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A novel model of type 2 diabetes mellitus based on obesity induced by high-fat diet in BDF1 mice

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Abstract

For experimental research on type 2 diabetes mellitus, a diet-induced obesity-dependent diabetes model developed using genetically normal animals is essential. However, attempts at feeding a high-fat diet (HFD) to major inbred strains of mice have not resulted in the establishment of an ideal model. Here, we show that BDF1 mice, the F_1 hybrids of C57BL/6 and DBA/2 normal strains, develop HFD-induced obesity-dependent diabetes. BDF1 mice fed a HFD gained weight rapidly and developed severe diabetes characterized by hyperglycemia, glucosuria, and elevation of hemoglobin A_{1C} levels in 3 to 4 months. The glucose tolerance of the diabetic mice was significantly impaired, and the elevation of plasma insulin after a glucose load was significantly reduced. Isolated pancreatic islets of HFD-fed BDF1 mice showed decreased insulin content and a reduced insulin secretory response to higher concentrations of glucose. Immunohistochemical analysis of the pancreas showed reduced staining intensity to insulin and aberrant distribution of glucagon-positive cells in diabetic BDF1 mice. These observations suggest the cause of the diabetes in HFD-fed BDF1 mice to be dysfunction of the pancreatic β-cells, which do not produce or secrete enough insulin to compensate for insulin resistance. BDF1 mice fed a HFD showing obesity-dependent diabetes are suggested to be an appropriate animal model of type 2 diabetes mellitus. This model would be useful for exploring the mechanism of obesity-dependent type 2 diabetes mellitus and evaluating antiobesity and antidiabetic drugs.

1. Introduction

Although the pathogenesis of type 2 diabetes mellitus is known to be complex, previous studies have shown that its process involves both polygenetic and environmental factors [1-3]. Until now, *db*/*db* mice and ZDF rats have been used in many studies as experimental models for type 2 diabetes mellitus. These models are reported to be useful for pharmacologic and genetic studies [4,5], but both models have some limitations on its pathogenesis of diabetes because both are monogenic models [6] and their diabetes is not based on environmental factors. Thus, their pathogenesis of diabetes may be quite far from that of clinically observed diabetes.

Recent epidemiologic studies indicated that the consumption of a high-caloric diet and the ensuing obesity are two of the principal causes of type 2 diabetes mellitus [3]. Thus, it would be worthwhile to establish an obesity-induced experimental model of type 2 diabetes mellitus by feeding a high-caloric diet to normal animals. Indeed, there are some reports about experimental models of obesity-induced diabetes; and to our knowledge, C57BL/6 mice fed a high-fat diet (HFD) are a representative model of obesity-dependent diabetes [7,8]. However, the utility of this model seems to be limited because the mice's expressed symptoms of diabetes are rather modest compared with those of the conventional genetic models; and more importantly, the glucose metabolism disorders in these mice are largely attributable to insulin resistance, and involvement of defects in insulin secretion is limited.

A number of clinical studies have indicated the importance of insulin secretory capability in the development and progression of type 2 diabetes mellitus [9,10]. As is the case with humans, the insulin secretory capability of inbred mice depends on their genetic backgrounds; C57BL/6 mice had a large capability of insulin secretion, whereas

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DBA/2 mice had a much smaller capability because of their higher susceptibility to β-cell dysfunction [11].

Taken together, the previous reports above suggested that DBA/2 mice fed an HFD would be a model of type 2 diabetes mellitus; however, there is one problem: DBA/2 mice are known to be rather resistant to HFD-induced obesity [12]. In the present study, to resolve this issue, we fed an HFD to BDF1 mice, F_1 hybrid mice of C57BL/6 and DBA/2. Our hypothesis is that DBA/2 mice may develop obesity-dependent diabetes when the obesity-prone characteristics are incorporated by breeding with C57BL/6 mice. Finally, we established a novel model of obesity-dependent type 2 diabetes mellitus that exhibits insulin resistance and distinctive defects in insulin secretion.

2. Materials and methods

2.1. Animals

Male BDF1 mice ([C57BL/6N × DBA/2N]:F_1), C57BL/6N mice, and DBA/2N mice (Charles River Japan, Yokohama, Japan) were purchased at 6 weeks of age and kept in a temperature- and humidity-controlled facility. The mice were housed in groups (6-8 mice per cage) and were given water and food ad libitum throughout the study except for during an oral glucose tolerance test, in which the mice were fasted overnight. All animal experiments were carried out in accordance with the guidelines provided by the Institutional Animal Care and Use Committee of Daiichi Sankyo (Tokyo, Japan).

2.2. Diet

The FR-2 pellet diet (Funabashi Farm, Funabashi, Japan) was used as the regular diet (control) and had a calorie ratio of protein-fat-carbohydrate of 23.2:12.0:64.8 with a metabolic calorie content of 3.6 kcal/g. An HFD was made by mixing FR-2 powder and lard (Funabashi Farm) at a ratio of 3:1 by weight; the HFD had a calorie ratio of protein-fat-carbohydrate of 12.6:52.1:35.3, with a metabolic calorie content of 4.9 kcal/g.

2.3. Feeding and monitoring mice

After the mice were habituated for a week, the feeding experiment was started. Mice were divided into the control and HFD groups, and the development of obesity and diabetes was monitored every 2 or 4 weeks. Blood and urine were collected between 9:00 and 10:30 AM from the mice in a nonfasting condition, if not otherwise specified. At 14 to 18 weeks of feeding, selected or whole groups of mice were used for several studies.

2.4. Oral glucose tolerance test

Overnight-fasted mice were given a glucose solution orally (2.0 g/kg); and blood was collected from a tail vein at 0, 10, 15, 30, 45, and 60 minutes after the glucose load.

2.5. Plasma and urine chemistry

Plasma and urine glucose, plasma free fatty acids, triglyceride, and cholesterol levels were determined using the commercially available diagnostic kits (Wako Chemicals, Osaka, Japan). Enzyme immunoassay kits for mouse insulin, mouse leptin (Morinaga Institute of Biological Science, Yokohama, Japan), and mouse adiponectin (Otsuka Pharmaceutical, Tokyo, Japan) were used to measure plasma hormone levels. The lower limit of detection of insulin immunoassay was 0.16 ng/mL, and all samples were measured above this value. Hemoglobin A_{1C} (HbA_{1C}) levels were determined with a DCA 2000 system (Bayer Medical, Tokyo, Japan).

2.6. Isolation of pancreatic islets

The mice were decapitated, and the pancreata were perfused via the common bile duct with 2.5 mL of Hanks balanced salt solution containing 4 mg/mL of type XI collagenase (Sigma Aldrich Japan, Tokyo, Japan) and 2 mg/mL of bovine serum albumin. The pancreata were dissected, incubated for 3.5 minutes at 37°C, and dispersed in 30 mL of Hanks balanced salt solution containing 2 mg/mL of bovine serum albumin. After 2 minutes of incubation on ice, the precipitated islets were manually picked up under a stereoscopic microscope.

2.7. Insulin secretion from isolated islets

After 30 minutes of equilibration with Krebs-Ringer bicarbonate buffer containing 5.6 mmol/L glucose at 37°C, 5 islets per assay were incubated with different concentrations of glucose (5.6, 11.1, and 22.2 mmol/L) and a high concentration of potassium (50 mmol/L) for 60 minutes. The supernatant was collected, and the insulin concentration was measured. The remaining islets were homogenized by sonication, and the DNA content was determined with a PicoGreen DNA quantitation kit (Molecular Probe, Eugene, OR). To standardize the insulin secretion from pancreatic islets of different sizes, insulin secretion was expressed as nanogram of insulin per nanogram of DNA of the islets used [13].

2.8. Insulin content of islets

Five islets per assay were homogenized by sonication, and the DNA content was determined. Aliquots of homogenates were incubated overnight with 4 vol of acid-ethanol solution (EtOH-H_2O-HCl = 150:7:3), and the insulin concentrations were measured.

2.9. Histologic analysis of pancreas

Whole pancreata were fixed in Bouin solution (saturated picric acid–formalin–acetic acid = 15:5:1) and were embedded in paraffin according to the standard protocol. Serial sections (3 μm thick) were stained for insulin and glucagon with a rabbit anti-insulin (H-86) antibody (SC-

9168; Santa Cruz Biotechnology, Santa Cruz, CA) and a rabbit anti-glucagon antibody (18-0064; Zymed, South San Francisco, CA) as primary antibodies in dilutions of 1:300 and 1:1000, respectively. Biotinylated goat anti-rabbit immunoglobulin G (BA-1000; Vector Laboratories, Burlingame, CA) at the dilution of 1:200 was used as a secondary antibody. The dye was developed with a Vectastain Elite ABC kit (PK-6100, Vector Laboratories) and aminoethylcarbazol (00-2007, Zymed). Four animals per group were examined for quantitative analyses of staining density. Images of randomly assigned 12 islets per each section were captured, and staining densities were analyzed by Image J image processing software (National Institutes of Health, Bethesda, MD).

2.10. Statistical analysis

The statistical significance for the differences between the 2 groups was tested by Student or Welch *t* test after the F test. The statistical significance for the differences among the 3 groups was tested by Dunnett test.

3. Results

3.1. HFD-induced obesity in BDF1 mice

There was no statistically significant difference in initial body weights between control and HFD-fed groups (Table 1). During 14 weeks of HFD feeding, the HFD-fed BDF1 mice became severely obese compared with the control mice (Fig. 1A). The difference in body weight between the 2 groups reached 10.7 g at 8 weeks of feeding and was maintained thereafter. Autopsy at 14 weeks of feeding showed that weights of both mesenteric and inguinal fat and the liver were significantly increased in HFD-fed mice (Fig. 1B).

Plasma leptin and insulin levels of the HFD-fed mice were significantly higher than those of the control mice (Table 1), coincident with the increase in fat tissue and resulting insulin resistance. In contrast, changes in plasma adiponectin levels were minimal throughout the study. Among plasma lipids, total cholesterol levels were significantly elevated in HFD-fed mice, whereas triglyceride levels were significantly decreased. Plasma levels of free fatty acid remained almost unchanged in both groups.

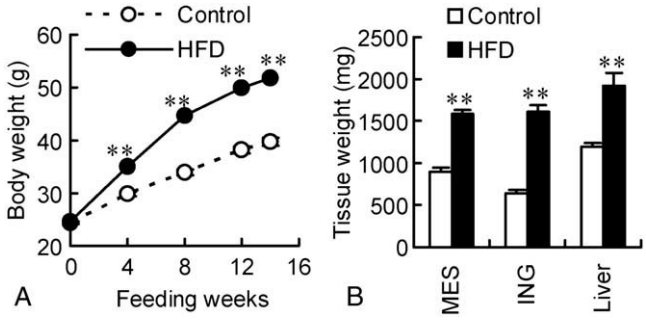

Fig. 1. High-fat diet–induced obesity in BDF1 mice. A, Body weight changes of control (open circle) and HFD-fed (closed circle) mice. B, Tissue weights of control (open bar) and HFD-fed (closed bar) mice at autopsy after 14 weeks of feeding. Data are the means ± SEM of 16 animals. $^{*}P < .05$, $^{**}P < .01$ vs control group. MES indicates mesenteric fat; ING, inguinal fat.

3.2. Development of diabetes in HFD-fed BDF1 mice

In parallel with the development of obesity, the HFD-fed BDF1 mice developed severe diabetes. Although none of the control mice showed plasma glucose levels greater than 230 mg/dL throughout the study, the HFD-fed BDF1 mice started to show hyperglycemia at 8 weeks of feeding; and the average plasma glucose level increased continuously and reached 335 ± 24 mg/dL (mean ± SEM) at 14 weeks of feeding (Fig. 2A). By that point, 11 of 16 mice (69%) showed hyperglycemia with glucose levels greater than 300 mg/dL. Urinary glucose levels also increased in the HFD-fed mice depending on the degree of hyperglycemia (Fig. 2B); and at 14 weeks of feeding, 9 of 11 hyperglycemic mice (82%) showed glucosuria with glucose levels greater than 50 mg/dL. The levels of HbA_{1C}, which represent

Table 1
Body weights and metabolic parameters of BDF1 mice before and after 14 weeks of a feeding study with control diet and HFD

	Control diet (n = 16)		HFD (n = 16)	
	0 wk	14 wk	0 wk	14 wk
Body weight (g)	24.4 ± 0.2	39.8 ± 0.8	24.6 ± 0.2	51.8 ± 0.7†
Plasma glucose (mg/dL)	188 ± 4	179 ± 4	188 ± 5	335 ± 24†
Urinary glucose (mg/dL)	ND	13 ± 1	ND	1596 ± 891†
Plasma total cholesterol (mg/dL)	125 ± 4	130 ± 3	124 ± 3	229 ± 2†
Plasma free fatty acid (mEq/L)	1.00 ± 0.04	1.02 ± 0.06	0.86 ± 0.05*	0.87 ± 0.04
Plasma triglyceride (mg/dL)	181 ± 8	172 ± 11	158 ± 14	91 ± 5†
Plasma insulin (ng/mL)	1.22 ± 0.10	5.00 ± 1.04	1.06 ± 0.17	30.7 ± 4.4†
Plasma leptin (ng/mL)	3.8 ± 0.5	25.4 ± 2.5	2.2 ± 0.37*	70.9 ± 2.5†
Plasma adiponectin (μg/mL)	11.7 ± 0.5	12.8 ± 0.4	10.7 ± 0.3	11.2 ± 0.5*

Data are the means ± SEM. ND indicates not determined.
* $P < .05$, $^{\dagger}P < .01$ vs control diet.

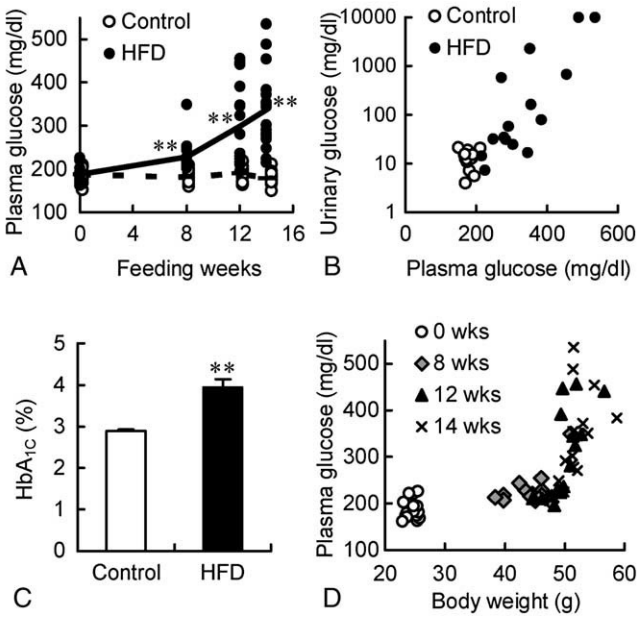

Fig. 2. High-fat diet–induced diabetes in BDF1 mice. A, Time course of individual plasma glucose levels in control (open circle, n = 16) and HFD-fed (closed circle, n = 16) mice. The mean changes in the control (dotted line) and HFD-fed (solid line) mice are also shown. $^{**}P < .01$ vs control. B, Relationship between plasma glucose and urinary glucose at 14 weeks of feeding. C, Hemoglobin A_{1C} concentrations at 14 weeks of feeding. Data are the means ± SEM (n = 16). $^{**}P < .01$ vs control. D, Relationship between body weight and plasma glucose levels in HFD-fed mice at successive time points (n = 16). Data at 0 (circles), 8 (diamonds), 12 (triangles), and 14 (crosses) weeks of feeding are plotted.

average blood glucose levels over the previous several weeks, were significantly elevated in the HFD-fed mice (Fig. 2C). The relation between body weight and plasma glucose levels of the HFD-fed mice at successive time points are shown in Fig. 2D. The steep increase in plasma glucose levels when the body weight is around 48 to 50 g indicates that the development of diabetes was dependent on the severity of obesity rather than the length of HFD feeding.

3.3. Impairment of glucose-induced insulin secretory response in diabetic BDF1 mice

To investigate the pathologic condition of diabetes in the HFD-fed BDF1 mice, glucose tolerance was examined at 14 to 18 weeks of feeding. Because the HFD-fed BDF1 mice varied in their body weights and the developmental status of diabetes, we divided the HFD-fed mice into 2 groups: diabetic mice that had already developed diabetes, and nondiabetic mice that had not yet developed diabetes. We diagnosed the mice with nonfasting plasma glucose levels greater than 300 mg/dL as diabetic. Table 2 summarizes the basal parameters of each group.

As expected, the diabetic HFD-fed mice showed severely impaired glucose tolerance. In contrast, the nondiabetic HFD-fed mice showed normal glucose tolerance (Fig. 3A); however, the highest plasma insulin levels among the 3 groups were observed in these nondiabetic HFD-fed mice (Fig. 3B), which suggests the prevalence of insulin resistance

Table 2
Body weights and plasma and urinary glucose levels of BDF1 mice used in glucose tolerance test at 14 to 18 weeks of feeding

	Control diet (n = 7)	HFD-non-DM (n = 6)	HFD-DM (n = 7)
Body weight (g)	41.6 ± 0.4	48.0 ± 0.7*	53.7 ± 0.7*,‡
Plasma glucose (mg/dL)	193 ± 7	225 ± 17	440 ± 29*,‡
Urinary glucose (mg/dL)	ND	47 ± 13	1764 ± 591†

Data are the means ± SEM.
* $P < .01$ vs control diet.
† $P < .05$, ‡$P < .01$ HFD-DM vs HFD-non-DM.

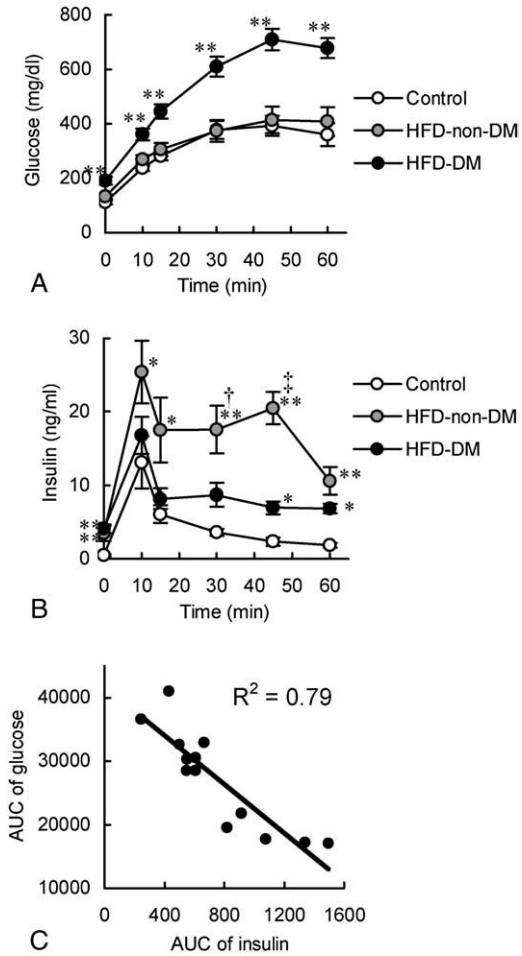

Fig. 3. Impaired glucose tolerance and insulin secretion in diabetic HFD-fed BDF1 mice. Before the oral glucose tolerance test at 14 to 18 weeks of feeding, the mice were divided into 3 groups according to diet and plasma glucose levels: control-diet fed (control, n = 7, open circles), nondiabetic HFD-fed (HFD-non-DM, n = 6, gray circles), and diabetic HFD-fed (HFD-DM, n = 7, closed circles) mice. A, Changes in plasma glucose levels after a glucose load. B, Changes in plasma insulin levels at the same time. Data are the means ± SEM. $^{*}P < .05$, $^{**}P < .01$ HFD-DM or HFD-non-DM vs control. †$P < .05$, ‡$P < .01$ HFD-non-DM vs HFD-DM. C, Relationship between the AUC of plasma insulin and the AUC of plasma glucose in HFD-fed animals (n = 13).

Table 3
Body weights and diabetes-related parameters of C57BL/6 mice used in glucose tolerance test at 18 weeks of feeding

	Control diet (n = 8)	HFD (n = 8)
Body weight (g)	34.8 ± 1.0	50.1 ± 0.2*
Plasma glucose (mg/dL)	200 ± 6	217 ± 19
Urinary glucose (mg/dL)	13.4 ± 1.0	17.1 ± 2.6
Plasma insulin (ng/mL)	4.6 ± 0.6	42.3 ± 5.3*

Data are the means ± SEM.
* $P < .01$ vs control diet.

and sufficient compensatory insulin secretion in these mice. On the other hand, plasma insulin levels of the diabetic HFD-fed mice were lower than those of the nondiabetic HFD-fed mice. In the correlation analysis among all HFD-fed mice, the area under the curve (AUC) of plasma glucose and plasma insulin levels showed a strong negative correlation (Fig. 3C), suggesting that reduced insulin secretion is a causal factor of diabetes in HFD-fed BDF1 mice.

3.4. No prevalence of diabetes in HFD-fed DBA/2 or C57BL/6 mice

To compare BDF1 mice with DBA/2 and C57BL/6 mice, the parental strains of BDF1 mice, we fed the same HFD to both strains and examined the development of obesity and diabetes. DBA/2 mice fed a HFD gained weight slowly, and the body weight difference from the control mice was only 4.9 g at 16 weeks of feeding (the means ± SEM for 8 animals of HFD-fed and control groups were 41.6 ± 2.0 and 36.7 ± 0.8 g, respectively). They showed normal plasma glucose levels (187 ± 8 mg/dL, mean ± SEM) and normal glucose tolerance (data not shown).

In contrast, HFD-fed C57BL/6 mice became as severely obese as BDF1 mice. Their body weight difference from the control mice was 10.4 g at 15 weeks of feeding. They however did not show hyperglycemia or glucosuria even though this was examined in selected heavier mice at 18 weeks of feeding (Table 3). In the oral glucose tolerance test, the glucose tolerance of the HFD-fed C57BL/6 mice remained normal except for the hyperglycemia seen before the glucose load (Fig. 4A), which was probably derived from severe insulin resistance in the liver under the fasting condition. The plasma insulin levels of the HFD-fed C57BL/6 mice were as much as 4-fold higher than those of the control mice after the glucose load (Fig. 4B); and the AUC of plasma glucose and insulin did not show a negative correlation (Fig. 4C), which is distinct from the observation for the BDF1 mice.

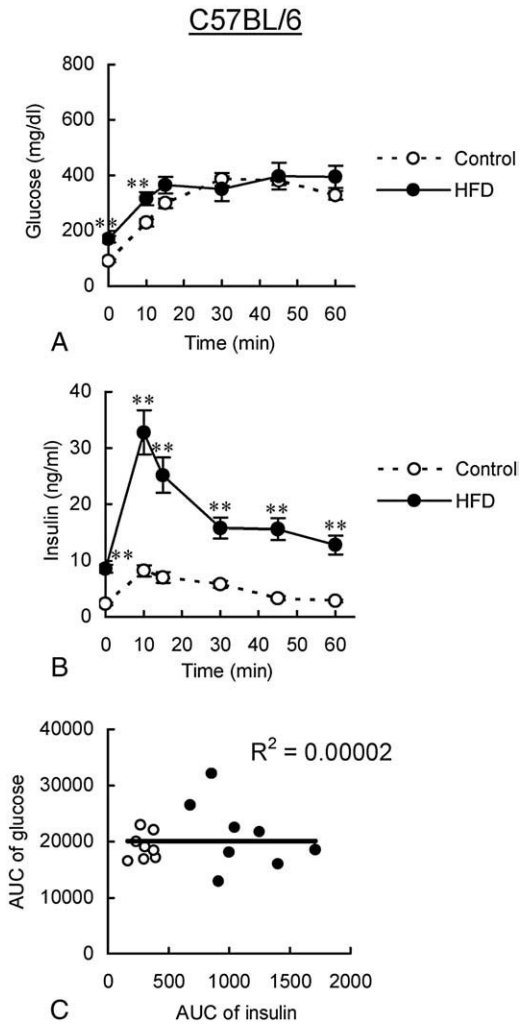

Fig. 4. Conserved glucose tolerance and insulin secretion in HFD-fed C57BL/6 mice. A, Changes in plasma glucose levels with the oral glucose tolerance test. B, Changes in plasma insulin levels at the same time. C57BL/6 mice fed a control diet (open circles, n = 8) or an HFD (closed circles, n = 8) for 18 weeks were tested. Data are the means ± SEM. **$P < .01$ vs control. C, Relationship between the AUC of plasma insulin and the AUC of plasma glucose in all animals tested.

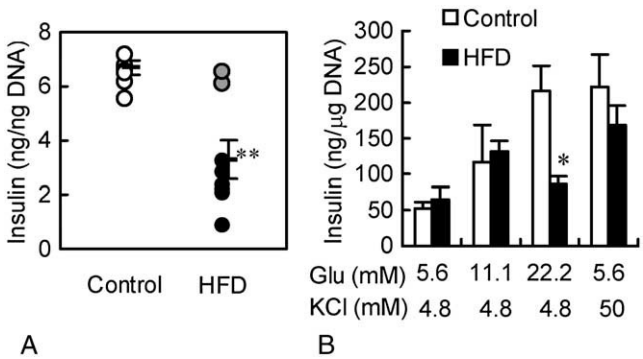

Fig. 5. Functional impairment of the pancreatic islets of HFD-fed BDF1 mice. A, Insulin contents of pancreatic islets isolated from BDF1 mice at 14 weeks of feeding. On the basis of plasma glucose levels, HFD-fed mice were classified as being nondiabetic (gray circles) or diabetic (closed circles). Means ± SEM are also depicted (n = 8). B, Insulin secretion from pancreatic islets isolated from control (open columns) and HFD-fed (black columns) mice. Islets were incubated in Krebs-Ringer bicarbonate buffer containing variable concentrations of glucose and KCl. Data are the means ± SEM of 6 animals. *$P < .05$, **$P < .01$ vs control.

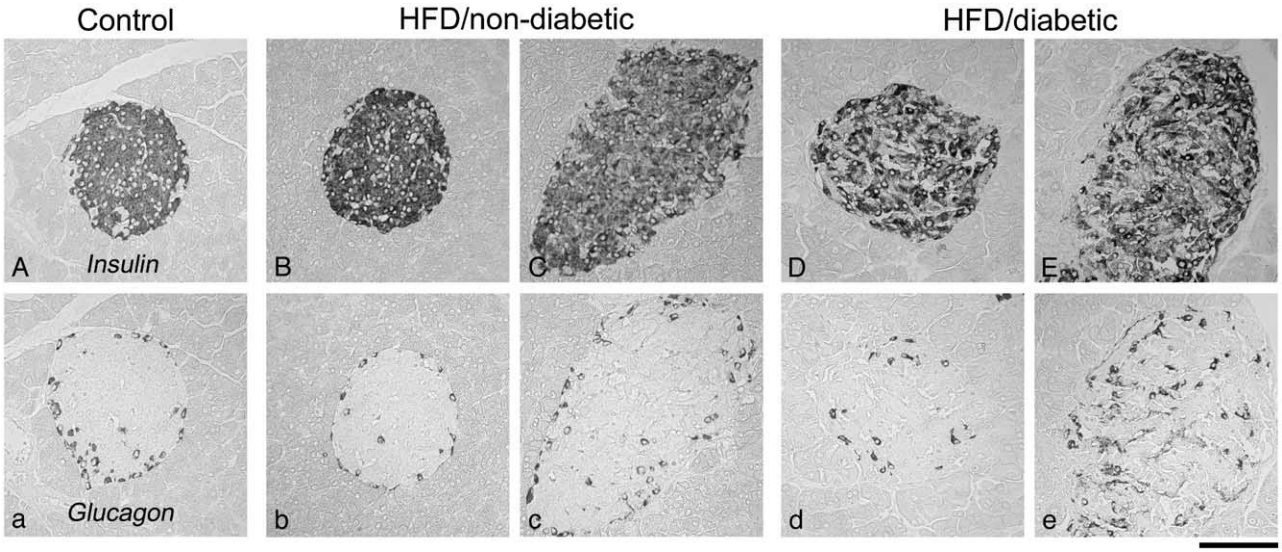

Fig. 6. Morphologic defects of the pancreatic islets of diabetic HFD-fed BDF1 mice. After 15 weeks of control diet or HFD feeding, pancreata isolated from control (A/a), nondiabetic HFD-fed (B/b, C/c), and diabetic HFD-fed (D/d, E/e) mice were examined immunohistochemically. Serial sections were stained for insulin (A, B, C, D, and E) and glucagon (a, b, c, d, and e), and representative islets are shown. For the specimen of HFD-fed mice, both the normal size (B/b, D/d) and the larger size (C/c, E/e) of islets are shown. Bar represents 100 μm.

3.5. Functional and morphologic defects of pancreatic islets in HFD-fed BDF1 mice

To further investigate the pathogenesis of the diabetes observed in the HFD-fed BDF1 mice, we examined their pancreatic islets in different ways. First, pancreatic islets were isolated from the BDF1 mice at 14 weeks of feeding to examine the insulin content and insulin secretory function. The mean islet insulin contents of the HFD-fed mice were significantly lower than those of the control mice (Fig. 5A). It was noticeable that 2 mice in the HFD-fed group showed normal plasma glucose levels (data not shown), and their islet insulin contents (gray circles in Fig. 5A) were comparable with those of the control mice. The glucose-induced insulin secretion from pancreatic islets was almost the same between the HFD-fed and the control mice for up to 11.1 mmol/L of glucose (equivalent to 200 mg/dL). At a higher glucose concentration of 22.2 mmol/L (equivalent to 400 mg/dL), however, the islets from the HFD-fed mice showed significantly lower insulin secretion than did those in the control mice (Fig. 5B). Maximal insulin secretions under a high potassium condition were comparable between 2 groups, suggesting that the reduced insulin secretion observed in the islets of HFD-fed mice is caused by the functional defects of the islets rather than the lower viability of the islets.

Immunohistochemical examination of the pancreata of the BDF1 mice was performed at 15 weeks of feeding. In the specimens from control mice, the bodies of the islets were densely stained with an anti-insulin antibody; and glucagon-positive cells were observed only on the periphery, which is a typical pattern in mouse islets (Fig. 6A and a). Nondiabetic HFD-fed mice showed many enlarged islets, but their staining densities and patterns were comparable with those of the normal islets of the control mice (Fig. 6B, C, b, and c). By contrast, the diabetic HFD-fed mice showed strikingly different appearance; the staining densities for insulin were reduced, and many glucagon-positive cells were observed (Fig. 6D, E, d, and e). The calculated staining densities for insulin and glucagon were 64% ± 3% and 360% ± 35% of those of the control mice, respectively; and both levels were significantly different from the control levels (both $P < .01$). Glucagon-positive cells were observed particularly in the enlarged islets, which had a majority in the pancreas of HFD-fed mice.

4. Discussion

In the present study, we tested the hypothesis that hybrid mice of an obesity-prone strain and a diabetes-prone strain would be useful for establishing a novel animal model of obesity-induced type 2 diabetes mellitus. Our results showed that BDF1 mice, the F_1 hybrid mice of the C57BL/6 and DBA/2 strains, develop HFD-induced obesity and the resultant severe diabetes characterized by hyperglycemia, glucosuria, and a significant increase in HbA_{1C} levels. Our results also showed that this diabetes in HFD-fed BDF1 mice is at least partly based on impaired insulin secretion in response to glucose to compensate for insulin resistance. The reduced insulin content along with impaired insulin release by isolated pancreatic islets further supports this notion.

The features of diabetes observed in HFD-fed BDF1 mice are similar to those of human type 2 diabetes mellitus in several points. First, in etiology, the BDF1 mice have no specific gene mutations related to glucose metabolism, to

our knowledge; and they developed diabetes as a result of diet-induced obesity. These etiologic features are similar to those of most human patients [14]. Second, in the disease process, HFD-fed BDF1 mice initially show simple obesity and, after a certain period, develop severe diabetes. The transition from simple obesity to diabetes is considered to be the key step in developing type 2 diabetes mellitus in humans [15]. Third, in pathogenesis, the diabetes in HFD-fed BDF1 mice is based on reduced insulin secretion from the pancreas in addition to insulin resistance. It is considered that the dysfunction of pancreatic β-cells plays a critical role in the pathogenesis of type 2 diabetes mellitus in humans [16]. In addition, a morphologic abnormality of the pancreatic islets of diabetic BDF1 mice—an increase in glucagon-positive cells in islets—is also reported in human type 2 diabetes mellitus patients [17,18].

Despite the above similarities of our mice to human type 2 diabetes mellitus patients, there also are some differences; and one of the most important points is a sex difference in development of diabetes. Female BDF1 mice fed the same HFD showed neither hyperglycemia nor glucosuria even though they had become as severely obese as male mice (data not shown). Sex differences have also been reported in most of the rodent models of diabetes [19], and involvements of sex hormones have been suggested [20]. The mechanism and the significance of this phenomenon need to be clarified in the future studies.

Previously, HFD-fed C57BL/6 mice have been frequently used as a diet-induced type 2 diabetes mellitus model [7,21]. In the present study, we could reproduce severe insulin resistance and elevated fasting plasma glucose levels in HFD-fed C57BL/6 mice; but we could not observe an increase in nonfasting plasma glucose levels and impaired glucose tolerance in these mice. These results suggest that C57BL/6 is a rather diabetes-resistant strain with a strong capability of secreting insulin to compensate insulin resistance. It is possible that the control C57BL/6 mice used in this study had already become diabetic because of the longer feeding period; and therefore, we could not observe impaired glucose tolerance in the HFD-fed C57BL/6 mice compared with the control mice. However, the observation that the HFD-fed C57BL/6 mice showed the same insulin content in their pancreatic islets as did young and lean control mice (data not shown) strongly supports our views.

As mentioned earlier, the genetic backgrounds of animals have significant effects on the development of diabetes. This study demonstrated the importance of obesity susceptibility as well as diabetes susceptibility in diet-induced diabetes. Although a previous genetic study reported that DBA/2 mice were diabetes-prone mice [11], DBA/2 mice fed an HFD did not develop diabetes in this study. This is probably because they did not become sufficiently obese to induce diabetes. In contrast, BDF1 mice fed an HFD became sufficiently obese and developed severe diabetes. These results could be considered to mean that DBA/2 mice incorporated the obesity-prone characteristics of C57BL/6 mice and demonstrated their original characteristic: susceptibility to obesity-induced diabetes. This approach of crossing obesity-prone mice may be useful for other diabetes-prone strains of mice to induce obesity-dependent diabetes.

Other than our study, only a limited number of reports have examined glucose metabolism in BDF1 mice; and to our knowledge, this is the first study to demonstrate that HFD-fed BDF1 mice develop severe diabetes. Hull et al [22] recently reported a study with BDF1 mice fed an HFD. However, their mice did not show hyperglycemia or impaired glucose tolerance; and they could only show the relatively reduced insulin secretion from the pancreatic islets of the HFD-fed mice. One possible explanation for the difference between these results and ours could be differences in diet composition. We used an HFD prepared by mixing regular feed and fat; hence, the nutrients other than fat were diluted in the HFD compared with those in the regular diet, which might have affected the glucose metabolism of the mice in the present study. However, this possibility seems unlikely because severe diabetes was also developed when BDF1 mice were fed a nutritionally controlled HFD (D12492; Research Diets, New Brunswick, NJ) containing the same amount of nutrients, such as proteins, vitamins, and minerals, as the regular diet. A difference in the breeder of the mice may also be another factor to consider; however, we tested BDF1 mice from another breeder (CLEA Japan, Tokyo, Japan) and obtained almost the same results.

It is now considered that obesity is a central component of metabolic disorders such as diabetes, hypertension, dyslipidemia, and cardiovascular diseases [23,24]. Therefore, HFD-fed BDF1 mice may have other coexisting metabolic disorders. In fact, hypercholesterolemia was evident in HFD-fed BDF1 mice; and cholesterol metabolism in these mice is of interest. In addition, we observed microalbuminuria, an early symptom of kidney dysfunction [25], in the BDF1 mice fed an HFD for 14 weeks (data not shown). Careful examination of HFD-fed BDF1 mice should be made in the future studies.

In conclusion, the present study showed that HFD-fed BDF1 mice develop obesity-dependent diabetes presumably caused by a dysfunction of pancreatic islets for producing and/or secreting insulin. This is an obesity-induced type 2 diabetes mellitus model with normal animals, which could be useful for investigating the mechanisms of diabetes development after obesity and dysfunction in pancreatic islets. These mice would also be useful for evaluating antiobesity and antidiabetic drugs.

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