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ORIGINAL ARTICLE

Prenatal Food Restriction with Postweaning High-fat Diet Alters Glucose Metabolic Function in Adult Rat Offspring

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Background and Aims. The present study was designed to investigate the effects of prenatal food restriction (PFR) with postweaning high-fat diet (HFD) on glucose metabolic function in adult offspring.

Methods. Pregnant Wistar rats were given PFR treatment from gestational day 11 to spontaneous delivery. All pups were fed by HFD after weaning. Oral glucose tolerance test (OGTT) was conducted at postnatal week (PW) 20. Rats were decapitated in PW24 to collect liver and pancreas, and expression of hepatic insulin signaling genes were then quantified.

Results. Body weight from PW4 to PW24 in PFR males was lower than those in control males, whereas there was no distinct difference between females. However, body weight gain rates were higher from PW16 to PW24 in PFR males and females. Fasting serum glucose presented no changes, whereas fasting serum insulin decreased in PW20 in PFR pups. Moreover, glucose intolerance only appeared in PFR males, whereas no changes were shown in PFR females in relative values. Serum insulin increased in both PFR groups after OGTT. Remarkable pathological changes were also found in islets from PFR rats. There was an increase in the hepatic mRNA expression of IR in PFR females and of Glut2 in PFR males.

Conclusion. PFR with postweaning HFD induced a catch-up growth in body weight, especially in PFR females. Serum insulin decreased in both PFR groups in fasting status. Insulin resistance after OGTT only existed in PFR males, whereas PFR females showed no obvious changes in glucose metabolism.

Key Words: Intrauterine growth retardation, Diabetes, Prenatal food restriction, Pancreas growth, Glucose metabolic phenotype.

Introduction

Diabetes is reported to be the third most fatal disease after malignant neoplasm and cardiovascular disease (1). The conventional view held is that the generation of type 2 diabetes mellitus (T2DM) might be involved with an unhealthy lifestyle in adulthood (for instance, high-glucose diet, high-fat diet and lack of exercise) or congenital genetic defects (2). Previous studies have shown that the occurrence of T2DM is also related to status of early life (3). Adverse intrauterine environments will program pancreatic growth and function to be irreversibly weak, which is exactly the principal view of "developmental origin of adult diseases (DOHaD)" theory about the generation of T2DM (4). Further in adulthood, unhealthy diet will induce β-cell failure and insulin resistance, eventually leading to glucose intolerance and T2DM (5–7). Intrauterine growth retardation (IUGR) is defined as a developing fetus weighing 10% (or two standard deviations) less than the mean body weight of normal fetuses at the same gestational age (8). IUGR can be induced by multiple adverse intrauterine environments (9). Meanwhile, IUGR offspring are more susceptible to T2DM and

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glucose intolerance in later life (10,11), which suggests that exposure to unhealthy environments *in utero* might result in fetal hypoplasia via inducing IUGR, finally affecting glucose metabolic function in adulthood (12).

Prenatal food restriction (PFR) is one of the typical IUGR modeling approaches (13). Animal experiments indicated that fasting serum glucose was increased and glucose tolerance was decreased in PFR offspring (14). High-fat diet (HFD) is one of the inducing factors of hypertension, T2DM, insulin resistance, and other metabolic diseases (15). Our work aimed to verify that PFR with postweaning HFD could induce a glucose metabolic alteration in adult rat offspring.

Materials and Methods

Insulin radioimmunoassay kits were provided by the North Institute of Biological Technology (Beijing, China). Glucose oxidase assay kits were purchased from Mind Bioengineering Co. Ltd. (Shanghai, China). Isoflurane was obtained from Baxter Healthcare (Deerfield, IL). Primers were synthesized by Sangong Biotech (Shanghai, China). Reverse transcription PCR and real-time fluorescent q-PCR kits were obtained by TaKaRa (Dalian, China).

Animals and Treatment

This experiment was performed in the Centre for Animal Experiments of Wuhan University (Wuhan, China), which was accredited by the Association for Assessment and Accreditation of International Laboratory Animal Care. All experimental animal procedures were approved by and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee.

Pathogen-free female Wistar rats weighing 205–238 g and males weighing 263–297 g were purchased from the Experimental Centre of Hubei Medical Scientific Academy (No. 2008–0005, Hubei, China). After 7 days of acclimation, two female rats were mated with one male rat from 7:00 pm to 7:00 am. Mating was confirmed by appearance of sperm in the vagina and designated gestational day (GD) 0. Pregnant females were then transferred to individual cages. Pregnant rats were allowed food *ad libitum* or placed on a restricted diet (50% of the daily food intake of control rats, 60 g/kg body weight) from GD11 until term delivery.

At parturition, food-restricted dams and their pups were fed *ad libitum*. Pups from food-restricted dams were designated as PFR rats, whereas those from *ad libitum*-fed mothers were control rats. Finally, eight pregnant rats with 8–14 pups were selected in each group. To keep the uniformity of the model, we further selected eight pups of each litter randomly and balanced the gender ratio (male:female = 1:1) to ensure adequate and equal nutrition until weaning (16). All pups were weaned to an *ad libitum* HFD until PW24 before decapitation. Standard rodent chow was purchased from the Experimental Centre of Hubei Medical Scientific Academy, which contained 21% kcal from protein, 68.5% kcal from carbohydrate and 10.5% kcal from fat. The HFD was previously reported by our laboratory (17) and contained 88.0% corn flour, 11.5% lard, and 0.5% cholesterol, which provided 18.9% kcal from protein, 61.7% kcal from carbohydrate and 19.4% kcal from fat. Oral glucose tolerance test (OGTT) was carried out at PW20. Rats were anesthetized with isoflurane and decapitated at PW24. Liver and pancreas were collected on ice. We randomly put five liver tissues into liquid nitrogen for freezing and then transferred them to −80°C. The remaining three pancreas tissues were dissected and fixed in a 4% paraformaldehyde solution for histological examination.

Body Weight and Body Weight Gain Rate

We traced the body weight of rat offspring in PW1, PW4, PW8, PW12, PW16, PW20 and PW24 and calculated their body weight gain rate. The body weight gain rate was calculated as follows:

$$\text{Body weight gain rate (\%)} = (\text{PWX weight} - \text{PW1 weight}) / \text{PW1 weight} \times 100\%$$

OGTT

Based on Reference (18), rats had no access to food from 8 PM until 8 AM the next day before OGTT. Each rat was given 2 g/kg glucose solution. The interval time of administration to each animal was 2 min, and the total operating time was limited to 30 min. Glucose (2 g/kg wt) was given by gavage, and blood from tail veins (300 μL) was collected at 0, 30, 60, 120 min (19). Serum was separated by centrifugation at 17205 × g for 15 min at 4°C and then stored at 80°C until glucose and insulin were measured. Serum insulin and serum glucose were measured, respectively, with radioimmunoassay kits and biochemical assay kits following the manufacturer's protocol. Area under the curve (AUC) was calculated by the trapezoidal rule (20,21). To eliminate the interference induced by individual differences of fasting serum glucose and insulin at 0 min, we divided the concentration of serum glucose and serum insulin at 0, 30, 60 and 120 min by those at 0 min to obtain the percentage. $\text{Con.}_{\text{N min}}$ represented serum glucose at each time point. Time $_{\text{N min–N min}}$ represented time gap between two time points.

$$\text{Con.}_{\text{N min}}\,(\%) = \text{Con.}_{\text{N min}} / \text{Con.}_{\text{0 min}} \times 100$$