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RESEARCH ARTICLES

Antecedent intake of traditional Asian-style diets exacerbates pancreatic β-cell function, growth and survival after Western-style diet feeding in weaning male rats☆

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Abstract

The prevalence of type 2 diabetes has been rapidly increasing in conjunction with the westernization of diet patterns in Asia. We determined whether the antecedent consumption of traditional Asian-style diets (ADs) deteriorates insulin action, insulin secretion and pancreatic β-cell mass after subsequent imposition of the diabetogenic challenge of Western-style diets (WDs) in weaning male Sprague–Dawley rats. Rats were provided AD (a low-fat and plant protein diet), WD (a high-fat and animal protein diet) or a control diet (CD) (a low-fat and animal protein diet) for 12 weeks. After 12 weeks, the groups were divided into two subsets; one set of the groups continued to consume their previous diets of WD, AD and CD for another 12 weeks, and the second set was divided into three groups represented by a switch in their designated diets from WD to AD, AD to WD and CD to WD. Whole-body glucose disposal rates and GLUT4 contents in soleus muscles were lower in WD regardless of the antecedent protein sources. The first-phase insulin secretion was higher in the CD group than in the other groups, whereas the second phase was lowered with AD consumption as antecedent and/or present diets. Asian-style diet and AD-WD intake did not compensate for insulin resistance due to the failure of β-cell expansion via decreased proliferation. These findings suggest that the antecedent consumption of AD possibly accelerates and augments the development of glucose dysregulation via decreased insulin secretion capacity and pancreatic β-cell mass when the diets switch to WD.

Keywords: Protein source; Fats; Leptin; Insulin resistance; Insulin secretion; Apoptosis; Proliferation

1. Introduction

The prevalence of type 2 diabetes mellitus (DM) has been continuously and rapidly increasing in Asia, including Korea where the prevalence rate is currently around 10% of the population [1–4]. One possible reason of increasing rates is the westernization of eating behavior. Koreans traditionally consumed protein mainly from grains, and a small amount of fats from meats [5]. The westernization of their diet has resulted in an increase in the consumption of protein and fats from animal sources [6]. According to the National Nutrition Survey Report of Korea, fat and simple sugar intakes during 1995 increased about 2.8-fold from those in 1970 [2]. Currently, Koreans receive approximately 62%, 18% and 20% of their energy from carbohydrates, protein and fats, respectively, indicating that absolute fat consumption is not so high as in Western countries, yet. However, the rate of fat consumption has more than doubled from 20 years ago [2,3,7]. These phenomena have occurred in most Asian countries, not just in Korea.

Because previous studies have demonstrated that diets high in fat, sucrose and fructose contribute to insulin resistance, the composition of macronutrients in diets acts as an environmental factor that contributes to the development or exacerbation of the insulin-resistant state [8,9]. Kraegen et al. [10] demonstrated that a high-fat diet (59% of total energy from safflower oil) produced insulin resistance in the liver and adipose tissues within a few days in adult male Wistar rats and generated it later in skeletal muscles. A high-fat diet (more than 40 En% fat) is known to cause hyperinsulinemia with the expansion of islets, which creates transitory normoglycemia [11,12]. However, long-term high-fat diets lead to insufficient

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insulin release due to β-cell senescence and eventually induce type 2 DM [12]. Diabetes mellitus develops when insulin secretory function fails in an insulin-resistant state [11,13,14]. Most studies related to protein consumption, insulin resistance and secretion have focused on protein restriction and/or malnutrition [11,13,14]. Protein–calorie malnutrition impairs the insulin secretory response of pancreatic β-cells, and when imposed during pregnancy, mild protein restriction, even in the absence of calorie restriction, elicits a profound impairment in the structural and functional development of the fetal endocrine pancreas [15,16]. A moderate protein restriction of the offspring of dams after birth leads to impaired glucose-stimulated insulin secretion in adulthood [16]. Protein–calorie malnutrition in adult rats also shows a blunted response of insulin secretion to glucose challenge. The effects of antecedent and present sources of dietary protein on insulin resistance, insulin secretion and pancreatic β-cell mass have not yet been studied and identified.

The characteristics of Asian type 2 DM are different from those of Western type 2 DM [17,18]. For example, Korean type 2 diabetic patients did not become obese; rather, they showed a dramatic loss of weight during the progression of the disease [17]. Their serum insulin levels were within or below the normal ranges, and they did not show hyperinsulinemia. This implies that insulin may be insufficiently secreted to compensate for insulin resistance. Thus, in Korean type 2 DM, insulin deficiency, as well as insulin resistance, is a crucial factor for the prevalence of DM. Rapid insulin deficiency may be related to their traditional Asian-style diet (AD) of low fat and plant protein. The patients developed insulin resistance without weight gain, and insulin deficiency was an important factor in contracting DM and the progression through further symptoms [17]. The increased prevalence and symptoms of Asian DM may be related to not only in protein restriction and/or malnutrition in earlier periods but also protein sources. Thus, antecedent consumption of plant protein as a major protein source may have attenuated insulin action, insulin secretion and pancreatic β-cell mass, and subsequent imposition of the diabetogenic challenge may stimulate the development and progression of type 2 DM.

The purpose of this study was to determine whether antecedent intake of traditional ADs affect insulin action, insulin secretion and pancreatic β-cell mass after changing to Western-style diets (WDs) in weaning male Sprague–Dawley rats. Results revealed that switching from a traditional AD to a WD exacerbated insulin secretion capacity and pancreatic β-cell mass more than switching from a control diet (CD) to a WD. The CD was composed of animal protein instead of plant protein as in AD. The dietary pattern changes from AD to WD increased the potential to develop type 2 DM. Thus, the remarkable increase of type 2 DM prevalence in Asia is closely associated to the sudden change to a high-fat diet after antecedent consumption of plant protein.

2. Materials and methods

2.1. Experimental animals

Weaning male Sprague–Dawley rats weighing 93±16 g were purchased from the Korean Animal Resources Center, Seoul, Korea, and were randomly divided into three groups. They were housed individually in stainless steel cages in a controlled (23°C; 12 h light and dark cycle) environment and were allowed to acclimate for a week before the commencement of the study. All surgical and experimental procedures were approved by the Animal Care and Use Committee at Hoseo University, Korea.

Each group was assigned a different diet, such as a low-fat and plant protein diet (AD, $n=40$), a high-fat and animal protein diet (WD, $n=40$) or a low-fat and animal protein diet (CD, $n=40$). Rats in the AD, WD and CD groups consumed their assigned diet for 12 weeks. After 12 weeks, rats from each of the three groups were randomly subdivided into two further groups. One subgroup continued to consume the same diet until the 24th week, represented as WD ($n=20$), AD ($n=20$) and CD ($n=20$) groups. The other subgroups switched diets from WD to AD (WD-AD, $n=20$), AD to WD (AD-WD, $n=20$) and CD to WD (CD-WD, $n=20$), respectively. Experimental diets to the end of the 12th week in this research are classified as antecedent, whereas diets after the 12th week are classified as present.

2.2. Diets

Semipurified diets for each group were modified based on AIN-93 formulation for experimental diets [19]. Western-style diet and AD mimicked the source of protein and amount of fats in WDs and traditional ADs, respectively. The major protein and fat sources of AD was glutelin (grain protein; Samyang, Seoul) and corn oil (CJ, Seoul), whereas those of WD was casein (milk protein) and butter (Seoulmilk, Seoul). Asian-style diet consisted of 72 En% starch, 10 En% corn oil and 13 En% glutelin plus 5 En% casein, whereas WD was composed of 42 En% carbohydrates, 10 En% corn oil, 30 En% butter and 18 En% casein (Table 1). Glutelin is a major grain protein in wheat, rice and barley in the form of glutelin, oryzein and hordenin, respectively [20]. Glutelin contains most essential amino acids, but it has limited amino acids such as lysine, methionine, tryptophane and threonine, which can be supplemented with 5 En% casein and methionine [20]. Even though glutelin is a poor quality protein compared to casein, it does not develop protein deficiency. The diet composition of CD was the same as that of AD except for the protein source in order to determine the effects of protein sources. Control diet contained casein instead of glutelin. The animals were allowed free access to their respective diets for 24 weeks. During the entire supplementation period, overnight fasting blood was collected from the cut-tail tips of individual rats weekly at an assigned time to measure serum glucose levels, and simultaneously, food intake and body weight were measured.

Table 1
Diet composition (g/kg diet)

	A high-fat and animal protein diet (WD)	A low-fat and plant protein diet (AD)	A low-fat and animal protein diet (CD)
Starch	402	598	598
Sucrose	100	100	100
Casein	200	21	180
Glutelin	–	159	–
Corn oil	43	43	43
Butter	176	–	–
Cellulose	30	30	30
D,L-Methionine	2	2	2
AIN mineral mixture	35	35	35
AIN vitamin mixture	10	10	10
Choline bitartrate	2	2	2
Gross energy content (kJ/g diet)	20.1	16.3	16.3

AIN, American Institute of Nutrition.

2.3. Hyperglycemic clamp

A subset of animals (13 per group) in the study was catheterized in the jugular vein and carotid artery at the twenty-third week of the experimental period [21]. Six days after the insertion, a hyperglycemic (HG) clamp was performed to evaluate the insulin secretion capacity of the rats in an awake, unstressed and fasting state [22]. Bolus glucose (375 mg glucose/kg body weight) was infused through the cannula for the first 5 min of the clamp, and 25% glucose was administered through the cannula to maintain serum glucose levels at 11.5 mM (steady state). Serum glucose and insulin levels were measured from the blood collected from the carotid artery at 0, 5, 10, 40, 50 and 60 min during the clamp. Body weight did not change during the clamp and the next 2 days until a euglycemic hyperinsulinemic (EH) clamp was undertaken.

2.4. Euglycemic hyperinsulinemic clamp

Two days later, EH clamp was performed in the same rats in order to perform HG clamp in a fasting and free moving state to determine insulin resistance [23,24]. Insulin-stimulated whole-body glucose flux was estimated using a prime continuous infusion of [3-^{3}H]glucose (10 μCi bolus, 0.1 μCi/min, NEN Life Science, Boston, MA) throughout the clamps. Regular human insulin (Humulin, Eli Lilly and Co, Indianapolis, IN) was continuously infused at a rate of 20 pmol/kg per minute in order to raise plasma insulin concentration to approximately 1100 pM. Blood samples from arteries were collected at 10-min intervals for glucose estimation, and 25% glucose was infused at variable rates as needed to clamp glucose levels at approximately 6 mM. Serum insulin levels were also measured from the blood collected at the steady state (90 to 120 min) at EH clamp to verify that the designated serum insulin levels were reached. To estimate the rates of insulin-stimulated glucose uptake and metabolism in vivo in individual tissues, we administered 2-deoxy-D-[1-^{14}C] glucose (51.1 mCi/mmol, NEN) as a bolus (30 μCi) at 45 min before the end of the clamps. For the determination of plasma [3-^{3}H]glucose concentrations, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$ (Sigma, St. Louis, MO), dried to remove ^3H_2O and resuspended in water, and disintegrations per minute (dpm) of ^{3}H were noted. The plasma concentration of ^3H_2O was determined by the difference between ^{3}H counts without and with drying. Rates of whole-body glucose uptake and basal glucose turnover were determined as the ratio of the [^{3}H] glucose infusion rate to the specific activity of plasma glucose (dpm/μmol) during the final 30 min of the respective experiments. Hepatic glucose production during clamps was determined by subtracting the glucose infusion rate from the whole-body glucose uptake.

Glucose uptake in muscles was calculated as described by Kraegen et al. [10]. Glucose transport in skeletal muscles was calculated from steady-state serum glucose concentration, serum 2-[^{14}C]deoxyglucose-phosphate profile and the muscle content of 2-[^{14}C]deoxyglucose-6-phosphate using the equation from Kraegen et al. [10]. Serum 2-[^{14}C]deoxyglucose-6-phosphate profile was fitted with a double exponential curving using GraphPad Prism (GraphPad Software, San Diego, CA). Muscle 2-[^{14}C]deoxyglucose-6-phosphate contents were determined by separation of 2-[^{14}C]deoxyglucose from muscle homogenates using an ion-exchange column (Bio-Rad Laboratories, Hercules, CA).

After finishing the EH clamp study, the rats were immediately anesthetized with xylazine (10 mg/kg body weight) and killed by decapitation. Liver and soleus and quadriceps muscles were rapidly dissected, frozen in liquid nitrogen and stored at −70°C until further analysis could be performed. Soleus and quadriceps muscles were selected to determine glucose metabolism as a representative of red and white muscles, respectively, as skeletal muscle fibers are classified into three types on the basis of various structural and functional characteristics [25]. However, muscles can be divided into two types by color. Soleus muscles are type I fibers, also called slow twitch fibers. They are red and geared to generate ATP by aerobic metabolic processes. In contrast, quadriceps muscles are type II B fibers, called fast twitch fibers. They are white and adapted to generate ATP by anaerobic metabolic processes using glucose.

2.5. Biochemical measurements

Serum glucose levels were analyzed by a glucose analyzer II (Beckman, Fullerton, CA). Serum insulin and leptin levels were measured in duplicate 100 μl samples by rat radioimmunoassay (RIA), using guinea pig anti-rat insulin, mono-^{125}I-human insulin and rat insulin standard (Linco Research, St. Charles, MO). The limit of detection of insulin and leptin was 0.1 and 0.5 ng/ml, respectively. The intraassay and interassay coefficients of variation of both insulin and leptin were lower than 7%. The insulin RIA has specificity for rat and human insulin, whereas

leptin RIA was specific for rat only. In order to determine the glycogen levels in the liver and soleus and quadriceps muscle tissues, these tissues were homogenized and deproteinized with 1.5 N perchloric acid. The glycogen was digested into glucose with α-amyloglucosidase in an acid buffer. Glycogen levels were expressed as glucose levels derived from the glycogen in the soleus and quadriceps muscle and liver tissues [26]. Interstitial fat from the soleus and quadriceps muscles was removed, and muscle triacylglycerol was extracted with a chloroform–methanol solution (2:1, vol/vol). Subsequently, the extracted triacylglycerol was resuspended in pure chloroform [27]. Triacylglycerol concentration was determined using a Trinder kit (Sigma).

Total plasma membranes from the soleus and quadriceps muscle were prepared by the methods of Jiang et al. [28]. Briefly, skeletal muscles were homogenized in buffer A, consisted of 250 mM, sucrose, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4, supplemented with 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 1 mM aprotinin and 1 mM pepstatin. Muscle homogenates were centrifuged at 2000×*g* for 10 min to remove unhomogenized muscle fibers. The resulting supernatants were spun at 19,000×*g* for 20 min. The pellets were resuspended in 3 ml buffer B (20 mM HEPES, 1 mM EDTA, pH 7.4), supplemented with protease inhibitors, layered on 6 ml sucrose cushion (38% sucrose in buffer B) and then spun at 100,000×*g* for 60 min. The membranes recovered on top of the sucrose cushion were resuspended in buffer B and spun at 40,000×*g* for 20 min. The pellet was designated as total plasma membranes. The whole procedure was carried out at 4°C. Equal amounts of protein (30 μg) were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking the membrane with 10% nonfat milk, antibodies against GLUT4 (1:2500; Chemicon, Temecula, CA) and β-actin (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) were incubated for 16 h at 4°C. Secondary antibodies were conjugated to horseradish peroxidase and visualized by enhanced chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ). Membranes were exposed to Kodak XAR films and subjected to laser scanning densitometry (BioRad, Richmond, CA) for quantitation of results.

2.6. Islet morphometry

At the end of the experiment, 5-bromo-2-deoxyuridine (BrdU, 100 μg/g body weight in saline, Roche Molecular Biochemicals, Indianapolis, IN) was intraperitoneally injected into seven to eight rats that had not undergone a clamp. Six hours later, they were sequentially perfused with saline and 4% paraformaldehyde (pH 7.2) [14], and the dissected pancreas was further fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin to determine islet morphometry. To measure β-cell mass, we immunostained 5-μm sections for insulin using a guinea pig anti-porcine insulin antibody (Dako, Denmark) and a biotinylated goat anti-guinea pig IgG (Dako). Detection was performed by standard avidin/biotin immunohistochemical methods (ABC method), and positive staining was visualized with DAB. Finally, counterstaining was performed with hematoxylin and eosin. Apoptosis of β-cells was measured by TUNEL kit (Roche, Mannheim, Germany) in a paraffin section of pancreas and counterstained with hematoxylin and eosin to visualize islets. Sections also underwent immunostaining with mouse anti-BrdU (Roche Molecular Biochemistry) and counterstained with insulin. Quantitative evaluation was performed with a BH-2 microscope (Olympus, Japan) connected via a color video camera to a computer.

The β-cell area was measured by acquiring images from two sets of 8 to 10 distal, random, nonoverlapping images at ×20 of insulin-stained pancreatic sections. Results of the β-cell quantification are expressed as the percentage of the total surveyed area containing insulin-positive cells. Pancreatic β-cell mass was determined by multiplying the β-cell area by the pancreas weight. β-Cell proliferation was expressed in the number of BrdU-positive β-cells (red dots, Fig. 4E) per millimeter squared pancreas, and it was represented as BrdU-positive nuclei in total β-cells (insulin-positive cells) per pancreas section, two sections per animal and five animals per group. Apoptosis of β-cells was determined by counting apoptotic bodies (brown dots) in total β-cells, and the representation was the same as that of β-cell proliferation.

2.7. Statistical analysis

All results are expressed as a mean±S.D. Statistical analysis was performed using the SAS statistical analysis program [29]. Diet effects were determined by one-way analysis of variance in the measurements at the 12th week. From the 13th week, one main diet was switched to another diet, but not all possible diet combinations were generated. According to the main diets, the subsequent diets were changed and classified into a nested design. Nested analyses of variance were carried out to determine the main diet effect of WD, AD and CD, and the combination diet effect for measurements in the 24th week (the effect of switching diets; WD-AD, AD-WD and CD-WD). Comparison within groups was performed by contrast tests. Differences with a $P<.05$ were considered as statistically significant.

3. Results

3.1. Dietary fat contents, but not protein sources, in antecedent and present diets change body weight, caloric intake and serum leptin levels

The rats did not exhibit any significant difference in body weight among the diets until week 4 of the experimental

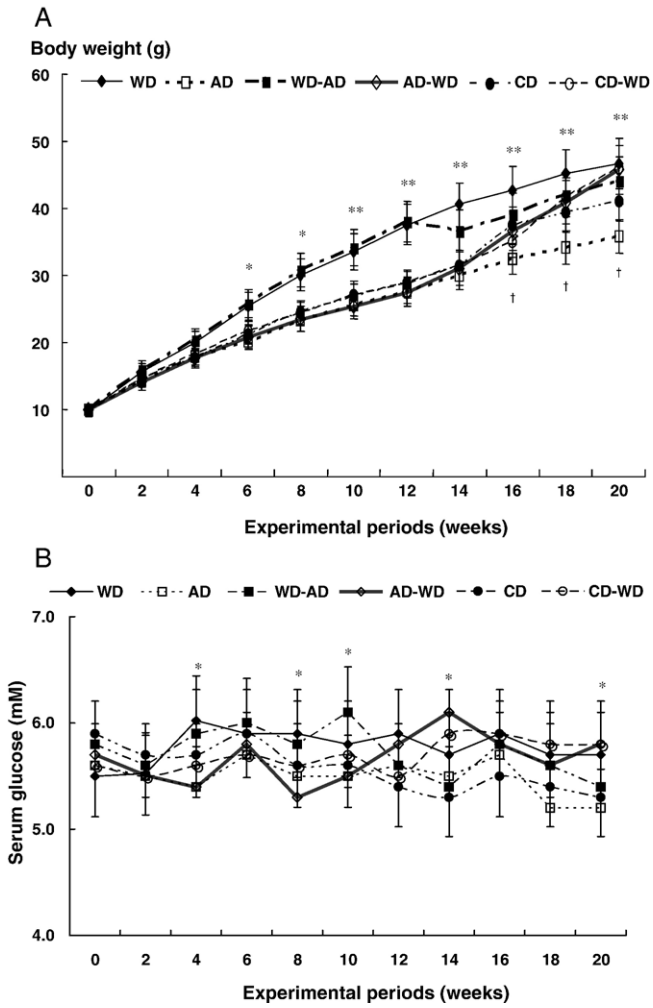

Fig. 1. Changes of body weight and serum glucose levels during experimental periods. Weekly changes of body weight (A) and fasted serum glucose concentrations (B) with WD (a high-fat and animal protein diet, $n=11$), AD (traditional AD, a low-fat and plant protein diet, $n=12$), CD (a low-fat and animal protein diet, $n=11$), WD-AD (switching diets from WD to AD, $n=11$), AD-WD (switching diets from AD to WD, $n=12$) and CD-WD (switching diets from CD to WD, $n=11$) intakes during the entire experimental period. Serum glucose concentrations were measured every Tuesday at 11 a.m. after overnight fasting. Values are mean±S.D. *$P<.05$ in main diet effect of WD, AD and CD. †$P<.05$ in effect of switching diets.

period (Fig. 1A). From week 5, body weight in the WD group was statistically and significantly higher than in the AD and CD groups until week 12, prior to switching the diets. Body weight did not change during the first 2 weeks after switching the diets from WD to AD, and after that, it increased to a level similar to the WD group. From the third week, the rate of weight gain was much higher in rats switching diets either from AD or CD to WD than those that consumed continuous WD ($P<.05$ by contrast test), leading to catching up the body weight of rats in both AD-WD and CD-WD groups with that of WD. Antecedent sources of protein did not influence body weight gain after a switch to a high-fat diet.

Changes of serum leptin levels were parallel with those of body weight. Serum leptin levels were higher in rats receiving WD compared to AD or CD ($P<.05$ by contrast test) (Table 2). In the WD-AD group, serum leptin levels were not as low as those of the AD group. On contrary, serum leptin levels in the AD-WD group reached the levels of the WD group. Increase of body weight in high-fat diets was explained by higher caloric intake in spite of increased serum leptin levels (Table 2). Consistent with the changes of body weight, caloric intake was higher in rats receiving WD compared to AD or CD ($P<.05$ by contrast test). It was remarkably increased after changes to WD regardless of antecedent protein sources.

3.2. Overnight fasted serum glucose and insulin levels increased by high-fat contents in present diets regardless of dietary protein sources

Overnight fasted serum glucose levels were maintained at near normal ranges in all groups during the experimental period (Fig. 1B). The glucose levels of the WD group were higher at 5, 9, 11, 15 and 20 weeks than those of the AD and CD groups ($P<.05$, by contrast test). However, the levels of the CD group did not differ from the AD group (Table 3). After switching the diets from AD to WD, serum glucose levels increased to levels similar to the WD group, whereas in the WD-AD group, they decreased to the levels of the AD group. They were significantly higher in the WD, AD-WD and CD-WD groups than in the other groups at the end of the experiment ($P<.05$ by contrast test). Dietary fat contents of the present diets, not the antecedent diets, increased serum glucose levels. However, protein subtypes did not alter serum glucose levels.

Overnight fasted serum insulin levels were altered by the fat contents of the present diet, but not those of the antecedent diets, unlike body weight. Similar to serum glucose levels, serum insulin levels were not changed by the protein sources of the antecedent and present diets (Table 3). Compared to the AD and CD groups, serum insulin levels were significantly higher in WD at the end of the experimental period ($P<.05$, by contrast test). They decreased after switching WD to AD, although they increased in the AD-WD and CD-WD groups ($P<.05$, by paired t test).

3.3. Insulin resistance was increased only by high fat in the present diets

As expected from the changes of overnight fasted serum glucose and insulin levels due to dietary fat contents and protein subtypes, peripheral insulin resistance was influenced by dietary fat contents in the present diets only. Table 4 shows whole-body glucose disposal rates at the EH clamp at end of the experimental period. Before the EH clamp, serum glucose and insulin levels at baseline demonstrated the same tendency as those of fasted serum insulin levels at the end of the experiment. Serum glucose and insulin levels at baseline were higher in the WD, AD-

Table 2
Body weight, serum leptin and food intake during the experimental period

	WD (n=11)	WD-AD (n=11)	AD (n=12)	AD-WD (n=12)	CD (n=11)	CD-WD (n=11)
Body weight (g)						
Initial	98±5	97±6	101±7	99±7	102±6	101±7
At the 12th week	394±23	387±22	299±15	293±23	308±24	305±19*
Final	461±35[a]	428±37	372±39[b,c]	452±35[a]	410±29[c]	465±33[a,*,†]
Serum leptin (ng/ml)						
Initial	5.4±0.8	5.3±0.9	5.4±0.8	5.2±0.7	5.5±0.9	5.4±0.9
At the 12th week	7.6±1.1	7.7±0.8	6.1±1.2	5.9±1.0	6.3±1.1	6.1±0.9*
Final	7.2±0.9[a]	6.3±0.7[b]	5.6±0.8[b,c]	7.0±0.9[a]	5.8±1.2[c]	7.2±1.1[a,*,†]
Caloric intake (kcal/day)	176±24[a]	146±29	129±18[b,c]	184±31[a]	134±25	182±33*

Values are mean±S.D. Abbreviations are the same as those in Fig. 1.
[a] P<.05 vs. CD by contrast test.
[b] P<.05 vs. WD by contrast test.
[c] P<.05 vs. CD–WD by contrast test.
* P<.05 in main diet effect of WD, AD and CD.
† P<.05 in effect of switching diets.

WD and CD-WD groups than the CD group (P<.05, by contrast test). Glucose disposal rates representing whole-body insulin sensitivity was higher in AD and CD, compared to WD (P<.05, by contrast test). Regardless of the types of antecedent diets, high fat in the present diets increased peripheral insulin resistance.

In the basal (fasted) state, hepatic glucose output to regulate fasting blood glucose levels was higher in the WD, AD-WD and CD-WD groups regardless of their previous diets than in the other groups, which is consistent with higher serum glucose levels in a fasting state (Table 4). Hyperinsulinemia in a fasting state occurred in WD, which was possibly associated with a higher hepatic glucose output as well. During a hyperinsulinemic clamped state, at about 1100 pM serum insulin levels, hepatic glucose output was suppressed by 75–83% from the basal state in all groups. However, the WD, AD-WD and CD-WD groups were less suppressed by insulin than in the other groups, similar to the hepatic glucose output in the basal state (Table 4). The antecedent and present dietary protein sources did not change hepatic glucose output at the basal and hyperinsulinemic clamped states.

The rate of insulin-stimulated glucose transport activity in skeletal muscle in vivo was estimated by measuring the muscle content of 2-deoxy-D-[1-^{14}C] glucose-6-phosphate using EH clamps in awake and fasted mice (Table 4). Insulin-stimulated glucose transport in soleus and quadriceps muscles was higher in the CD and AD groups (P<.05, by contrast test), and the switch to a high-fat diet lowered the glucose transport comparable to the results of whole-body glucose disposal rates.

Factors influencing whole-body glucose disposal rates such as glycogen and triacylglycerol deposits in muscles and GLUT4 contents were measured. Glycogen deposits in soleus muscles were lower in the WD and CD-WD groups compared to the CD group (P<.05, by contrast test), but deposit levels in quadriceps muscles did not show significant variance among all groups (Table 5). Triacylglycerol contents in soleus and quadriceps muscles were significantly higher in the WD, CD-WD and AD-WD groups than in the CD group. Rats consuming WD, CD-

Table 3
Overnight fasted serum glucose and insulin levels during the experimental period

	WD (n=11)	WD-AD (n=11)	AD (n=12)	AD-WD (n=12)	CD (n=11)	CD-WD (n=11)
Serum glucose (mM)						
Initial	6.1±0.7	5.9±0.6	5.8±0.8	6.0±0.8	5.9±0.7	5.8±1.0
At the 12th week	5.8±0.5	5.9±0.6	5.5±0.6	5.6±0.9	5.4±0.7	5.6±0.7
Final	5.7±0.6[a]	5.2±0.5	5.1±0.4[b,c]	5.8±0.6[a]	5.2±0.7	5.8±0.6[a,*]
Serum insulin (pM)						
Initial	257±78	248±98	277±65	278±91	259±81	265±85
At the 12th week	406±106	387±98	188±77	195±92	248±104	259±85*
Final	418±81[a]	259±78[b,c]	204±64[b,c]	378±93[a]	262±65[b,c]	383±87[a,*]

Values are mean±S.D. Abbreviations are the same as those in Fig. 1.
[a] P<.05 vs. CD by contrast test.
[b] P<.05 vs. WD by contrast test.
[c] P<.05 vs. CD–WD by contrast test.
* P<.05 in main diet effect of WD, AD and CD.

Table 4
Whole-body glucose disposal rate, hepatic glucose output and skeletal muscle glucose transport during EH clamp

	WD (n=11)	WD-AD (n=11)	AD (n=12)	AD-WD (n=12)	CD (n=11)	CD-WD (n=11)
Glucose (mM)						
Basal state	6.6±0.7	5.9±0.7	6.1±0.5	7.0±0.6	6.2±0.9	6.6±0.8*
Steady state	5.2±1.3	5.1±0.8	5.1±0.5	5.2±0.8	5.2±0.7	5.3±0.9
Insulin (pM)						
Basal state	401±109[a]	244±97[b,c]	182±88[b]	366±108[a]	235±97[b,c]	390±81[a,*]
Steady state	1180±678	1136±546	1128±643	1191±458	1147±548	1159±557
Glucose disposal rate (mg/kg per minute)	33.8±8.2[a]	44.0±9.7[b]	48.1±7.9[b,c]	36.1±8.5[a]	49.7±9.5[c]	37.5±8.2[a,*]
Hepatic glucose output (mg/kg per minute)						
Basal	45.5±6.4[a]	39.4±5.3[b]	37.6±4.5[b,c]	47.7±5.3[a]	36.3±4.8[c]	45.9±5.2[a,*]
Steady state	10.7±2.4[a]	7.3±2.1[b]	6.9±1.8[b,c]	11.5±2.2[a]	6.2±1.7[c]	10.9±2.1[a,*]
Muscle glucose transport (nmol/g per minute)						
Soleus muscle	148.4±33.5[a]	189.8±29.4[b]	207.5±39.9[b]	163.3±35.7[a]	211.4±31.9[c]	168.3±35.8[a,*]
Quadriceps muscle	131.4±32.9[a]	169.4±24.7[b]	194.3±35.6[b]	144.3±22.5[a]	204.3±33.6[c]	141.4±31.5[a,*]

Values are mean±S.D. The values at steady state were obtained when serum glucose concentrations were clamed at 6 mM during EH clamp in all rats. Abbreviations are the same as those in Fig. 1.

[a] P<.05 vs. CD by contrast test.

[b] P<.05 vs. WD by contrast test.

[c] P<.05 vs. CD–WD by contrast test.

* P<.05 in main diet effect of WD, AD and CD.

WD and AD-WD had lower GLUT4 contents in the soleus muscle membranes compared to the CD group (P<.05, by contrast test; Fig. 2), whereas GLUT4 contents in the quadriceps muscles showed tendencies similar to the soleus muscles, but it was not significant. Glucose utilization in skeletal muscles was not altered by antecedent and present dietary protein sources. Dietary fat amounts in the present diet were a crucial factor in determining glucose utilization in muscles.

3.4. Dietary protein subtypes as well as fat contents in antecedent and present diets modify pancreatic β-cell function and mass

Fig. 3 shows pancreatic β-cell function as the insulin secretion pattern and capacity during HG clamp. Insulin is secreted from pancreatic β-cells in a biphasic pattern, the acute (first) and steady-state (second) phases, against glucose challenge. Serum insulin levels at the acute and steady states represent insulin secretory pattern and capacity. Serum glucose levels were maintained at 11.5 mM during the steady state of HG clamp (Fig. 3A). After 5 min of bolus glucose infusion into the jugular vein, the absolute and relative ratio of first-phase insulin secretion from the basal state did not increase in the AD and WD-AD groups as much as in the CD group (Fig. 3B and C; P<.05, by contrast test). However, AD-WD increased the absolute first-phase insulin secretion and the relative ratio as much as CD-WD (P<.05, by contrast test), suggesting that antecedent protein subtypes did not play a crucial factor for first-phase insulin secretion. First-phase insulin secretion was modified by dietary fat contents and protein subtypes in present diets only. In contrast to first-phase insulin secretion, the absolute values of steady-state insulin secretion were lowered by plant protein in the antecedent and present diets, but not by a high-fat diet (Fig. 3B). WD-AD exhibited improvement in the relative ratios of second-phase insulin secretion from the basal state, compared to WD (P<.05, by contrast test). Compared to the CD-WD group, absolute serum insulin levels and the relative ratio from the basal state of the AD-WD group were lower at the steady state (P<.05, by contrast test). We questioned how AD lowered insulin secretion during first-phase as well as second phase, compared to CD, because whole-body

Table 5
Glycogen and triacylglycerol in soleus and quadriceps muscle

	WD (n=11)	WD-AD (n=11)	AD (n=12)	AD-WD (n=12)	CD (n=11)	CD-WD (n=11)
Glycogen (mg/g tissue)						
Liver	41.5±18.6	45.3±16.7	46.7±17.8	43.3±18.1	45.9±15.1	43.3±13.9
Soleus muscle	4.5±0.8	4.9±1.1	5.4±0.7	4.7±0.9	5.3±0.8	4.5±0.9*
Quadriceps muscle	4.1±0.7	4.3±0.5	4.6±0.8	4.2±0.9	4.7±0.8	4.2±0.8
Triacylglycerol (mg/g tissue)						
Soleus muscle	199.1±36.7	169.4±43.8	147.4±39.5	197.5±34.5	152.5±33.9	198.1±34.5*
Quadriceps muscle	511.4±53.5	458.8±98.5	416.8±89.1	501.8±78.8	428.7±74.5	483.3±86.3*

Values are mean±S.D. Abbreviations are the same as those in Fig. 1.

* P<.05 in main diet effect of WD, AD and CD.

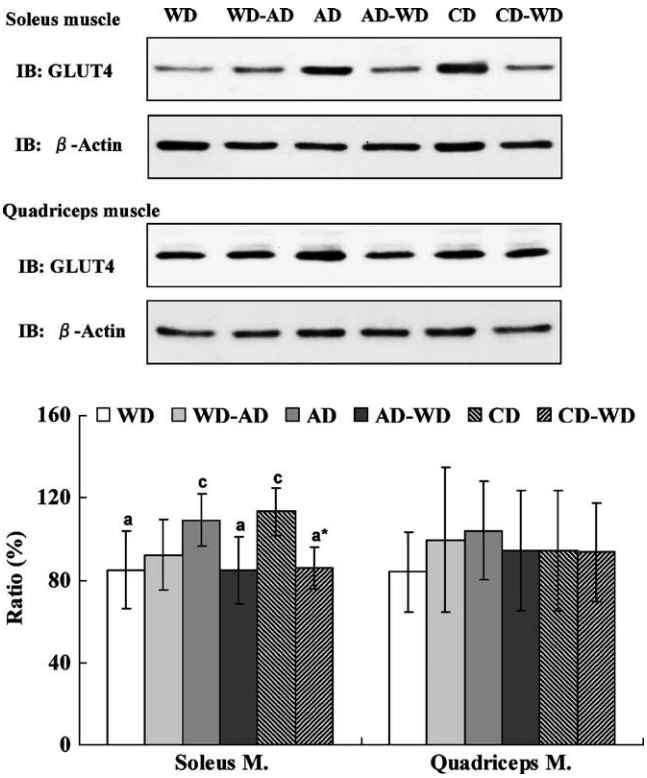

Fig. 2. GLUT4 contents in soleus and quadriceps muscles. At the end of the experiment, total plasma membranes were isolated from soleus and quadriceps muscle from the rats of each group by the methods of Jiang et al. explained in method section. The membranes were lysed with a lysis buffer, and the expression levels of GLUT4 and β-actin were determined by immunoblotting with anti-GLUT4 and β-actin antibodies, respectively. The intensity of protein expression was determined using laser scanning densitometry (BioRad) for quantitation of results. *$P<.05$ in main diet effect of WD, AD and CD. [a]$P<.05$ vs. CD by contrast test.

insulin resistance in AD was as low as CD. Antecedent consumption of a plant protein diet decreased steady-state insulin secretion capacity during conditions of increased insulin resistance such as occurring with consumption of high-fat diets, possibly via modulating pancreatic β-cell mass.

Relative area of pancreatic β-cells evaluated by insulin-stained areas increased with the WD diet, compared to AD and CD diets (Fig. 4A, B, C). After switching diets, the absolute area of β-cells was remarkably higher in the CD-WD group compared to the CD group, but not in the AD-WD group ($P<.05$, by contrast test). Absolute β-cell mass was calculated by the relative β-cell area multiplied by the pancreas weight. Because pancreas weight did not exhibit any significant differences among groups, absolute β-cell mass was revealed to be the same as the relative β-cell area. The differences of pancreatic β-cell mass can be explained by the net of β-cell proliferation and apoptosis. Proliferation of β-cells was influenced by main diets and switching diets (Fig. 4D and E). It was lower in the AD group compared to the WD and CD-WD groups ($P<.05$, by contrast test), but not in the AD-WD group. The red dots in Fig. 4E represent BrdU-incorporated nuclei, which in turn represent proliferating cells. In contrast to proliferation, apoptosis of β-cells was affected only by

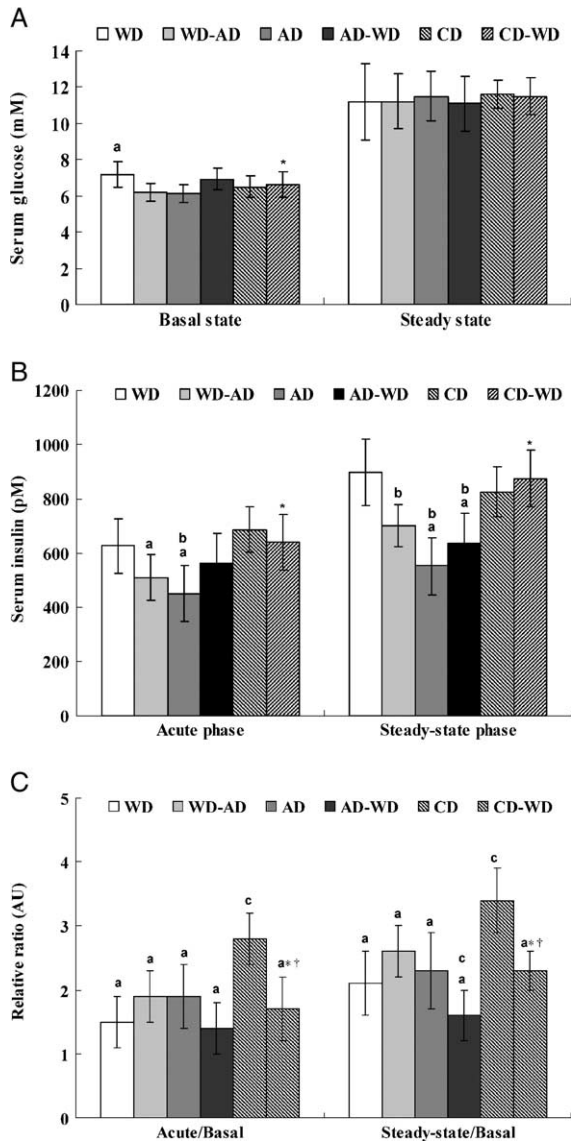

Fig. 3. Insulin secretion from pancreatic β-cells at HG clamp. During HG clamp, bolus glucose (375 mg glucose/kg body weight) was initially infused through the cannula for the first 5 min of the clamp, and 25% glucose was administered through the cannula to maintain serum glucose levels at 11.5 mM (steady state). Serum glucose and insulin levels were measured from blood collected from the carotid artery at 0, 5, 10, 40, 50 and 60 min during the clamp. (A) Serum glucose levels at basal state were the overnight fasted levels prior to the clamp. The steady-state serum glucose levels were maintained at 11.5 mM. (B) Acute insulin secretion was calculated by the average of serum insulin levels at 5 and 10 min at HG clamp. Steady-state insulin secretion was determined when serum glucose concentrations were maintained at 11.5 mM. The values at steady state were calculated by the average of serum insulin levels at 60 and 90 min of HG clamp when serum glucose levels were maintained at 11.5 mM. (C) The relative ratio of insulin secretion of acute state and basal state was determined and the relative ratio of steady state and basal state also measured. Values are mean±S.D. *$P<.05$ in main diet effect of WD, AD and CD. †$P<.05$ in effect of switching diets. [a]$P<.05$ vs. CD by contrast test. [b]$P<.05$ vs. WD by contrast test. [c]$P<.05$ vs. CD-WD by contrast test. Abbreviations are the same as those in Fig. 1.

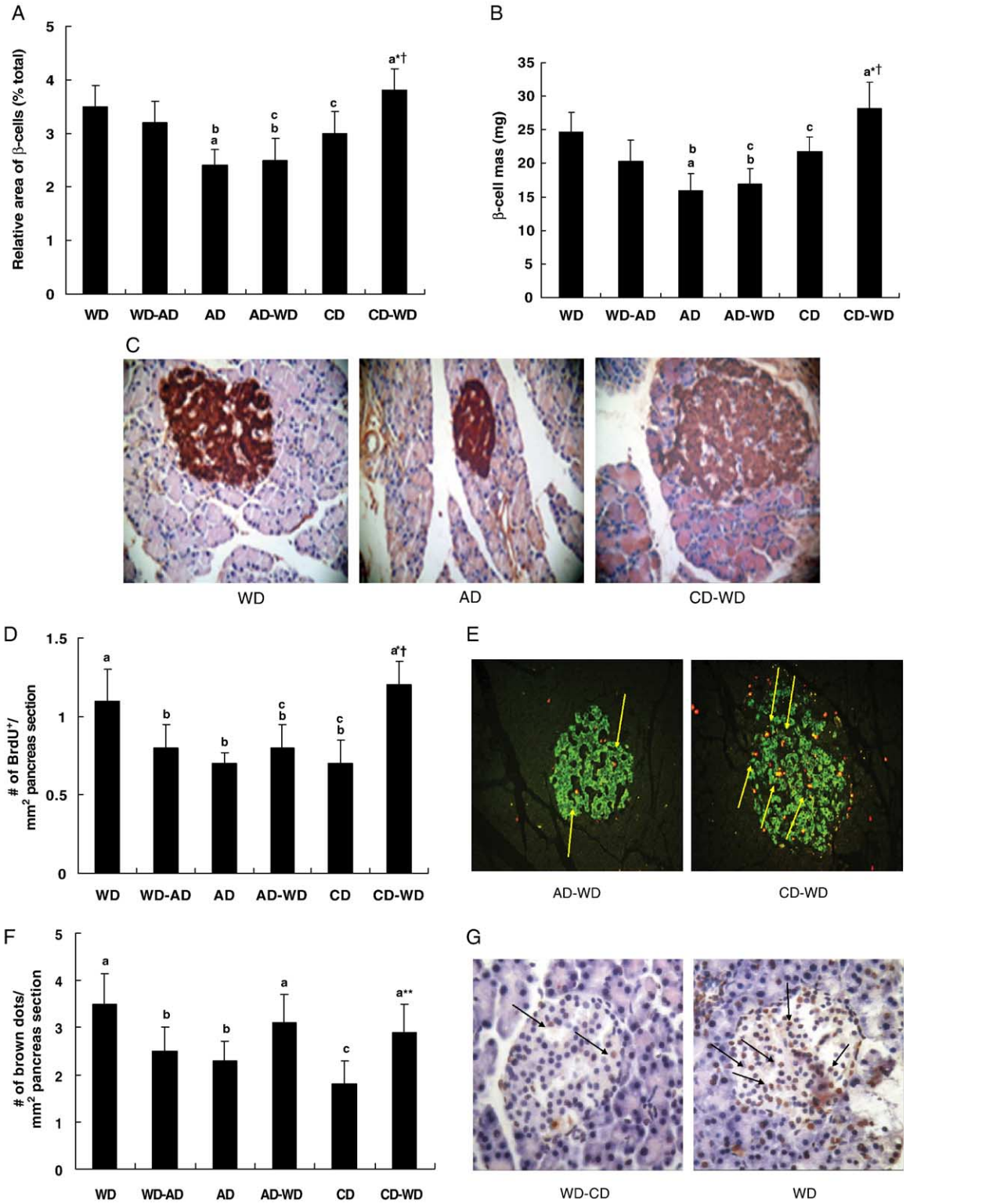

Fig. 4. Pancreatic β-cell area and mass, proliferation and apoptosis. (A, B) The relative area of β-cells was determined by the mean ratio of the area occupied by immunoreactive insulin-positive cells and that occupied by total pancreatic β-cells in pancreas paraffin sections of seven to eight rats. (C) Pancreatic β-cell mass was determined the β-cell area multiplied by the pancreas weight. (D, E) The β-cell proliferation was expressed in the number of BrdU-positive β-cells (red dots) per millimeter squared pancreas, and it was calculated as BrdU-positive β-cells in total β-cell nucleus per pancreas section, two sections per animal and five animals per group. Green color represents insulin-positive cells in E. (F, G) Apoptosis of β-cells was determined by counting apoptotic bodies (brown dots) in total β-cell nucleus, and the calculation was the same as that of β-cell proliferation. The values are mean±S.D. *$P<.05$ in main diet effect of WD, AD and CD. †$P<.05$ in effect of switching diets. [a]$P<.05$ vs. CD by contrast test. [b]$P<.05$ vs. WD by contrast test. [c]$P<.05$ vs. CD-WD by contrast test. Abbreviations are the same as those in Fig. 1.

the corresponding diets, not by the diets in a prior period (Fig. 4F and G). The brown dots in Fig. 4G are the apoptotic bodies in β-cells. It increased the most due to high fat in present diets, but antecedent and present plant protein diets did not affect apoptosis. A plant protein diet in the antecedent period influenced pancreatic β-cell mass mainly by β-cell proliferation, not by apoptosis, after a switch to a high-fat diet from a low-fat diet.

4. Discussion

Asians, including Koreans, have seen a remarkably high rate of development in impaired glucose tolerance and type 2 DM in the past 10 years. Scientists estimate that the rates will increase continuously to encompass approximately 20% of Asian population in 2030 [1–4]. Characteristics of Asian DM are different from those of Westerners. A possible reason is the sudden change in dietary patterns. Asians consumed protein mostly from grains with low fat until 30–40 years ago, but they recently increased their consumption of animal protein and fats [5,6]. We hypothesized that the antecedent plant protein and low-fat diet would accelerate the glucose dysregulation when the diets were switched to a high-fat diet, compared to the antecedent animal protein and low-fat diet. The present study design allowed us to assess insulin resistance, insulin secretion and pancreatic β-cell mass when protein subtypes and fat contents in diets were switched. In this study, peripheral insulin resistance was increased by high-fat contents in the present diet, regardless of protein sources in the antecedent and present diets. Insulin resistance is associated to impaired first-phase insulin secretion. However, plant protein in the present diets attenuated first-phase insulin secretion compared to animal protein, even though insulin resistance was not different between the diets with two protein subtypes. Second-phase insulin secretion was lowered by plant protein in antecedent and present diets and did not increase even after switching to high-fat diets. Thus, plant protein in antecedent diets increased the possibility to fail to compensate for insulin resistance and to develop DM. These changes in second-phase insulin secretion were involved in β-cell expansion via the net of proliferation and apoptosis of β-cells. Plant protein in antecedent and present diets failed to expand β-cells due to decreased proliferation. Antecedent and present consumption of plant protein possibly accelerates and augments the development of glucose dysregulation via decreased insulin secretion capacity and pancreatic β-cell mass when experimental diets are switched to WD. Thus, remarkably increased prevalence of type 2 DM in Asians is associated with a switch from a high-plant protein and low-fat diet to a high-fat diet.

In this study, the comparison between dietary fat subtypes for insulin resistance and insulin secretion was not performed because our previous study demonstrated that a high corn oil diet increased insulin resistance as much as a high butter diet in 90% pancreatectomized rats with mild diabetic symptoms [30]. Rather, we concentrated on whether changing AD to WD aggravated insulin resistance, insulin secretion and pancreatic β-cell mass compared to the continuous consumption of WD. Some studies showed that protein restriction during periods of pregnancy reduced fetal growth and impaired the development of tissues involved in regulating glucose metabolism [31,32]. This retardation was mediated through protein malnutrition, which developed insulin resistance and impaired pancreatic β-cell function and development in offspring [31]. However, it has not been studied whether different protein subtypes affect glucose metabolism in young rats. Because both WD and AD contained sufficient essential amino acids and proteins, the changes of glucose metabolism in AD were not affected by a lack of protein. Although the amino acid composition of glutelin and casein is different, both WD and AD contain the minimum of essential amino acids to avoid protein deficiency. Thus, the metabolic changes in AD were not a result of protein malnutrition. However, low-quality proteins such as grain protein may not be sufficient to modify endocrine function to maintain glucose homeostasis via β-cell growth and survival.

Studies related to the effect of dietary protein on glucose metabolism were mainly of protein restriction or malnutrition [15,16,33]. Low or restricted protein diets generally demonstrated normal or enhanced glucose tolerance and insulin action, but the diets had a blunted insulin secretion response to glucose challenges [33]. However, insulin-stimulated glucose disposal rates were remarkably reduced when protein-restricted diets were switched to high-fat diets. Unlike protein restriction, high-fat diets with different protein subtypes, soy protein and casein did not attenuate whole-body and skeletal muscle insulin resistance, compared to chow diets (a low-fat diet) [34]. These results were consistent with those of the present study. However, rats fed cod protein with a high-fat diet exhibited higher rates of insulin-mediated glucose disposal in muscles that were comparable to those of chow-fed rats. Thus, dietary protein subtypes weakly influenced peripheral insulin resistance. However, the effect of dietary protein subtypes on insulin secretion capacity and pancreatic β-cell mass has not yet been studied.

In general, hyperinsulinemia is thought to be induced to compensate for insulin resistance. However, the relationship between insulin resistance and insulin secretion is controversial. Kahn et al. [35] reported that the relationship between insulin sensitivity and β-cell measures was clearly curvilinear and reciprocal for fasting insulin, first-phase insulin response and β-cell secretory capacity. The curvilinear relationship between insulin sensitivity and β-cell measures could be explained as multiplying insulin sensitivity, and β-cell function is a constant. If the curvilinear relation is broken, in other words, β-cell function did not

increase in a low insulin sensitive state, type 2 DM occurs. Based on Kahn's results [35], rats that consumed AD-WD have a higher probability to develop diabetes than those which continuously consumed WD in our study.

Several studies have showed that insulin secretion capacity is affected by pancreatic β-cell mass [14,36]. Insulin secretion increased in an insulin-resistant state until β-cell mass was sufficiently maintained. Mice lacking insulin receptor substrate-2 (IRS2) gene developed diabetes at 5 weeks of age, which progressed rapidly to severe diabetes and death after 10 to 15 weeks, owing to reduced proliferation and increased apoptosis of β-cells [14]. $IRS2^{-/-}$ mice developed diabetes mainly from the lack of insulin secretion due to decreased β-cell mass when the mice displayed significant insulin resistance [29,30,32]. Increased induction of IRS2 in pancreatic β-cells alleviated diabetes in IRS2 knockout mice, obese mice and streptozotocin-induced diabetic mice [36]. The enlarged β-cell mass along with enhanced insulin secretion is important to prevent the development of diabetes in insulin resistance states and delay its progression. Thus, failure of β-cell growth and its survival can easily lead to DM under insulin-resistant circumstances. This also implies that AD-WD possibly increases the chance to develop diabetes and accelerate the progression.

Our study demonstrates that the antecedent consumption of low-quality protein accelerates and augments the development of impaired glucoregulation after high-fat feeding, possibly due to low pancreatic β-cell mass [15,16,33]. Dietary protein sources, as well as amounts during childhood, are an important factor for establishing insulin sensitivity, pancreatic β-cell mass and insulin secretion for later life. This may explain why the prevalence of diabetes is expected to be much higher in 2030 in Asia compared to Western countries, even though the extent of obesity is much lower in Asia, including Korean [2,4]. A low-fat diet is beneficial for reducing insulin resistance regardless of dietary protein sources. However, the consumption of low-quality proteins, such as plant protein, shows a higher probability to develop and progress into type 2 DM when faced with the circumstances induced by insulin resistance due to a low capacity of insulin secretion and β-cell growth and survival. Therefore, the sufficient consumption of animal protein throughout life, especially during childhood, is important to prevent the development and progression of type 2 DM.

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