF diet is consumed by pregnant or pregnant-and-lactating females, the baby mice develop irreversible type 2 diabetes, which is also transmissible to their progeny.

Our data confirm previous demonstrations that critical events during sensitive periods of development may "program" the long-term or lifetime structure or function of the organism. In particular, both the quantity and the quality of nutrition during early development have lifetime consequences, supporting the concept of the metabolic programming of early adaptive responses into adulthood (20). Recent research provides compelling evidence that health in adulthood is mainly determined by the conditions under which an organism develops in the womb.

Cerf et al. (28) showed that neonates exposed to a HF diet for the entire duration of pregnancy were hyperglycemic, with reduced β-cell volume and number. However, they mentioned in their study only the percentage of fat in the diet (40%) without specifying the nature of the fats, i.e., whether they were saturated, unsaturated, or a mixture. The lack of this information renders it difficult to compare the results of their study with our results. Furthermore, only fasting glucose and insulin concentrations are reported (28), whereas in our study, the insulin dynamic response to a glucose load as well as the glucose time course are shown. Moreover, in our experimental sessions, the inclusion of a group of suckling mice fed a HF diet has allowed us to evaluate the effects of this diet in a formative period of life other than pregnancy. In this regard, the effect of the HF diet in the suckling period alone seems to have a smaller impact on β-cell function than a HF diet administered during either the fetal stage or the suckling stage. The latter, in fact, seems to greatly contribute to β-cell failure.

Evidence for this kind of "metabolic programming" also occurs in humans (17), where the small size of a newborn caused by maternal malnutrition correlated with an increased risk for type 2 diabetes, hypertension, and cardiovascular disease in adult life (51). A possible biological mechanism underlying the nutritional environment effect on fetus development might include adaptive changes in gene expression capable of influencing the metabolic system (16), but further studies are needed in order to identify the biological mechanism underlying this phenomenon.

Our study gives evidence that an acquired form of diabetes mellitus due to a HF diet is inheritable. Of paramount importance is the observation that a β-cell insult during fetal life, particularly if combined with the same insult also during the suckling period, not only can induce the phenotype of type 2 diabetes, but also transmits this characteristic to the progeny, even in the absence of additional dietary treatment. The present findings support the theory that a close regulation of alimentary habits, which should start from very early infancy, might be able to reduce type 2 diabetes onset also in humans. Saturated fat consumption should be monitored, at least in adult women with a family history of type 2 diabetes, both during pregnancy and lactation, in order to reduce the harmful effects of a HF diet during fetal development and lactation. Furthermore, it is advisable that the offspring of these women, when not breast-fed during the suckling period, receive a low-saturated-fat milk formula.

The excellent technical assistance of Mr. Giuliano Carlesso, Dr. Luciano Giacomelli, and Mrs. Anna Caprodossi was greatly appreciated.

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