Maternal high-fat diet induces insulin resistance and deterioration of pancreatic β-cell function in adult offspring with sex differences in mice

Hisashi Yokomizo,1 Toyoshi Inoguchi,1,2 Norimuku Sonoda,1,2 Yuka Sakaki,1 Yasutaka Maeda,1 Tomoaki Inoue,1 Eiichi Hirata,1 Ryoko Takei,1 Noriko Ikeda,1 Masakazu Fujii,1 Kei Fukuda,3 Hiroyuki Sasaki,3 and Ryoichi Takayanagi2

1Department of Internal Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; 2Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan; and 3Division of Epigenomics, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

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Obesity has increased at an alarming rate and is now a worldwide public health problem (12). If current worldwide trends continue, the number of overweight people is projected to increase from 1.3 billion in 2005 to nearly 2.0 billion by 2030 (23). Obesity is a risk factor for a variety of chronic conditions, including diabetes, hypertension, high cholesterol, stroke, and heart disease (11, 35). Diabetes may be most closely linked to obesity, and the increasing incidence of diabetes worldwide is of considerable concern. Furthermore, the steady increase in overweight reproductive-age women may be correlated with increases in rates of childhood and adult obesity (34).

Genetic and/or environmental factors such as the high-fat diet (HFD), common to the Western lifestyle, are thought to contribute to the prevalence of obesity and diabetes (12). Western-type diabetes and Asian-type diabetes have some similarities, although they have important differences in pathophysiology and clinical phenotypes (23, 50). For example, compared with Caucasians, Japanese patients with impaired glucose tolerance and diabetes have less insulin resistance and more insulin insufficiency, especially in the early phase after glucose load (13). Therefore, it has been postulated that the genetic background for diabetes may be different between the countries. Interestingly, the recent success of genetics has identified a number of common genetic variants associated with type 2 diabetes. Approximately 20 genetic variants demonstrate genome-wide significance, with most genetic factors related to type 2 diabetes associated with having a role in pancreatic β-cell function (38) but that do not distinguish patients among different ethnic groups (22, 43). For example, recent reports have revealed that almost all of the novel type 2 diabetes susceptibility genes found in Caucasians are replicated in Japanese (22, 49). This indicates that these DNA variants do not provide strong predictive power and suggests that environmental influences such as HFD may underlie modern type 2 diabetes.

Recently, epidemiological and experimental evidence has indicated that early life events between the fetal and neonatal period play an important role in influencing later susceptibility to certain chronic diseases such as diabetes in postnatal offspring (3, 9, 16). For example, epidemiology studies have indicated that malnutrition in dams during pregnancy produces postnatal offspring with elevated risk of developing obesity and diabetes (26, 28). These observations have led to the fetal programming hypothesis that malnutrition during fetal and neonatal periods causes permanent changes in the structure, physiology, and metabolism needed to adapt to a restricted nutritional supply and increases the risk of chronic diseases. These “developmental origins of health and disease” concepts have important biological, medical, and socioeconomic implications (14, 15). Additionally, there are increasing studies on the impact of maternal HFD on diabetes in offspring (42). Considering that about one-third of all pregnant women are obese (10) and that the period from conception to birth is a time of rapid growth, cellular replication, and differentiation and functional maturation of organ systems, maternal obesity may...
have long-lasting effects on the development of obesity and diabetes in adult offspring.

Several studies have suggested the involvement of various mechanisms in developmental origins of health and disease (51). It has been hypothesized that the molecular mechanisms underlying this phenomenon may be related in part to epigenetic modulation of expression in key developmental genes (36). Epigenetic states can also be modified by environmental factors, which may contribute to the development of abnormal phenotypes (19, 37). When the nutritional supply is excessive, such as during exposure to HFD in the Western lifestyle, thrifty traits by fetal programming may become maladaptive; that is, they contribute to the development of obesity and metabolic syndrome. Although it has been shown that glucose metabolism can be programmed with a maternal HFD, there have been few reports on whether excessive maternal nutrition affects glucose metabolism and pancreatic β-cell function with sex differences (7, 32, 44, 48). Considering that estrogens protect against HFD-induced insulin resistance and glucose intolerance (5, 39), the aim of the present study was to investigate in mice what maternal HFD effects during gestation and lactation are induced between male and female offspring and whether a correlation between estradiol and pancreatic β-cell function exists in offspring.

MATERIALS AND METHODS

Animals and Diets

Female and male C57/BL6J mice, aged 7 wk, and all diet food were purchased from Oriental Yeast (Tokyo, Japan). All mice were maintained under standard pathogen-free conditions with free access to water and standard chow at Kyushu University Animal Center (Fukuoka, Japan). One week after arrival, one male and one female were allowed to breed naturally, and pregnancy was confirmed by the presence and expulsion of a vaginal plug. After the mice became pregnant, they were fed either a control diet (CD) or HFD during gestation and lactation. The CD contained 11.5, 70.3, and 18.2% calories from fat, carbohydrate, and protein, respectively, and a total of 3.53 kcal/g. The HFD contained 62.2, 19.6, and 18.2% calories from fat, carbohydrate, and protein, respectively, and a total of 5.06 kcal/g. Both the CD and HFD had the same micronutrient composition and differed only in the macronutrient and caloric content (Table 1). After weaning, all pups were fed the CD from 4 to 6 wk of age and then the CD or HFD from 6 to 20 wk of age, resulting in four experimental littermate groups: CD-CD, CD-HFD, HFD-CD, and HFD-HFD, combinations of maternal and offspring diets (Fig. 1A). This study was reviewed and approved by the Committee on the Ethics of Animal Experiments, Graduate School of Medical Science, Kyushu University.

Measurement of Body Weight and Food Consumption

Body weight was assessed once/wk during gestation for dams and assessed at birth and between 4 and 20 wk of age for offspring. Food consumption was assessed once/wk during gestation for dams and at 6, 14, and 20 wk of age for offspring. Food consumption was determined for each group by weighing the total amount of food given at the start of each week and then subtracting the amount of food remaining at the end of the week. The average food consumed per mouse was then obtained by dividing by the number of mice.

Assays for Biochemical Parameters and Plasma Estradiol Levels

Blood samples were obtained from the dorsal tail vein. Plasma glucose and insulin concentrations and liver triglyceride content were determined by the glucose oxidase method, using enzyme-linked immunosorbent assays (ELISA; Morinaga Institute of Biological Science, Yokohama, Japan) and a Triglyceride Quantification Kit (BioVision, Mountain View, CA), respectively, according to the manufacturer’s protocols. Fasting plasma monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP) levels were measured by Mouse CCL2/JE/MCP-1 Immunoassay (R & D Systems, Minneapolis, MN) and Mouse High-Sensitive CRP ELISA (Kamiya Biomedical, Seattle, WA) for dams at 3 wk after pregnancy. Additionally, fasting plasma estradiol levels were measured by competitive Estradiol ELISA Kit (Cayman Chemical, Ann Arbor, MI) for offspring at 20 wk according to the manufacturer’s protocol.

Intraperitoneal Glucose Tolerance Tests and Insulin Tolerance Test

Glucose tolerance was assessed at 6, 14, and 20 wk following a 16-h fast. Offspring were administered glucose intraperitoneally (2 g/kg body wt) and plasma glucose and insulin levels determined. The area under the curve was calculated by the trapezoidal rule. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined as described previously (4, 31). For the insulin tolerance test (ITT), offspring were injected with 0.5 U/kg of human biosynthetic insulin (Novo Nordisk, Bagsvaerd, Denmark) at 14 and 20 wk, and blood glucose levels were measured.

Histological Analysis and Immunohistochemistry of Islet

The average area of islets from each group of mice was determined at 20 wk. Briefly, the pancreas was removed and fixed in 10% formaldehyde and embedded in paraffin. Five-micrometer-thick paraffin sections were cut, deparaffinized, and stained with hematoxylin and eosin (H & E). Every 30th section (10 sections/pancreas), including representative sections of the head, body, and tail of the pancreas, was used. Every islet from one section on each slide was viewed using a bright-field illumination microscope (model BZ-9000; Keyence, Osaka, Japan). Average area of >200 islets/mouse was evaluated. Immunostaining for 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was carried out as described previously (24).

Histological Analysis of Liver and White Adipose Tissue

Liver sections were prepared and stained with H & E. Fresh-frozen livers in Tissue-Tek OCT compound (Sakura Finetechnical, Tokyo, Japan) were sectioned and stained with hematoxylin and eosin (H&E) (Bio-Optica, Milan, Italy) and Oil Red O (Sigma-Aldrich, St. Louis, MO) for lipid accumulation.
Japan) were stained with Oil Red O to identify neutral lipids, as described previously (25). Adipose tissue was collected and weighed from the intra-abdominal perigonadal fat pad, which others have shown to be metabolically significant in regard to adipocyte biology and inflammation (41). Bilateral perigonadal fat pads were dissected and weighed, and fat pad weight was calculated as a percentage of body weight. To measure adipocyte area (46), formalin-fixed, paraffin-embedded adipose tissue sections were stained with H&E, and stained images of 100 adipocytes in the adipose tissue of each mouse were used for quantitative evaluation by microscopy. Immunostaining of F4/80, a marker of tissue macrophages, was carried out for adipose tissue (45). Briefly, after endogenous peroxidase activity was blocked, deparaffinized adipose sections were incubated with the primary antibody rat monoclonal anti-mouse F4/80 antibody (4.0 μg/ml).

Fig. 1. Experimental protocol and body weight in offspring. A: experimental protocol. One week after arrival, C57/BL6J mice were allowed to breed naturally and fed either control diet (CD) or high-fat diet (HFD) during gestation and lactation. After weaning, all pups were fed CD from 4 to 6 wk of age and then fed CD or HFD from 6 to 20 wk of age, resulting in 4 groups of littersmates: CD-CD, CD-HFD, HFD-CD, and HFD-HFD combinations of maternal and offspring diets. B: fasting plasma monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP) levels in dams at 3 wk after pregnancy. Results are expressed as means ± SE; n = 8 mice/group. *P < 0.05; **P < 0.01 vs. CD-fed dams. C: body weight at birth. CD represents offspring of CD-fed dams, and HFD represents offspring of HFD-fed dams. Results are expressed as means ± SE; n = 4 littersmates/group. D: changes in body weight in offspring of CD-fed dams (○) and those of HFD-fed dams (■) between 4 and 6 wk. Results are expressed as means ± SE; n = 12 mice/group. *P < 0.05; **P < 0.01 vs. offspring of CD-fed dams. E: changes in body weight in CD-CD (C-C; ○), CD-HFD (C-H; □), HFD-CD (H-C; ●), and HFD-HFD (H-H; ■) between 6 and 20 wk; n = 6 mice/group. *P < 0.05; **P < 0.01 vs. female CD-HFD. NS, not significant.
RESULTS

Dams

Body weight, fasting blood glucose levels, and food consumption in HFD-fed dams were greater than in CD-fed dams during gestation (Table 2). In addition, fasting plasma MCP-1 and CRP levels increased significantly in HFD-fed dams compared with CD-fed dams at 3 wk after pregnancy (Fig. 1B). However, there were no significant differences in occasional blood glucose levels between CD-fed and HFD-fed dams (data not shown).

Offspring

Effect of maternal HFD on body weight and food consumption in offspring. Body weight in males and females of HFD-fed dams was greater than those of CD-fed dams at birth (Fig. 1C) and from 4 to 6 wk (Fig. 1D) despite the same CD consumption at 6 wk (Table 3). From 6 to 20 wk, there was a significant difference in body weight between CD-HFD and HFD-HFD females while on maternal HFD (Fig. 1E). Similar results were observed in food consumption at 14 and 20 wk (Table 3).

Effect of maternal HFD on glucose tolerance and insulin resistance in offspring. To test whether maternal HFD affects glucose tolerance in offspring, we performed intraperitoneal glucose tolerance test (IPGTT) at 6, 14, and 20 wk. At 6 wk, after a bolus injection of glucose, blood glucose levels were significantly higher in offspring of HFD-fed dams than in those of CD-fed dams (Fig. 2, A and B). In addition, HOMA-IR was also markedly increased in offspring of HFD-fed dams (Table 3). At 14 and 20 wk, IPGTT showed that blood glucose levels were higher in maternal HFD in males (Fig. 2A), whereas they were higher in HFD-fed dams than in CD-HFD females (Fig. 2B). HOMA-IR also increased in maternal HFD (Table 3).

Additionally, to test whether maternal HFD affects insulin sensitivity in offspring, we performed ITT at 14 and 20 wk. As shown in Fig. 2, C and D, insulin sensitivity was markedly worse in maternal HFD at both 14 and 20 wk.

Effect of maternal HFD on liver steatosis in offspring. To determine whether maternal HFD can cause peripheral insulin resistance, such as adipose tissue inflammation and liver steatosis in adult offspring, we investigated liver morphology and triacylglycerol content. H & E staining of the liver revealed a severe steatosis, with almost all cells affected and male HFD-
Table 3. Fasting blood glucose, HOMA-IR, fat pad weight, and food consumption in offspring at 6, 14, and 20 wk

<table>
<thead>
<tr>
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<th>Offspring of CD-Fed Dams</th>
<th>Offspring of HFD-Fed Dams</th>
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<tbody>
<tr>
<td><strong>Males at 6 wk</strong></td>
<td></td>
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<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>83.1 ± 6.1</td>
<td>95.7 ± 3.0^*</td>
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<tr>
<td>HOMA-IR</td>
<td>1.39 ± 0.18</td>
<td>3.21 ± 0.58^*</td>
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<tr>
<td>Fat pad weight, %</td>
<td>1.35 ± 0.11</td>
<td>1.81 ± 0.10^*</td>
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<tr>
<td>Food consumption, kcal/day</td>
<td>10.1 ± 0.56</td>
<td>9.3 ± 0.22</td>
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<tr>
<td><strong>Females at 6 wk</strong></td>
<td></td>
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<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>80.0 ± 2.9</td>
<td>88.1 ± 5.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.52 ± 0.20</td>
<td>3.61 ± 1.47^*</td>
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<tr>
<td>Fat pad weight, %</td>
<td>0.72 ± 0.13</td>
<td>1.26 ± 0.11^*</td>
</tr>
<tr>
<td>Food consumption, kcal/day</td>
<td>7.8 ± 0.47</td>
<td>7.3 ± 0.24</td>
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| **Males at 14 wk**   |                          |                           |
| Fasting blood glucose, mg/dl | 80.7 ± 10.7             | 131.3 ± 13.2^f            |
| HOMA-IR              | 0.78 ± 2.16              | 3.94 ± 2.41               |
| Food consumption, kcal/day | 9.9 ± 0.73               | 14.2 ± 0.99^-g            |
| **Females at 14 wk** |                          |                           |
| Fasting blood glucose, mg/dl | 72.6 ± 5.2               | 101.6 ± 5.2^c             |
| HOMA-IR              | 0.47 ± 0.47              | 2.22 ± 0.50^-g            |
| Food consumption, kcal/day | 9.0 ± 1.1               | 14.9 ± 0.89^-g            |

| **Males at 20 wk**   |                          |                           |
| Fasting blood glucose, mg/dl | 107.3 ± 12.6             | 153.5 ± 15.4^b            |
| HOMA-IR              | 4.52 ± 1.84              | 13.4 ± 2.60^-g            |
| Fat pad weight, %    | 1.6 ± 0.4                | 4.2 ± 0.43^-d             |
| Food consumption, kcal/day | 9.7 ± 0.61               | 14.7 ± 0.77^-g            |
| **Females at 20 wk** |                          |                           |
| Fasting blood glucose, mg/dl | 107.3 ± 12.6             | 153.5 ± 15.4^-c           |
| HOMA-IR              | 3.32 ± 1.36              | 5.55 ± 1.44               |
| Fat pad weight, %    | 1.3 ± 0.2                | 5.2 ± 0.2^-e              |
| Food consumption, kcal/day | 9.7 ± 4.1                | 21.1 ± 4.3               |

Data are means ± SE; n = 6 mice/group. HOMA-IR, homeostasis model assessment of insulin resistance. ^p < 0.05 vs. CD-fed dams (paired t-test); ^p < 0.05; ^p < 0.01 vs. CD-CD (ANOVA); ^p < 0.05; ^p < 0.01 vs. CD-HFD (ANOVA); ^p < 0.05; ^p < 0.01 vs. HFD-CD (ANOVA). Nonsignificant P values are not indicated.

HFD containing large fat vacuoles, whereas staining of female HFD-HFD showed a mild to moderate steatosis (Fig. 3A) (45). Oil Red O staining of the liver also revealed a dramatic increase in lipid staining in male HFD-CD and HFD-HFD, whereas females displayed increased lipid staining in HFD-HFD (Fig. 3B). Furthermore, liver triacylglycerol content also increased in maternal HFD in both males and females (Fig. 3C).

Effect of maternal HFD on adipose tissue inflammation, adipocyte area, and fat pad weight in offspring. To investigate the effect of maternal HFD on adipose tissue inflammation, we conducted several studies in WAT. At 6 and 20 wk, fat pad weight markedly increased in maternal HFD (Table 3). Similarly, the average adipocyte area increased in maternal HFD at 20 wk (Fig. 4, A–C). We next examined mRNA levels of TNFα and IL-6, proinflammatory markers produced by macrophages and adipocytes (46). We also examined MCP-1 (a member of the small inducible cytokine family, which plays a role in monocyte recruitment to sites of injury and infection) (53), F4/80, and CD11c (a marker of M1 macrophages). All assays were conducted on 20-wk white adipose tissue (WAT) using RT-PCR.

As shown in Fig. 4, D–F, mRNA levels of TNFα, MCP-1, and CD11c were significantly higher in HFD than in CD-HFD and slightly but not significantly higher in HFD-CD than in CD-CD. Similarly, IL-6 and F4/80 mRNA levels increased in HFD-HFD compared with CD-HFD in males, whereas those mRNA levels were slightly but not significantly increased in HFD-HFD compared with CD-HFD in females (Fig. 4, G and H). Tissue macrophages in WAT were evaluated by immunostaining of F4/80, a classical macrophage-restricted surface glycoprotein (53). F4/80 immunohistochemical analysis showed a significant increase in F4/80 crown-like structures in maternal HFD compared with maternal CD (Fig. 4).

Effect of maternal HFD on plasma insulin levels in offspring. To test whether maternal HFD affects plasma insulin levels in their offspring, we determined insulin levels by the IPGTT. At 6 wk, plasma insulin levels were higher at 0 and 30 min in males of HFD-fed dams (Fig. 5). At 14 wk, insulin levels were higher in maternal HFD (Fig. 5, A and B). Moreover, the proportion of small islet areas (<5,000 μm²) increased in maternal HFD in males, whereas it increased in HFD-HFD compared with CD-HFD in females (Fig. 6).

Effect of maternal HFD on islet area. We also assessed the impact of maternal HFD on islet area at 20 wk. Consistent with the insulin level data, the average islet area decreased in maternal HFD in males, whereas it increased in maternal HFD compared with CD-HFD in females (Fig. 6).
Fig. 2. Effects of maternal HFD on blood glucose in offspring based on intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT). A and B: changes in blood glucose levels and the area under the glucose concentration curve (AUC) after an IPGTT at 6, 14, and 20 wk in males (A) and females (B). C and D: changes in blood glucose levels after an ITT at 14 and 20 wk in males (C) and females (D). Blood glucose levels are expressed as % basal blood glucose levels. CD (△) represents offspring of CD-fed dams, and HFD (▲) represents offspring of HFD-fed dams at 6 wk. C-C (○), C-H (□), H-C (●), and H-H (■). Results are expressed as means ± SE; n = 6 mice/group. *P < 0.05; **P < 0.01.
regulator of insulin promoter activity, were evaluated in isolated islets. Similarly, islet PDX-1 mRNA levels decreased in maternal HFD in males, whereas they increased in maternal HFD in females (Fig. 6).

Effect of maternal HFD on oxidative stress in islets and on plasma estradiol levels in offspring. To address the potential effect of oxidative stress on the sex differences observed in pancreatic β-cells, we evaluated oxidative stress in islets. First, we measured expression of NAD(P)H oxidase gp91phox, a major source of superoxide production in isolated islets (24).

![Image of oxidative stress measurement](image_url)

The mRNA levels of gp91phox in islets increased in HFD-HFD compared with CD-HFD in males, whereas there were no significant differences in females (Fig. 7A). Similar results were observed for mRNA levels of NOX4, another NAD(P)H oxidase component gene (Fig. 7B), and Gpx, an oxidative stress-related gene (Fig. 7C). Furthermore, islet oxidative stress was evaluated by immunostaining of 8-OHdG. As shown in Fig. 7, D and E, staining intensity of 8-OHdG was significantly higher in maternal HFD in males, whereas there were no intensity differences in 8-OHdG in females. To evaluate...

Fig. 3. Effects of maternal HFD on liver steatosis in C-C, C-H, H-C, and H-H at 20 wk. A and B: hematoxylin and eosin (H & E; A) and Oil Red O (B) staining of liver sections. Scale bar, 100 µm. Original magnification, ×200. C: liver triacylglycerol content. Data are expressed as means ± SE; n = 6 mice/group. *P < 0.05; **P < 0.01.
Fig. 4. Effects of maternal HFD on adipose tissue inflammation in C-C, C-H, H-C, and H-H at 20 wk. A and B: average area of adipocytes. Representative photomicrographs (A) and average area (B) of adipose tissue sections stained with H&E. Staining images of 100 adipocytes in the adipose tissue of each mouse were used for quantitative evaluation. Results are expressed as means ± SE. Scale bar, 100 μm; n = 3 mice/group. Original magnification, ×200. C: histogram of adipocyte area. Staining images of 100 adipocytes in the adipose tissue of each mouse were used for quantitative evaluation. C-C (○), C-H (□), H-C (●), and H-H (●); n = 3 mice/group. D–H: the mRNA expression levels of TNFα, MCP-1, CD11c, IL-6, and F4/80 in WAT. Total RNA was extracted from white adipose tissue (WAT). The mRNA levels were measured by real-time RT-PCR and normalized to β-actin levels. Results are expressed as means ± SE percentages of the levels in C-C; n = 6 mice/group. I: detection of tissue macrophages in adipose tissue evaluated by immunostaining of F4/80. Representative photomicrographs of adipose tissue sections from animals as labeled and immunostained with anti-F4/80 antibody. Arrows, crowns surrounding adipocytes. Scale bar, 100 μm. Original magnification, ×200. *P < 0.05; **P < 0.01.
whether a correlation between estradiol and pancreatic β-cell function exists, we performed plasma estradiol measurements in offspring at 20 wk. Overall, plasma estradiol levels were lower in males than in females and decreased in offspring fed HFD. Additionally, they were also decreased in maternal HFD (Fig. 7F).

**DISCUSSION**

It has been shown previously that maternal HFD during gestation and lactation causes glucose intolerance, insulin resistance, hepatic steatosis, and adipose tissue inflammation. In the present study, we have confirmed and extended these observations. We also found sex differences in pancreatic β-cell function and suggest that oxidative stress may play an important role in the sex differences observed. Furthermore, we found a possible correlation between estradiol and pancreatic β-cell function and suggest that estrogens (estradiol) may protect pancreatic β-cells from oxidative injury in female.

Pregnant HFD-fed dams were obese and had increased adiposity, although they did not develop an overt diabetic state. Interestingly, our results show that males and females are programmed differently in body weight at 20 wk. Although males had no difference in body weight at 20 wk, maternal HFD can cause not only glucose intolerance and insulin resistance but also liver steatosis and adipose tissue inflammation in offspring. These findings are partly consistent with previous reports indicating that mice offspring on HFD during fetal life had increased pancreatic β-cell mass, replication, and neogenesis, leading to hyperglycemia in adult life (17). In contrast, and extending previous observations, our results suggest that males and females are programmed differently with respect to insulin levels, and females may be protected from deficient insulin levels and deterioration of pancreatic β-cell function.

Recent data from human and animal studies suggest that the ovarian estrogen estradiol protects insulin production in diabetic states. First, although there are more elderly women than men with diabetes, diabetes prevalence is higher in men (52). Second, in most diabetes rodent models, females are protected from β-cell death and hyperglycemia. Conversely, males develop overt insulin-deficient diabetes (30). Furthermore, when used in pharmacological concentrations, estradiol protects human pancreatic islets from apoptosis induced by proinflammatory cytokines in vitro (6). The mechanism underlying the effects of estrogen on insulin level is uncertain. Estrogen receptors ERα and ERβ are found in pancreatic β-cells and are important molecules involved in glucose metabolism (1), but their role in pancreatic β-cell physiology is still poorly understood. However, direct effects of estrogen on the pancreas via steroid receptors or indirect effects via estrogen-induced glucagon antagonism and subclinical increases in glucocorticoids and growth hormone could all contribute to preserve insulin response to glucose (18). Further studies will be needed to resolve these possibilities and to establish how these effects are mediated at the molecular level.

Interestingly, our results are also supported by studies that have demonstrated that HFD exposure decreases plasma estradiol levels in diet-induced obese (DIO) animals (2, 5). The reason for the reduction in plasma estradiol levels is not clear.

Fig. 5. Effects of maternal HFD on insulin secretion after an IPGTT in C-C, C-H, H-C, and H-H at 6, 14, and 20 wk. A: changes in plasma insulin levels after an IPGTT at 6 wk. CD (○) represents offspring of CD-fed dams, and HFD (●) represents offspring of HFD-fed dams. B and C: changes in plasma insulin levels after an IPGTT in C-C (○), C-H (●), H-C (■), and H-H (▲) at 14 (B) and 20 wk (C). D: AUC after an IPGTT at 14 and 20 wk. Results are expressed as means ± SE; n = 6 mice/group. *P < 0.05; **P < 0.01.
However, Balasubramanian et al. (2) reported that HFD exposure increases serum leptin levels in the DIO group and that elevated leptin levels can impair ovulation and cause a reduction in estradiol synthesis in the ovary, resulting in inversely decreased estradiol levels. Further studies are needed to prove that HFD-induced increase in leptin levels is responsible for the decrease in estradiol levels. Considering that estrogens protect against HFD-induced insulin resistance and glucose intolerance (39), our results suggest that decreased plasma estradiol levels in male offspring in maternal HFD may contribute to the mechanism of sex differences in pancreatic β-cell function.

Since chronic oxidative stress decreases insulin gene expression via downregulation of PDX-1 and accelerates β-cell apoptosis (40), we evaluated oxidative stress in islets to investigate the mechanism underlying the sex differences in pancreatic β-cell function. Because our results showed that oxidative stress in islets increased in maternal HFD only in males, one potential mechanism for the effects of maternal HFD on glucose intolerance in adult offspring with sex differences might be due, at least partly, to increases in oxidative stress in male islets. Considering that estrogens may protect pancreatic β-cells from oxidative injury and prevent glucose intolerance (21, 27), these results suggest that females might be protected from insulin deficiency by inhibiting oxidative stress despite glucose intolerance, whereas, conversely, males develop overt insulin-deficient diabetes.

Fig. 6. Effects of maternal HFD on islet area, insulin content, and pancreatic and duodenal homeobox factor-1 (PDX-1) mRNA levels in isolated islets in C-C, C-H, H-C, and H-H at 20 wk. A and B: average islet area. Representative photomicrographs (A) and average area (B) of pancreatic islet sections stained with H & E. Staining images of every islet in 10 consecutive cross-sections, including representative sections of the head, body, and tail in the pancreas of each mouse, were used for quantitative evaluation. An average area of >200 islets/mouse was evaluated. Scale bar, 100 μm. Results are expressed as means ± SE; n = 4 mice/group. C: the proportion of islet areas; n = 4 mice/group. D and E: insulin content evaluated by ELISA (D) and mRNA expression levels of PDX-1 (E) in isolated islets. Total RNA was extracted from isolated islets, and insulin levels were adjusted for DNA content (D). The mRNA levels were measured by real-time RT-PCR and normalized to β-actin levels (E). Results are expressed as means ± SE percentages of the levels in C-C; n = 6 mice/group. *P < 0.05; **P < 0.01.
Our study suggests that not only undernutrition in dams during pregnancy but also overnutrition may produce adult offspring at risk of developing diabetes. This finding is consistent with previous reports in which meta-regression and categorical analyses showed a significant U-shaped relation between birth weight and later-life risk of type 2 diabetes (20). From a public health point of view, it is important to remember that adult diseases are not programmed, but the tendency toward these unfavorable health outcomes does seem to be programmed. This developmental model of the origins of chronic disease may offer a new way forward. The mechanisms responsible for these effects are not clearly understood but may represent an area of intense research spanning animal models to human epidemiological studies and, more recently, epigenetic research (29). Because epigenetic modifications provide a mechanism that allows the stable propagation of gene activity states from one generation of cells to the next (7), the intrauterine environment of diabetic or obese women may influence the epigenome of the offspring and lend support to further studies in this area. For example, Park et al. (36) reported that Pdx-1 expression is permanently reduced in intrauterine growth retardation β-cells and that CpG islands in...
the proximal promoter are methylated after adult-onset diabetes, resulting in permanent silencing of the Pdx-1 locus. Recently, Ehara et al. (8) showed that maternal HFD leads to decreased glycerol-3-phosphate acyltransferase-1 (GPAT1; Gpam) promoter methylation, with increased GPAT1 expression and triacylglyceride content in the pup liver. Because GPAT1 plays an important role in the regulation of triacylglyceride biosynthesis and GPAT1 expression levels are highest in adipose tissue and liver (47), we asked whether maternal HFD may have an effect on GPAT1 expression and Gpam promoter methylation in adipose tissue between CD-CD and HFD-CD at 20 wk. Although GPAT1 mRNA expression increased in HFD-CD compared with CD-CD only in males, there were no significant differences in Gpam promoter methylation between the two groups (data not shown). However, because DNA methylation and histone modifications represent the major epigenetic mechanisms implicated in regulating gene transcription, additional epigenetic studies are needed to explore the mechanisms through which maternal HFD influences growth, body composition, and glucose metabolism in the offspring.

In conclusion, after confirming the previously reported results in which maternal HFD during gestation and lactation caused glucose intolerance, insulin resistance, hepatic steatosis, and adipose tissue inflammation, we have demonstrated for the first time that maternal HFD induces marked sex differences in pancreatic β-cell function in offspring. In addition, our results suggest that potential mechanisms underlying sex differences in pancreatic β-cell function may be related partially to increases in oxidative stress in male islets. Furthermore, we found a possible correlation between estradiol and pancreatic β-cell function and suggest that estradiol may protect pancreatic β-cells from oxidative injury in females. Appropriate interventions may have long-term multigenerational effects that reduce the risk of chronic disease and could benefit the child, the mother, her future pregnancies, and subsequent generations.

GRANTS

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DISCLOSURES

The authors report no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS


REFERENCES


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