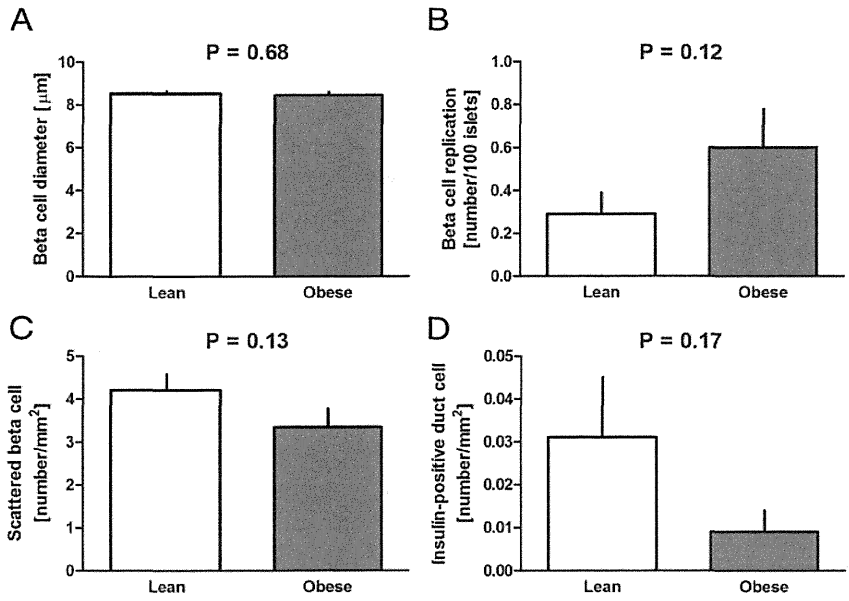

Figure 3. Comparisons of individual β-cell diameter (A), β-cell replication (B), scattered β-cells (C), and insulin-positive duct cells (D) between lean and obese subjects. None of them was significantly changed in obese subjects compared with lean subjects.

8.45 ± 0.79 μm, $P = .68$, Figure 3A). There was no significant difference in β-cell replication between the groups (0.29 ± 0.61 vs 0.60 ± 1.01 per 100 islets, $P = .12$, Figure 3B). Neither scattered β-cells nor insulin-positive duct cells were increased in obese subjects compared with lean subjects (3.4 ± 2.5 vs 4.2 ± 2.4/mm^2, $P = .13$ and $P = .009$ ± 0.029 vs 0.031 ± 0.088/mm^2, $P = .17$, respectively, Figure 3, C and D). β-cell apoptosis (ie, ssDNA positive β-cell) was not detected in either group. The rarity of the β-cell apoptosis was also confirmed by immunostaining for cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase-1 (data not shown).

α-Cell to β-cell ratio

Similarly to β cell area, there was no significant increase in %ACA in obese compared to lean subjects (0.79 ± 0.67 vs 1.06 ± 0.75%, $P = .14$, Figure 4A). The ratio of %ACA to %BCA was not significantly different between the 2 groups (0.91 ± 1.09 vs 0.75 ± 0.57, $P = .47$, Figure 4B). There was a significant positive correlation between the %ACA and the %BCA ($r = 0.46$, $P < .01$, Figure 5).

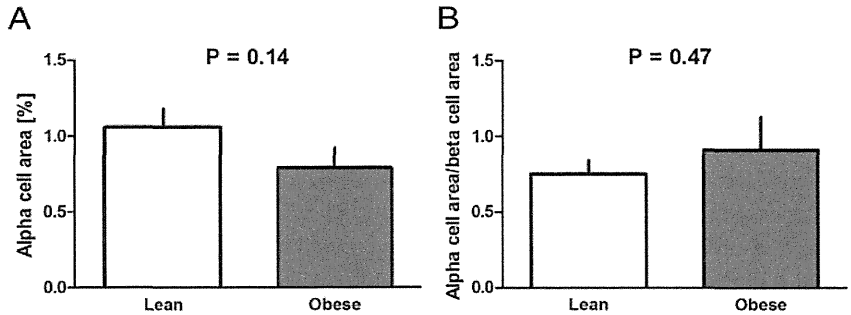

Figure 4. The %ACA (A) and the ratio of %ACA to %BCA (B) in lean and obese subjects are shown. There was no significant difference in %ACA and the ratio of %ACA to %BCA between lean and obese subjects.

Discussion

In this study we demonstrated that in Japanese nondiabetic obese individuals, we found the following: 1) there was no significant increase in BCM, 2) there was no detectable change in β-cell turnover, ie, β-cell replication, β-cell apoptosis, and β-cell neogenesis, and 3) there was no significant change in α-cell to β-cell ratio compared with that in lean individuals.

Type 2 diabetes is characterized by a deficit of BCM (1–4). However, the physiological change in BCM in humans remains largely unknown because of the difficulty accessing the pancreas. To date, BCM can be reasonably measured only by immunohistochemical analysis of the pancreas.

Obesity is an established risk factor for type 2 diabetes (17–19). Insulin sensitivity is decreased in the face of obesity, and insulin secretion is increased to compensate for this decreased insulin sensitivity to maintain normal glucose tolerance (7, 20, 21). However, there are limited data available as to the change in BCM in the presence of obesity in humans. It has been reported that BCM is significantly increased in obese individuals compared with lean individuals, and there is a significant positive correlation between BMI and BCM (2, 4, 8). To our knowledge, however, there are no data available in the Japanese population.

In this study, unexpectedly, BCM was not significantly increased in obese subjects compared with lean subjects. Because we used estimated pancreas volume to assess BCM, this might have affected estimated BCM in this study. However, most importantly, %BCA was significantly decreased in obese subjects compared with lean subjects, which is a striking difference from previous reports in the Caucasian population showing a significant increase in %BCA in obese individuals (8).

To exclude possible confounding factors that could affect BCM, we

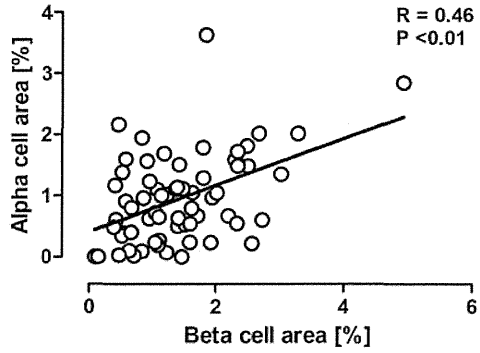

Figure 5. Correlation between %ACA and %BCA. There was a significant positive correlation between α-cell and β-cell area.

performed several subanalyses. It has been reported that the proportion of β-cells is different in the ventral portion of the pancreas head compared with other parts of the pancreas (3). However, we confirmed consistent results in a subanalysis excluding samples obtained from the pancreas head. It has been reported that the pancreas volume starts to decline after 60 years of age (16). Because of the difficulty of obtaining eligible cases, we included subjects aged 20–69 years in this study. Thus, the use of the equation for estimating pancreas volume in those aged older than 60 years might affect our findings. However, the results were consistent in the subanalysis excluding those aged older than 60 years.

We also observed that there was no significant change in β-cell turnover, ie, β-cell replication, β-cell neogenesis, and β-cell apoptosis. We used surrogate markers (ie, scattered β-cells and insulin positive duct cells) to assess β-cell neogenesis because it is not possible to directly assess β-cell neogenesis in humans (1, 22). BCM is regulated by input (ie, β-cell replication and neogenesis) and output (ie, mostly apoptosis) of β-cells (23–25). Butler et al (22) reported a significant (~50%) increase in BCM in pregnant women, accompanied by an increase in small islets and scattered β-cells, suggesting that β-cell neogenesis may contribute to the increase in BCM during pregnancy. Thus, the absence of a significant change in β-cell turnover in obese subjects in this study is also suggestive of no change in BCM in Japanese obese individuals, although it is possible that we were not able to detect any change in β-cell turnover in the obese subjects because the change in insulin sensitivity with obesity is more gradual compared with that with pregnancy.

An absence of increase in BCM in obese subjects was also confirmed by assessing the ratio of α-cell area to β-cell area. It has been reported that α-cell mass and α-cell to β-cell ratio are increased in subjects with type 2 diabetes (2, 3, 26). Whether α-cell to β-cell ratio is changed in the presence of obesity would be of interest. Henquin and Rahier (27) have reported that α-cell mass was not increased in obese subjects compared with lean subjects. In the present study, there was no increase in %ACA in obese subjects, and the ratio of α-cell to β-cell area was not significantly changed in those subjects. Thus, our and previous studies strongly suggest that there is no increase in α-cell mass in the presence of obesity.

Taken together, our findings consistently suggested that BCM was not increased in Japanese nondiabetic obese subjects compared with lean subjects. Although this result is distinct from those of previous reports, most of those studies have been conducted in Caucasian populations (4, 8). There has been one study from Korea reporting a positive correlation between %BCA and BMI; however, the positive correlation was seen in only a small number of nondiabetic subjects (n = 9), and the correlation between BCM and BMI was not reported (2). Recent studies have revealed that there is ethnic variability in the pathophysiology of type 2 diabetes. It is now widely appreciated that Asian populations with type 2 diabetes are leaner than Caucasian populations (28, 29). However, the underlying mechanism of this difference remains unknown. One possible explanation is that there is less β-cell functional capacity in Asians compared with Caucasians (9–11). An increase in insulin secretion in the face of obesity has also been shown in the Japanese population, but the degree of increment may be limited (30, 31). Our findings suggest that lower β-cell functional capacity in Asians may be associated with less β-cell regenerative capacity in response to obesity.

As with other autopsy studies, our study was not free of limitations. First, the cause of death or treatment prior to death might have affected BCM in our study population. Also, we did not have information on the changes in nutritional status or body weight prior to death. However, this should affect both the lean and obese groups. Likewise, illness prior to death, especially malignancy, might cause a loss of body weight at the time of death, which might result in underestimation of BMI. However, because the mean β-cell area of the lean subjects in this study was comparable with that of Caucasians (8), underestimation of BMI was unlikely to be the cause of the comparable BCM in lean and obese subjects observed in this study. We also confirmed our findings in a subanalysis of subjects without malignancy in whom the change in nutritional status prior to death was relatively small.

Mean BMI of obese subjects in this study was lower than that in previous studies in Caucasian populations (4, 8). It can be speculated that the undetectable change in BCM in this study was because of less adiposity in this study population. Although we were not able to assess insulin sensitivity in our study population, it has been reported that Asian people often have a greater degree of

visceral adiposity and higher risk of type 2 diabetes for the same BMI (28, 29, 32, 33), indicating that insulin sensitivity deteriorates at a lower BMI in Japanese compared with Caucasians. Thus, it is likely that the obese subjects in our study had lower insulin sensitivity compared with the lean subjects. Furthermore, previous studies have demonstrated a positive linear correlation between BMI and β-cell area or mass in the range of BMI of our study population (4, 8). In contrast, the correlation between β-cell area and BMI in this study rather tended to be negative. It is possible that BCM may increase in Japanese with more severe obesity comparable with that in Caucasians (ie, BMI $\geq$ 30 kg/m^2), although individuals with BMI $\geq$ 30 kg/m^2 comprise only approximately 2% of the Japanese population (12).

Although we obtained information on the presence of diabetes from medical records, if there were more subjects with undiagnosed diabetes in the obese group than in the lean group, this might result in underestimation of BCM in obese subjects. However, in the subjects in whom we were able to obtain glycated hemoglobin (HbA1c) level within 1 year prior to death (n = 38), none was apparently diabetic [ie, HbA1c $\geq$ 6.5% and random plasma glucose $\geq$ 200 mg/dL (34)] and there was no significant difference in HbA1c level between obese and lean subjects [5.2 $\pm$ 0.5 vs 5.4 $\pm$ 0.8%, $P = .41$, data are expressed as the National Glycohemoglobin Standardization Program value (35)]. Even in the subgroup of subjects whose HbA1c levels were less than 6.0% (n = 31), there was no increase in %BCA and BCM in obese subjects compared with lean subjects (1.16 $\pm$ 0.74 vs 1.92 $\pm$ 1.12%, $P = .03$, and 0.59 $\pm$ 0.37 vs 0.87 $\pm$ 0.51 g, $P = .14$, respectively).

Finally, although we carefully chose a similar study design to that of the previous study in a Caucasian population (8), it should be noted that our findings cannot be directly compared with the findings reported in that study. Technically, the use of a different scanning device or antibodies and the involvement of different investigators might have affected the different findings between the 2 studies. Moreover, because the subjects had been selected from different institutions, a difference in background characteristics of the study populations between the studies may exist. Thus, our findings should be confirmed in another Asian cohort. On the other hand, although we used the same equation to estimate pancreas parenchymal volume, the equation was estimated in a cohort in which the majority were Caucasian (16). Therefore, if the change in pancreas volume in Japanese differs from that in Caucasians, this might have affected our findings. However, a comparable change in pancreas mass or volume with obesity in Japanese has been reported in both anatomical (36) and imaging studies (37). In our previous study mentioned above (16), we also confirmed a similar change in pancreas parenchymal volume with obesity in Asians compared with Caucasians (Saisho, Y., unpublished data). Nonetheless, future investigations to clarify the effect of obesity on pancreas mass in the Japanese population are clearly warranted.

In this study, %BCA was decreased in obese subjects, and there was a significant negative correlation between %BCA and BMI. Based on this finding, one might assume that BCM decreased with obesity in Japanese. However, because of the facts that the significance of the difference between obese and lean subjects disappeared after adjusting for pancreas volume and that there was not an increase in apoptosis, a decrease in β-cell replication, or a decrease in neogenesis in obese subjects, we assume this possibility is unlikely and further studies are needed to address this question.

In conclusion, there was no increase in BCM and no detectable change in β-cell turnover in Japanese nondiabetic obese individuals. Our findings suggest the possibility that β-cell regenerative capacity in Japanese may differ from that in the Caucasian population. To test this hypothesis, we encourage further studies on BCM in different Asian cohorts, and if our findings are confirmed, direct comparison among different ethnicities will be warranted. Further comparison of islet morphology among different ethnic groups may provide an insight into the mechanism of β-cell regeneration in humans.

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The authors made the following contributions: YS and HI conceived and designed the experiments: K.K., Y.S., and T.Y. performed the experiments; K.K. and Y.S. analyzed the data; K.K., Y.S., S.S., T.Y., and H.I. contributed to the discussion; K.K. and Y.S. wrote the manuscript; and K.K., Y.S., S.S., T.Y., and H.I. reviewed/edited the manuscript.

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Clinical Study

Relationship between Stage of Diabetic Retinopathy and Pulse Wave Velocity in Japanese Patients with Type 2 Diabetes

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Objectives. We investigated the relationship between the stage of diabetic retinopathy and pulse wave velocity (PWV). *Methods.* This was a cross-sectional study of 689 patients (406 men and 283 women) with type 2 diabetes who were admitted to our hospital from 2004 to 2007. Brachial-ankle pulse wave velocity (baPWV) was measured by an arterial pressure measurement device as PWV/ABI. Diagnosis of diabetic retinopathy was made by ophthalmologists based on the Davis classification: no diabetic retinopathy (NDR), simple retinopathy (SDR), pre-proliferative retinopathy (pre-PDR), and proliferative retinopathy (PDR). *Results.* There was a significant difference in PWV between patients without diabetic retinopathy (1657.0 ± 417.9 m/s (mean ± SD)) and with diabetic retinopathy (1847.1 ± 423.9 m/s) ($P < 0.001$). In addition, the stage of diabetic retinopathy was associated with aortic PWV (1657.0 ± 417.9 m/s in NDR ($n = 420$), 1819.4 ± 430.3 m/s in SDR ($n = 152$), 1862.1 ± 394.0 m/s in pre-PDR ($n = 54$), and 1901.1 ± 433.5 m/s in PDR ($n = 63$) ($P < 0.001$)). *Conclusions.* In patients with diabetic retinopathy, even in those with SDR, PWV was higher than that in patients without diabetic retinopathy. Physicians should therefore pay attention to the value of PWV and macroangiopathy regardless of the stage of diabetic retinopathy.

1. Introduction

Pulse wave velocity (PWV) has been used as a noninvasive clinical index of aortic stiffness. It is reported that PWV of patients with diabetes is higher than that of healthy subjects [1]. In a Japanese report of more than 10,000 healthy subjects (age 30 to 74 years), it is reported that the mean ± standard deviation values of PWV are 1331.0 ± 242.0 m/s in male and 1207.0 ± 245.0 m/s in female [2]. It is considered that chronic hyperglycemia causes the progression of arterial stiffness. Chronic hyperglycemia also causes progression of diabetic microangiopathy including diabetic retinopathy. Previous studies have shown that two-hour plasma glucose, glycated hemoglobin, and fasting plasma glucose concentrations are predictors of the development of retinopathy and nephropathy [3, 4]. It was reported that the association of hyperglycemia with retinopathy is stronger than that with nephropathy [3]. In addition, microangiopathy is a strong predictor of the development of the more serious long-term complications of diabetes such as blindness, end-stage renal disease, amputation [5], and cardiovascular disease [6]. Previous studies have shown that PWV, a marker of arterial stiffness, is associated with the presence of diabetic retinopathy [7–11].

Retinal capillary microaneurysms are the hallmark of diabetic retinopathy and its earliest reliable sign, and individual acellular capillaries are usually visible histologically in the earliest stages of diabetic retinopathy. As retinopathy becomes more severe, larger patches of acellular capillaries are seen. When lesions like cotton-wool spots, intraretinal microvascular abnormalities, venous beading, and retinal hemorrhages are prominent, diabetic retinopathy is considered pre-proliferative, and new vessels are likely to appear soon on the surface of the retina or optic disc. When new

vessels appear on the surface of the retina or optic disc, diabetic retinopathy is said to have entered the proliferative stage [12].

To our knowledge, no study has compared PWV with the stage of diabetic retinopathy. Therefore, we investigated the relationship between increased PWV and the stage of diabetic retinopathy.

2. Methods

2.1. Subjects. From January 2004 to December 2007, 732 Japanese patients with type 2 diabetes who were admitted to Keio University Hospital (Tokyo, Japan) were consecutively observed. Among them, 43 patients with acute illness (e.g., cardiovascular event, stroke, infection, etc.) were excluded from the evaluation. Consequently, a total of 689 patients with type 2 diabetes who were admitted due to having poor glycemic control were enrolled in this study. All of their purposes of admission were to control glucose metabolism and education for diabetes. The study protocol was approved by the ethical committee of the hospital. Informed consent was obtained from all patients.

2.2. Measurements. The diagnosis of diabetic retinopathy was made by ophthalmologists based on the Davis classification: no diabetic retinopathy (NDR); simple retinopathy (SDR); pre-proliferative retinopathy (pre-PDR); and proliferative retinopathy (PDR).

During hospitalization, fasting plasma glucose (FPG), 2-hour plasma glucose (PG), C-peptide (CPR), hemoglobin A1c (HbA1c), glycated albumin (GA), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen (UN), creatinine (Cr), uric acid (UA) in blood, and 24-hour urine microalbumin were measured. HbA1c was determined by high-performance liquid chromatography (Toso, Tokyo, Japan) and presented as the equivalent National Glycohemoglobin Standardization Program (NGSP) value [13]. Furthermore, we measured systolic/diastolic blood pressure, height, weight, BMI, waist, and hip circumference.

Measurements of brachial-ankle PWV (baPWV) were carried out using an automatic waveform analyzer (Colin Medical Technology Corporation, Japan). Patients lay in the supine position during the test, and occlusion and monitoring cuffs were placed around both the upper and lower extremities. PWV was calculated using the formula: baPWV = $(D1 - D2)/T1$, where $D1$ is the distance from the heart to the left ankle, and $D2$ is the distance from the heart to the right upper arm. These distances were calculated automatically on the basis of the patient's height. The pressure waveforms obtained at two different sites were simultaneously recorded, and the time interval between the initial rise in the brachial and tibial pressure waveforms was determined as $T1$. ABI was calculated using the formula ABI = ankle systolic BP/brachial systolic BP.

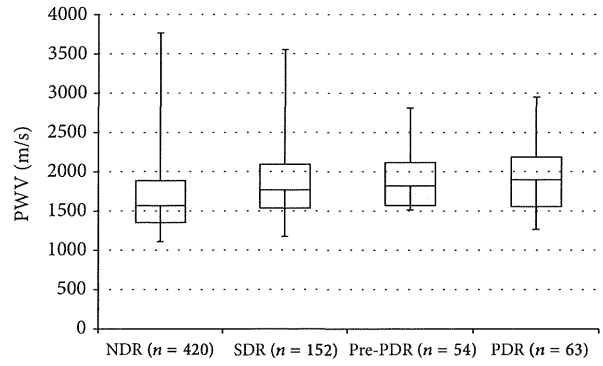

FIGURE 1: Pulse wave velocity (PWV) in NDR (n = 420), SDR (n = 152), pre-PDR (n = 54), and PDR (n = 63). Significant difference among groups ($P < 0.001$) was detected by the Kruskal-Wallis test. NDR: no diabetic retinopathy, SDR: simple diabetic retinopathy, pre-PDR: pre-proliferative diabetic retinopathy, and PDR: proliferative diabetic retinopathy.

2.3. Statistical Analysis. Demographic factors and baseline characteristics were summarized by diabetic retinopathy (DR) and NDR groups. They were compared between the DR and NDR groups using Mann-Whitney U test. Next, the patients were divided into four groups according to the stage of diabetic retinopathy (NDR, SDR, pre-PDR, or PDR) to investigate the relationship between each stage and the value of PWV by the Kruskal-Wallis test. The relationship between the PWV and each factor was evaluated with Spearman's correlation coefficient. The selected variables, which were statistically significant and clinically important, were included in nonparametric multiple regression models to evaluate the association between PWV and each stage of diabetic retinopathy adjusted for some covariates. The purpose of these multivariate analyses was to show the robustness of the results from the univariate analysis.

Data are presented as mean ± standard deviation (SD) in the text and tables. The significance level for all tests was two-sided, at 5%. All analyses were performed using SPSS 17.0 (SPSS; Chicago, IL, USA) and SAS 9.2 (SAS; Cary, NC, USA).

3. Results

Demographic factors and clinical baseline characteristics of patients are shown in Table 1. Among patients, durations of diabetes, age, SBP, and PWV were significantly higher in patients with retinopathy than in those without.

PWV in patients with diabetic retinopathy (1847.1 ± 423.9 m/s) was significantly higher than that in patients without diabetic retinopathy (1657.0 ± 417.9 m/s) ($P < 0.001$). Furthermore, there was a significant positive association between the stage of diabetic retinopathy and PWV. PWV was 1657.0 ± 417.9 m/s in NDR (n = 420), 1819.4 ± 430.3 m/s in SDR (n = 152), 1862.1 ± 394.0 m/s in pre-PDR (n = 54), and 1901.1 ± 433.5 m/s in PDR (n = 63) ($P < 0.001$) (Figure 1).

Some sensitivity analyses were performed to evaluate robustness of the results from the univariate analysis. Factors significantly correlated with the PWV by means of

Table 1: Clinical characteristics of patients with type 2 diabetes mellitus.

	Total	NDR	DR	P value
N (male/female)	689 (406/283)	420	269	
Duration (years)	12.0 ± 10.0	10.0 ± 9.5	16.0 ± 10.0	<0.001
Age (years)	62.2 ± 13.4	61.0 ± 14.3	65.0 ± 11.6	0.002
BMI (kg/m^2)	25.0 ± 5.5	25.6 ± 5.8	24.3 ± 4.7	0.001
SBP (mmHg)	132.0 ± 20.9	130.0 ± 20.0	135.0 ± 22.0	0.009
DBP (mmHg)	76.9 ± 14.0	77.0 ± 13.6	76.0 ± 14.5	0.907
HbA1c (%)	9.6 ± 2.0	9.7 ± 2.2	9.5 ± 1.7	0.233
LDL-C (mg/dL)	127.2 ± 37.1	128.0 ± 37.2	125.9 ± 37.2	0.295
PWV (m/s)	1731.2 ± 430.0	1657.0 ± 417.9	1847.1 ± 423.9	<0.001

Data are shown as mean ± SD. Comparison between patients without diabetic retinopathy (NDR) and with DR by Mann-Whitney's U test.
BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, LDL-C: low-density lipoprotein cholesterol, and PWV: pulse wave velocity. HbA1c is presented as the National Glycohemoglobin Standardization Program (NGSP) value.

Table 2: Relationship between PWV and clinical factors by Spearman's correlation.

	Correlation coefficient	P value
BMI (kg/m^2)	−0.19	<0.001
Age (years)	0.61	<0.001
SBP (mmHg)	0.35	<0.001
DBP (mmHg)	−0.01	0.73
FPG (mg/dL)	−0.14	<0.001
HbA1c (%)	−0.20	<0.001
LDL-C (mg/dL)	−0.05	0.20
HDL-C (mg/dL)	0.00	0.98
TG (mg/dL)	−0.01	0.73
TC (mg/dL)	−0.04	0.33
ABI	0.07	0.06

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, TC: total cholesterol, and ABI: ankle brachial index.

Spearman's correlation coefficient were BMI, age, SBP, FPG, and HbA1c (Table 2). However, we decided that FPG and HbA1c should not be included in the multivariate analysis because they fluctuate by control of diabetes. We evaluated the association between the stage of diabetic retinopathy and PWV adjusted for the above-mentioned covariates by using nonparametric multiple regression analyses. As a result, when taking account of the covariates that have an effect on PWV, PWV tended to increase as the stage of diabetic retinopathy progression ($P < 0.001$).

4. Discussion

Measurement of aortic PWV is considered the gold-standard evaluation of arterial stiffness [14]. Values of PWV in patients with diabetes are higher than those in healthy people in the same generation [15]. The prevalence of arterial stiffness is increased in patients with type 2 diabetes, and these patients are at particularly higher risk for cardiovascular morbidity and mortality. Several studies have shown that diabetic retinopathy is associated with cardiovascular complications [16–18].

In the present study, PWV was significantly higher in patients with diabetic retinopathy than in those without. This finding supports the report that diabetic retinopathy is the microvascular complication with the strongest association with increased aortic stiffness [7]. In addition, there was a relationship between PWV and stage of diabetic retinopathy in Japanese patients with type 2 diabetes, in our study. We showed that the values of PWV in patients with diabetic retinopathy, even in those with SDR, were higher than those in patients without diabetic retinopathy. Henricsson et al. reported that the severity of diabetic retinopathy might be associated with survival, primarily owing to cardiovascular death in patients with diabetes [19]. While the severity of diabetic retinopathy might be important for prediction of macroangiopathy, physicians should pay more attention to macroangiopathy in patients with diabetic retinopathy, regardless of the stage.

Increased arterial stiffness is thought to be related to not only hyperglycemia but also to carbonyl and oxidative stress, chronic inflammation, endothelial dysfunction, and formation of advanced glycation end products (AGEs) [7]. It is reported that PWV is associated with the duration of diabetes and with the accumulation of fluorescent AGEs [20]. Besides, several reports indicate that the stage of diabetic retinopathy correlates with the accumulation of AGEs [21, 22]. Therefore, there is a possibility that the stage of diabetic retinopathy is associated with PWV through the accumulation of AGEs.

Several limitations should be taken into account when considering the results of this study. First, the cross-sectional study design and small sample size for each stage of diabetic retinopathy in our study make it difficult to infer the association between PWV and retinopathy. Second, we could not consider the effects of prescribed medication, for instance, anti-platelet agents, which could influence the state of both retinopathy and arterial stiffness. Third, the raw data might have deviated slightly because there was more than one PWV

technician and ophthalmologist. However, it was thought that the influence of bias was small because the technicians and ophthalmologists were experts and were not aware of this study when they carried out the examinations. Lastly, the patients with type 1 diabetes were not included in this study. In a recent meta-analysis of observational studies, diabetic retinopathy predicted all-cause mortality and cardiovascular events in patients with type 2 diabetes and also type 1 diabetes [18]. Based on these findings, physicians should pay attention to latent macroangiopathy in patients with not only type 2 diabetes but also type 1 diabetes who have diabetic retinopathy, even SDR.

In conclusion, this study suggested that PWV is significantly higher in patients with diabetic retinopathy than in those without, and that there is a relationship between the stage of diabetic retinopathy and PWV in Japanese patients with type 2 diabetes. Physicians should pay attention to latent macroangiopathy in patients with type 2 diabetes who have diabetic retinopathy, even SDR.

Conflict of Interests

The authors declare that they have no conflict of interests.

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ORIGINAL

Marked decline in beta cell function during pregnancy leads to the development of glucose intolerance in Japanese women

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Abstract. The aim of this study is to investigate glucose metabolism longitudinally during pregnancy to explore mechanisms underlying gestational diabetes mellitus (GDM). We reviewed a total of 62 pregnant Japanese women who underwent a 75g oral glucose tolerance test (OGTT) twice during pregnancy (median: early, 13; late, 28 weeks' gestation) because of positive GDM screening. All showed normal OGTT results in early pregnancy. Based on late OGTT, 15 had GDM (late-onset GDM) and 47 normal glucose tolerance (NGT). In early pregnancy, there were no significant differences in insulin sensitivity (insulin sensitivity index derived from OGTT [IS_{OGTT}] and homeostasis model assessment for insulin resistance [HOMA-IR]) and insulin secretion (a ratio of the total area-under-the-insulin-curve to the total area-under-the-glucose-curve [$AUC_{ins/glu}$] and insulinogenic index [IGI]) between the NGT and late-onset GDM groups. In each group, insulin sensitivity significantly decreased from early to late pregnancy, most in the late-onset GDM group (each $p < 0.05$). The insulin secretion showed no significant changes with advancing pregnancy in both of the groups, although late-onset GDM showed significantly lower IGI compared with NGT in late OGTT ($p < 0.05$). When assessed beta cell function by OGTT-derived disposition index (*i.e.* Insulin Secretion-Sensitivity Index-2 and IGI/fasting insulin), the indices significantly decreased from early to late pregnancy in the both groups (each $p < 0.05$). Women with late-onset GDM showed significantly lower indices compared with NGT (each $p < 0.05$). The failure of beta cell to compensate for decreased insulin sensitivity could contribute to the development of the late-onset GDM.

Key words: Insulin sensitivity, Insulin secretion, Disposition index, Glucose metabolism, Pregnancy

IT HAS BEEN widely recognized that insulin sensitivity decreases as pregnancy advances, reaching the nadir in the third trimester [1]. When insulin secretion fails to compensate for the escalated insulin needs during pregnancy, pregnant women are diagnosed to have gestational diabetes mellitus (GDM)[2]. To date, studies on glucose metabolism in pregnant women have shown impaired beta cell function in GDM [3, 4, 5]. As a consequence, beta cell dysfunction is thought to be a potential etiology of GDM [6].

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Several prospective studies in Caucasian population have demonstrated that beta cell function could deteriorate from early to late pregnancy in women with normal glucose tolerance as well as GDM [1, 7]. Especially, women diagnosed with GDM in late pregnancy (*i.e.* late-onset GDM) showed marked decline in beta cell function during pregnancy [3, 5]. This observation might be one explanation that women with a history of GDM are at high risk for the future glucose intolerance (*i.e.* type 2 diabetes) on a background of chronic insulin resistance. However, data on longitudinal changes in glucose metabolism of pregnant Japanese women are unavailable because only cross-sectional studies have been reported [5].

In the current study, we retrospectively examined the glucose metabolism of pregnant Japanese women. Using a cohort of pregnant women undergoing oral

glucose tolerance test (OGTT) twice in early and late pregnancy, alterations in indices of insulin sensitivity, insulin secretion, and beta cell function were examined. Furthermore, the indices in early and late pregnancy were compared between those with and without late-onset GDM.

Methods

Subjects

We conducted a retrospective cohort study of 62 consecutive pregnant Japanese women who underwent the diagnostic OGTT between 2004 and 2010. Each woman met the following criteria: 1) normal OGTT results after the universal early testing based on high-risk characteristics (*i.e.* early OGTT), 2) positive GDM screening using glucose challenge test (GCT) between 24 and 27 weeks of gestation. All women were cared for at the perinatal unit of Keio University Hospital. The gestational age was confirmed in the first trimester by crown-rump length measurements. Excluded from this study were women with multiple pregnancies and women whose neonates exhibited congenital anomalies. The research was performed in accordance with the Declaration of Helsinki and informed consent was obtained from patients where appropriate. The institutional review board at Keio University School of Medicine approved the study.

GDM screening and glucose tolerance test

In our hospital, each woman underwent a two-step screening for GDM: universal testing and a standard 1 h, 50-g GCT in early and late pregnancy, respectively. The universal early testing included the clinical risk factors, as follows: 1) pregestational obesity (BMI $\geq$ 25), 2) past history of gestational diabetes, 3) past history of macrosomia (birth weight $\geq$ 4,000g), and 4) family history of diabetes. If woman has any of the clinical risk factors at early prenatal visit, the diagnostic 75-g OGTT (*i.e.* early OGTT) was performed as soon as feasible after confirming that the random plasma glucose level did not exceed 200 mg/dL. The OGTT was performed after a 12 h overnight fast. Venous blood samples for measurement of plasma glucose levels and insulin concentrations were drawn in the fasting state and at 30 min, 1 h and 2 h after ingestion of the glucose drink. Women with the negative early testing or normal OGTT results underwent a standard 1 h, 50-g GCT between 24 and 27 weeks of gestation as a universal screening. If the GCT result exceeded 140 mg/dL, the diagnostic 75-g OGTT (*i.e.* late OGTT) was then performed.

During the study period between 2004 and 2010, GDM was diagnosed according to the former criteria defined by the Japan Diabetes Society (JDS) if two or more values reached or exceeded the following thresholds: fasting, 100 mg/dL; 1 h, 180 mg/dL; 2 h, 150 mg/dL [8]. Plasma glucose and insulin levels were measured by a glucose oxidase method and enzyme immunoassay, respectively. The normal glucose tolerance (NGT) group comprised women with normal OGTT results in spite of positive GDM screen.

Assessment of insulin sensitivity, insulin secretion and beta cell function

Insulin sensitivity and insulin secretion were evaluated using measurements from the diagnostic OGTT. The insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) and the whole-body insulin sensitivity index derived from the OGTT (IS_{OGTT}). The HOMA-IR was calculated as fasting plasma glucose (mg/dL) x plasma insulin (mU/L) / 405, and the IS_{OGTT} was calculated by the following formula: 10,000 / square root $\{Glu_0 \times Ins_0 \times (Glu_0 + Glu_{60} \times 2 + Glu_{120}) \times 0.5 \times (Ins_0 + Ins_{60} \times 2 + Ins_{120}) \times 0.5\}$, where Glu_y and Ins_y represent plasma glucose (mg/dL) and insulin values (mU/L), respectively, at time y min during the OGTT[9]. Insulin secretion was assessed by the insulinogenic index (IGI: $\{Ins_{30} - Ins_0\} / \{Glu_{30} - Glu_0\}$) and the ratio of the total area under the insulin curve to the total area under the glucose curve ($AUC_{ins/glu}$) during the OGTT. To evaluate beta cell function, we calculated the OGTT-derived disposition index (DIo) using the following measures: Insulin Secretion-Sensitivity Index-2 (ISSI-2: the $AUC_{ins/glu}$ multiplied by IS_{OGTT}) and IGI/fasting insulin [5, 10-12].

Statistical analysis

Data were presented as mean ± SD in text and tables, and illustrated as mean ± SEM in figures. Continuous variables were tested for normality of distribution and were compared between the groups using the unpaired Student's *t* test. Changes in indices of insulin sensitivity, insulin secretion, and beta cell function between the early and late OGTT within each study group (*i.e.* the NGT and late-onset GDM) were assessed by the paired Student's *t* test. Categorical variables were presented as proportions and were assessed with the χ^2 test or

Fisher's exact test. Statistical analysis was performed using the SPSS (version 19.0, IBM, Chicago, IL, USA). $p < 0.05$ was considered as statistically significant.

Results

Maternal demographic characteristics and 75g-OGTT profiles

Of 62 women, 15 were diagnosed to have GDM with late OGTT (*i.e.* the late-onset GDM group) and 47 showed the normal OGTT results (*i.e.* the NGT group). There were no significant differences in maternal age, a history of GDM, family history of diabetes, and gestational weeks at OGTT between the NGT and late-onset GDM groups (Table 1). Maternal body weight gain was comparable between the two groups, although pregravid body weight and BMI in the late-onset GDM group were significantly lower than those in the NGT group ($p < 0.05$).

In early OGTT, plasma glucose levels at 60 and 120 min in the late-onset GDM group was significantly higher than those in the NGT group (Table 2). With regard to late OGTT, the late-onset GDM group showed significantly higher levels of plasma glucose at all time points, compared with the NGT group. When analyzed the insulin profiles, fasting insulin levels in late OGTT significantly increased compared with early OGTT in both of the NGT and late-onset GDM groups. In late OGTT, levels of plasma insulin concentration at 120 min were significantly higher in the late-onset GDM group than those in the NGT group.

Changes in insulin sensitivity, insulin secretion and beta cell function during pregnancy

In early OGTT, the IS_{OGTT} and HOMA-IR were comparable between the NGT and late-onset GDM groups. The IS_{OGTT} significantly decreased from early to late OGTT in the NGT as well as late-onset GDM

Table 1 Maternal demographic characteristics

	NGT (n = 47)	Late-onset GDM (n = 15)
Age (years)	37 ± 5	38 ± 4
Parous (%)	25.5	33.3
Prior GDM (%)	6.8	7.1
Family history of diabetes (%)	31.1	40.0
Pregravid body weight (kg)	63.5 ± 11.1	50.6 ± 11.3*
Pregravid BMI	25.2 ± 4.4	20.3 ± 4.6*
Gestational weeks at early OGTT (weeks)	14 ± 4	14 ± 4
Gestational weeks at late OGTT (weeks)	28 ± 3	29 ± 3
Body weight at late OGTT (kg)	68.5 ± 9.9	56.4 ± 9.9*
Body weight gain by late OGTT (kg)	5.2 ± 4.4	4.3 ± 1.9

NGT; normal glucose tolerance; GDM; gestational diabetes mellitus. * $p < 0.05$ *vs.* the NGT group.

Table 2 Plasma glucose and insulin profiles of early and late OGTT

	NGT (n = 47)		Late-onset GDM (n = 15)	
	early OGTT	late OGTT	early OGTT	late OGTT
Plasma glucose (mg/dL)				
0 min	84 ± 7	84 ± 7	85 ± 7	91 ± 9*#
30 min	141 ± 18	140 ± 17	150 ± 13	156 ± 12*
60 min	143 ± 25	155 ± 20§	159 ± 12*	189 ± 11*#
120 min	129 ± 21	130 ± 17	150 ± 23*	176 ± 26*#
Insulin (mU/L)				
0 min	8.0 ± 4.7	9.2 ± 3.8§	8.7 ± 8.1	11.7 ± 8.8#
30 min	64.8 ± 26.2	65.7 ± 27.2	60.7 ± 33.4	53.1 ± 23.8
60 min	72.9 ± 37.5	79.8 ± 34.5	69.2 ± 39.3	77.5 ± 42.4
120 min	69.3 ± 54.2	72.2 ± 42.0	75.3 ± 48.5	104.4 ± 64.9*#

NGT; normal glucose tolerance, GDM; gestational diabetes mellitus, * $p < 0.05$ *vs.* the NGT group, § $p < 0.05$ for late *vs.* early OGTT of the NGT group, # $p < 0.05$ for late *vs.* early OGTT of the GDM group.

groups ($p < 0.05$, Fig. 1A). Consistent with this observation, HOMA-IR significantly increased in late OGTT compared with early OGTT in both of the groups ($p < 0.05$), most in the late-onset GDM group (Fig. 1B). In the NGT group, pregravid obese women (n = 27) showed significantly lower levels of IS_{OGTT} and higher levels of HOMA-IR compared with non-obese subjects in early and late OGTT (each $p < 0.05$). With regard to early and late OGTT results of late-onset GDM, levels of IS_{OGTT} and HOMA-IR in pregravid obese women (n = 5) were significantly lower and higher than those in non-obese subjects, respectively (each $p < 0.05$).

There were no significant differences in $AUC_{ins/glu}$ and IGI between NGT and late-onset GDM in early OGTT. The $AUC_{ins/glu}$ was comparable between early and late OGTT in the NGT as well as late-onset GDM groups (Fig. 1C). The IGI showed no significant differences between early and late OGTT in both of the NGT and late-onset GDM groups, although the late-onset GDM group showed significantly lower IGI compared with the NGT group in late OGTT (Fig. 1D).

Beta cell function was assessed by validated DIo (*i.e.* ISSI-2 and IGI/fasting insulin, Fig. 1E and F). The ISSI-2 and IGI/fasting insulin at late OGTT signif-

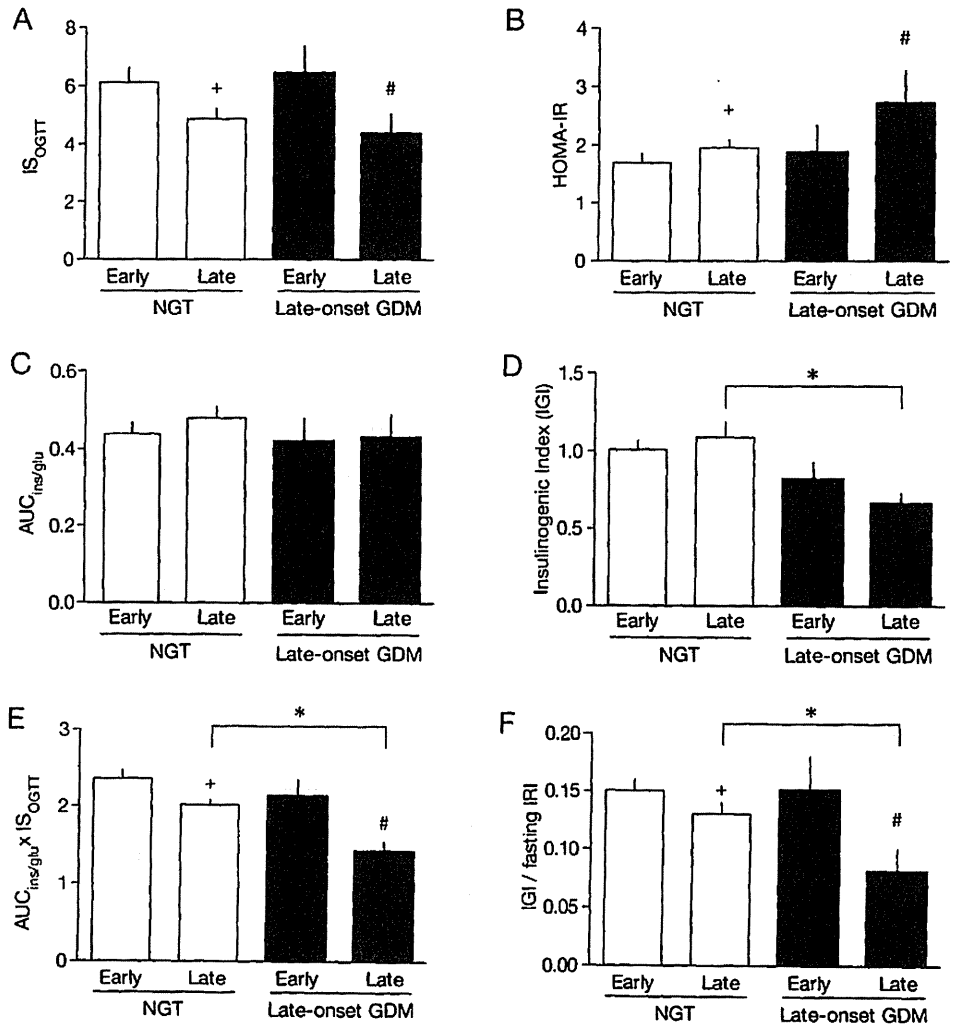

Fig. 1 Insulin sensitivity (A and B), insulin secretion (C and D) and beta cell function (E and F) of early and late OGTT in women with normal glucose tolerance (NGT) and those with late-onset gestational diabetes (late-onset GDM). * $p < 0.05$ *vs.* the NGT group, + $p < 0.05$ for late *vs.* early OGTT of the NGT group, # $p < 0.05$ for late *vs.* early OGTT of the late-onset GDM group.

icantly decreased compared with those at early OGTT in the NGT as well as late-onset GDM groups ($p < 0.05$). Women with late-onset GDM showed significantly lower levels of ISSI-2 and IGI/fasting insulin compared with NGT ($p < 0.05$).

Discussion

The present study demonstrated that 1) beta cell function evaluated using DIo significantly decreased from early to late pregnancy, most in women with late-onset GDM, 2) the possible mechanism of decline in beta cell function during pregnancy could be ascribed to insufficient compensatory increase in insulin secretion against marked decrease in insulin sensitivity. To date, no studies on longitudinal assessment of glucose metabolism during pregnancy in Japanese women have been reported.

Insulin sensitivity decreases with advancing gestation, especially in late pregnancy [1]. It has also been reported that women with GDM have lower insulin sensitivity than those with body weight-matched normal glucose tolerance [2, 3, 5]. In this study, insulin sensitivity assessed by IS_{OGTT} and HOMA-IR significantly deteriorated in late OGTT compared with early OGTT in both of the late-onset GDM and NGT group. There were no significant differences in maternal baseline characteristics between the late-onset GDM and NGT groups, except that pregravid BMI in the late-onset GDM group were significantly lower than those in the NGT group. However, body weight gain from early to late OGTT was comparable between the two groups. Peripheral tissues, probably skeletal muscle, are primarily responsible for disposal of glucose [13, 14]. Therefore, the reduced skeletal muscle mass could be possible contributors to decreased insulin sensitivity. This might be associated with our findings that insulin sensitivity in early pregnancy was comparable between the NGT and late-onset GDM groups, although women with late-onset GDM were leaner than those with NGT. Further studies will be needed to clarify factors related to alterations in insulin sensitivity in late-onset GDM.

Albeit decreased insulin sensitivity in late pregnancy, insulin secretion assessed by $AUC_{ins/glu}$ and IGI did not change from early to late OGTT in the NGT as well as late-onset GDM groups. Consistent with our findings, several studies have shown the minimal increase in insulin secretion from early to late pregnancy[1, 2, 7]. Of interest, women with late-onset GDM showed lower levels of IGI compared with those with NGT at late OGTT. In the Caucasian population, studies on insulin secretion using the intravenous glucose tolerance test revealed that a decrease in early-phase insulin response contributes to the development of late-onset GDM [15]. The IGI is one of the OGTT-derived measures for the early-phase insulin secretion [10]. Similar to the Caucasian population, defective early phase of insulin response could be associated with late-onset GDM in Japanese women.

The beta cell function assessed by DIo significantly decreased from early to late OGTT in the NGT and late-onset GDM groups, with greater deterioration in the late-onset GDM group. In our previous investigation, beta cell dysfunction was demonstrated in Japanese women with late-onset GDM [5], which is similar to Caucasian population [6]. However, alterations in beta cell function during pregnancy in women with late-onset GDM were not investigated. Therefore, we examined the longitudinal changes in ISSI-2 and IGI/fasting insulin in the current study. In this investigation, both of two measures of beta cell function significantly deteriorated during pregnancy in late-onset GDM. As was found in the assessment of insulin secretion, women with late-onset GDM showed lower levels of IGI compared with NGT. Both of the defective initial insulin response and impaired beta cell function seemed associated with late-onset GDM, as are reported in type 2 diabetes [16]. Additionally, we found beta cell dysfunction in women with GDM detected early pregnancy using DIo (unpublished data). Taken all together, beta cell dysfunction seems characteristic of early- and late-onset GDM.

Similar to women with late-onset GDM, those with NGT showed decline in beta cell function from early to late OGTT. In this study, the NGT group comprised of women with normal OGTT results in early and late pregnancy. However, those have positive screen for GDM. It has been reported that a milder degree of glucose intolerance in pregnancy (*i.e.* abnormal GCT with normal OGTT) is related with the future risk of pre-diabetes or diabetes [17]. Our results suggest that those with positive GDM screen are at risk of beta cell dysfunction on a background of decreased insulin sensitivity.

The main limitation of this study is that the number of women examined was small. Since we reviewed clinical data of women who underwent the diagnostic OGTT twice during pregnancy because of positive GDM screening, the number of subjects was lim-

ited. To confirm our findings, studies using a larger cohort of pregnant Japanese women should be performed. The second limitation was that this study was conducted using a cohort of tertiary hospital patients in urban area of Japan. Therefore, most women examined were over the age of 35. Since beta cell function could decline with advancing age [18, 19], some may argue that advanced maternal age could have influence on the results. With regard to analysis performed in this study, maternal age was comparable between those with NGT and late-onset GDM. However, we should be cautious in interpreting absolute values of index examined. It might be of interest to investigate changes in metabolic phenotype of younger pregnant women. Finally, beta cell function (*i.e.* ISSI-2 and IGI/fasting insulin) at early OGTT was not associated with the development of GDM at late OGTT in our study population (data not shown). Because of the observational nature of this study, it is difficult to determine whether beta cell dysfunction is a cause or consequence of the development of late-onset GDM.

The DIo is valid when the relationship between insulin sensitivity and insulin secretion is expressed as a hyperbolic curve [12]. Using a model of log (secretion measures) = constant + $\beta \times$ log (sensitivity measures), a hyperbolic relationship can be established if β is approximately equal to -1, with 95% CI excluding 0. In our previous study cohort including a part of the present study population, mathematical measures have shown the hyperbolic relationship between insulin secretion ($AUC_{ins/glu}$) and sensitivity (IS_{OGTT}) in both of the NGT and late-onset GDM groups in pregnant Japanese women [5, 11], as was found in pregnant Caucasian women [20, 21]. Consistent with previous findings [10, 12], the relationship between IGI and fasting insulin was also hyperbolic in a study cohort of our previous report, although ISSI-2 showed more satisfactory results about the hyperbolic criteria (unpublished data). The hyperbolic relationship of ISSI-2 was reproducible in this study population (*i.e.* β: NGT, -0.8, 95% CI -0.6 to -0.9; GDM: -0.8, 95% CI -0.5 to -0.9). Nonetheless, because of the small sample size of this study, further investigation using a larger cohort is needed.

To our knowledge, this is the first report on longitudinal alterations in glucose metabolism during pregnancy in Japanese women. We have demonstrated a marked decline in beta cell function in women who developed the late-onset GDM, with the underlying mechanism of inadequate increase in insulin secretion against decreased insulin sensitivity. Additionally, adaptive increase in insulin secretion was minimal and beta cell function could deteriorate during pregnancy in women with positive screen for GDM. Our data imply that women with gestational glucose intolerance are likely to develop beta cell dysfunction on a background of chronic insulin resistance.

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ORIGINAL

Association between beta cell function and future glycemic control in patients with type 2 diabetes

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Abstract. The aim of this study was to clarify the association between C-peptide immunoreactivity (CPR), a marker of beta cell function, and future glycemic control in patients with type 2 diabetes. We conducted a retrospective analysis of 513 consecutive patients with type 2 diabetes who were admitted to our hospital between 2000 and 2007 and followed up for 2 years. Serum and urinary CPR levels were measured during admission, and CPR index was calculated as the ratio of CPR to plasma glucose. The associations between these markers at baseline and glycemic control after 2 years were assessed by means of logistic regression models. After 2 years, 167 patients (32.6%) showed good glycemic control (HbA1c <6.9%). Baseline serum and urinary CPR indices were significantly associated with good glycemic control after 2 years, and the postprandial CPR to plasma glucose ratio (postprandial CPR index) showed the strongest association (odds ratio (OR) 1.29, 95% confidence interval (CI) 1.12–1.50, $P = 0.001$) among CPR indices. Multivariate analyses showed consistent results (OR 1.23, 95%CI 1.03–1.48, $P = 0.021$). In conclusion, preserved beta cell function at baseline was associated with better glycemic control thereafter in patients with type 2 diabetes.

Key words: Type 2 diabetes, Glycated hemoglobin, Beta cell function, C-peptide

TYPE 2 DIABETES is characterized by failure of beta cells to compensate insulin demand, which is usually increased with the presence of insulin resistance [1]. The UK Prospective Diabetes Study (UKPDS) has shown that progressive decline of beta cell function was associated with worsening of glycemic control in patients with type 2 diabetes, irrespective of the treatment strategy [2, 3]. In A Diabetes Outcome Progression Trial (ADOPT), patients with type 2 diabetes who failed to maintain adequate glycemic control with initial monotherapy also showed marked deterioration of beta cell function during the trial [4, 5].

Although these studies have revealed the importance of preserving beta cell function to maintain glycemic control in patients with type 2 diabetes, the predictive value of baseline beta cell function for future glycemic control in patients with type 2 diabetes remains unclear. Serum and urinary C-peptide immunoreactivity (CPR) are markers of beta cell function widely used in clinical settings [6, 7]. Since CPR is not extracted by the liver, it reflects endogenous insulin secretion more accurately than does insulin. Therefore, in this study we conducted a retrospective chart review to examine the relationship between baseline CPR values and future glycemic control in patients with type 2 diabetes.

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Abbreviations: CPR, C-peptide immunoreactivity; JDS, Japan Diabetes Society; JNS, Japan Nephrology Society; eGFR, estimated glomerular filtration rate; CV, coefficient of variation; BG, biguanide; TZD, thiazolidinedione; α-GI, α-glucosidase inhibitor; CAD, coronary artery disease; UKPDS, United Kingdom Prospective Diabetes Study; ADOPT, A Diabetes Outcome Progression Trial; NGSP, National Glycohemoglobin Standardization Program; GAD, glutamic acid decarboxylase; IA-2, insulinoma-associated antigen-2; CI, confidence interval; OHA, oral hypoglycemic agent; ROC, receiver operating characteristic; AUC, area under curve; OR, odds ratio

Subjects, Materials and Methods

Initially, we conducted a chart review of 896 patients with type 2 diabetes who were admitted to our hospital between 2000 and 2007, as described elsewhere [8, 9]. Most patients had been admitted to our hospital because of poor glycemic control, and initially received basal-bolus insulin therapy during admission, usually for 1 – 2 weeks. We excluded 3 subjects positive for glutamic acid decarboxylase (GAD) or insulinoma-associated antigen-2 (IA-2) antibody. We also excluded 41 subjects with renal failure defined as serum creatinine level ≥ 2 mg/dL, as renal insufficiency affects CPR level. Beta cell function may be transiently impaired by marked hyperglycemia, so-called glucose toxicity. It has been reported that the beta cell response to glucose is blunted above a plasma glucose level of 180 mg/dL [10]. Therefore, 163 subjects with fasting plasma glucose (FPG) level ≥200 mg/dL on the day of CPR measurement were also excluded, and 689 subjects were judged to be eligible for this analysis. Out of the 689 subjects, 513 (74.5%) who were able to be followed up for 2 years were analyzed in this study (321 men and 192 women, age 63 ± 12 years (mean ± SD), duration of diabetes 12.5 ± 9.6 years, BMI 24.4 ± 4.1 kg/m^2, Table 1). There was no significant difference in clinical characteristics between the patients included in this study and those excluded from this study because the follow-up period was <2 years (N = 176, data not shown). The patients were treated according to the Japan Diabetes Society (JDS) guidelines for treatment of diabetes [11]. Before admission, 50.3% of subjects were treated with a sulfonylurea (SU), 1.8% with a glinide, 15.0% with a biguanide (BG), 8.4% with a thiazolidinedione (TZD), 28.9% with an α-glucosidase inhibitor (α-GI) and 17.0% with insulin (Table 1), and 29.0% were receiving no medication.

This study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the ethical review committee of Keio University School of Medicine, Tokyo, Japan.

Table 1 Baseline characteristics of subjects

N	513
Male (%)	62.6
Age (years)	63 ± 12
Duration of diabetes (years)	12.5 ± 9.6
Family history of diabetes (%)	50.9
BMI	24.4 ± 4.1
HbA1c (%)	9.9 ± 1.8
eGFR (mL/min/1.73m^2)	70.9 ± 24.8
CPR indices	
Fasting plasma glucose (mg/dL)	144 ± 30
Fasting CPR (ng/mL)	1.73 ± 1.00
Fasting CPR index	1.23 ± 0.72
Postprandial plasma glucose (mg/dL)	235 ± 62
Postprandial CPR (ng/mL)	4.31 ± 2.51
Postprandial CPR index	1.94 ± 1.32
Urinary CPR (μg/day)	65.6 ± 51.7
Urinary CPR index	0.47 ± 0.35
Complications	
Diabetic retinopathy (%)	39.2
Diabetic nephropathy (%)	37.2
Diabetic neuropathy (%)	56.7
Hypertension (%)	51.3
Dyslipidemia (%)	42.1
Coronary artery disease (%)	20.1
Stroke (%)	13.1
Medication before admission	
SU (%)	50.3
Glinide (%)	1.8
BG (%)	15.0
TZD (%)	8.4
α-GI (%)	28.9
Insulin (%)	17.0
Medication at discharge	
SU (%)	18.9
Glinide (%)	6.2
BG (%)	12.5
TZD (%)	0.6
α-GI (%)	34.6
Insulin (%)	67.1

eGFR, estimated glomerular filtration rate; CPR, C-peptide immunoreactivity; SU, sulfonylurea; BG, biguanide; TZD, thiazolidinedione; α-GI, α-glucosidase inhibitor. Data are expressed as mean ± SD or percentage.

Measurements

All measurements except CPR measurements were performed on admission by the Department of Laboratory Medicine, Keio University School of Medicine with routine automated laboratory methods. HbA1c was measured by HPLC and expressed as the National Glycohemoglobin Standardization Program (NGSP) value according to the JDS statement [12].

Baseline plasma glucose and serum CPR levels were measured after overnight fasting and 2 h after breakfast during admission, within a few days after admission under basal-bolus insulin therapy using regular and NPH insulin. Insulin therapy was started at a dose of 0.2 - 0.3 U/kg, and then titrated to achieve good glycemic control (FPG <130 mg/dL and postprandial plasma

glucose <180 mg/dL) without hypoglycemia according to the guidelines of the JDS [11]. During basal-bolus insulin therapy, insulin secretagogues (SU and glinides) were discontinued, but insulin non-secretagogues (BG, TZD and α-GI) were usually continued. All patients were receiving the ideal dietary calorie intake calculated from their ideal body weight (*i.e.*, height $(m)^2$ x 22 x 25 kcal/kg; carbohydrate 50-60%, protein 15-20% and fat 20-25% based on a meal-exchange plan) at the times of collection of blood samples. Plasma glucose was measured by glucose oxidase method, and CPR was measured by EIA. Coefficient of variation (CV) of the within-run and between-day precision of CPR was 2.39% and 2.97%, respectively. Fasting and postprandial CPR indices were calculated as follows: fasting or postprandial serum CPR (ng/mL) / fasting or postprandial plasma glucose (mg/dL) x 100, respectively. In addition, 24 h urinary CPR was also measured. Urinary CPR index was calculated as 24 h urinary CPR (μg/day) / FPG (mg/dL).

Estimated glomerular filtration rate (eGFR: mL/min/1.73 m^2) was calculated according to the Statement of the Japan Nephrology Society (JNS) as follows: 194 x serum creatinine (mg/dL) $^{-1.094}$ x age (years) $^{-0.287}$ (x 0.739 for women)[13].

Diabetic complications were evaluated in detail during admission. Diagnosis of diabetic retinopathy was performed by ophthalmologists.

Statistical analysis

Descriptive statistics were calculated for the baseline characteristics. Glycemic control was classified into two groups according to the JDS guidelines as follows: good; HbA1c <6.9% and inadequate; HbA1c ≥6.9% [11]. Homogeneity of the distributions of the baseline factors between the groups was examined with unpaired *t*-test or Fisher's exact test. The association between CPR levels and glycemic control after 2 years was evaluated with a logistic regression model for each CPR parameter. The CPR parameters were standardized (SD = 1, mean = 0) in the analysis to compare the effect on HbA1c after two years among these parameters. Multivariate logistic regression analysis was used to evaluate the effect of postprandial CPR index on glycemic control after 2 years adjusted for other confounders. The association between postprandial CPR index and HbA1c after 2 years was also estimated with Pearson's correlation coefficient. All data are expressed as mean ± SD, and significance levels for all tests were $\alpha = 0.05$ (two-sided). All statistical analyses were conducted using the Statistical Package for the Social Sciences (version 19.0; SPSS, Chicago, IL, USA).

Results

Comparison of baseline characteristics according to glycemic control after 2 years

At discharge, 18.9% of the subjects were being treated with a SU, 6.2% with a glinide, 12.5% with a BG, 0.6% with a TZD, 34.6% with an α-GI and 67.1% with insulin, and only 3.5% were receiving no medication (Table 1).

Mean HbA1c level was 9.9 ± 1.8% on admission and improved to 7.6 ± 1.4% after 2 years (P <0.001 by paired *t*-test). Comparison of baseline characteristics according to glycemic control at 2 years after admission is shown in Table 2. One-third (32.5%) of the subjects had good glycemic control after 2 years (*i.e.*, HbA1c <6.9%).

Serum and urinary CPR indices were higher in those with good glycemic control (all P <0.05). The proportion of male subjects, duration of diabetes, family history of diabetes, and BMI were also significantly different between the groups (all P <0.05). Fewer subjects used medication before admission in those with good control, while there was no significant difference in the proportion of subjects using medication at discharge between the groups. There was no significant difference in frequency of micro- and macrovascular complications between the groups (all P >0.1, data not shown).

Associations between CPR indices and glycemic control

Univariate logistic regression analysis was performed to know the relation of variables shown in Table 2 to the good or inadequate glycemia 2 years later. Results for 6 CPR-related variables were shown in Table 3: none of other variables listed in Table 2 was significantly related to the glycemic outcome 2 years later by the logistic regression analysis. Higher fasting and postprandial CPR indices, and urinary CPR index were significantly related to good glycemic control after 2 years (all P <0.05, Table 3). When the variables were standardized by SD to compare the odds ratios, postprandial CPR index showed the highest odds ratio (OR) among the indices (OR 1.40 for 1 SD increase, 95% confidence interval (CI) 1.15–1.70, P = 0.001, Table 3). There was a significant negative correlation between postprandial CPR index and HbA1c after 2

Table 2 Comparison of baseline characteristics according to glycemic control after 2 years

	Good HbA1c <6.9%	Inadequate HbA1c ≥6.9%	*P* value
N (%)	167 (32.6)	346 (67.4)	-
Male (%)	70.1	59.0	0.015
Age (years)	63 ± 12	63 ± 13	0.972
Duration of diabetes (years)	10.2 ± 9.2	13.5 ± 9.6	<0.001
Family history of diabetes (%)	41.9	55.2	0.006
BMI	23.8 ± 3.6	24.7 ± 4.3	0.015
HbA1c (%)	9.8 ± 2.1	9.9 ± 1.6	0.701
eGFR (mL/min/1.73m^2)	67.9 ± 24.8	72.3 ± 24.6	0.056
CPR indices			
Fasting plasma glucose (mg/dL)	139 ± 31	146 ± 30	0.020
Fasting CPR (ng/mL)	1.83 ± 1.04	1.68 ± 0.97	0.109
Fasting CPR index	1.37 ± 0.80	1.16 ± 0.67	0.005
Postprandial plasma glucose (mg/dL)	227 ± 60	239 ± 62	0.032
Postprandial CPR (ng/mL)	4.77 ± 2.92	4.09 ± 2.27	0.009
Postprandial CPR index	2.24 ± 1.52	1.79 ± 1.18	0.001
Urinary CPR (μg/day)	71.1 ± 56.1	62.9 ± 49.4	0.092
Urinary CPR index	0.52 ± 0.36	0.44 ± 0.35	0.029
Medication before admission			
SU (%)	42.5	54.1	0.018
Glinide (%)	1.2	2.0	0.725
BG (%)	9.6	17.6	0.017
TZD (%)	4.8	10.1	0.042
α-GI (%)	24.6	30.9	0.146
Number of OHA	0.8 ± 1.0	1.1 ± 1.0	0.001
Insulin (%)	6.0	22.3	<0.001
Medication at discharge			
SU (%)	16.8	19.9	0.470
Glinide (%)	6.6	6.1	0.847
BG (%)	12.0	12.7	0.887
TZD (%)	0.0	0.9	0.554
α-GI (%)	30.5	36.5	0.198
Number of OHA	0.7 ± 0.8	0.8 ± 0.8	0.184
Insulin (%)	62.9	69.1	0.162

eGFR, estimated glomerular filtration rate; CPR, C-peptide immunoreactivity; SU, sulfonylurea; BG, biguanide; TZD, thiazolidinedione; α-GI, α-glucosidase inhibitor; OHA, oral hypoglycemic agent. Data are expressed as mean ± SD or percentage.

Table 3 Crude and standardized odds ratios of baseline CPR indices for good glycemic control (HbA1c <6.9%) after 2 years

Variables	Odds ratio (95%CI)	Odds ratio (95%CI) for 1 SD increase	*P* value
Fasting CPR (ng/mL)	1.16 (0.97–1.39)	1.16 (0.97–1.39)	0.110
Fasting CPR index	1.46 (1.14–1.88)	1.32 (1.10–1.58)	0.003
Postprandial CPR (ng/mL)	1.11 (1.03–1.19)	1.30 (1.08–1.56)	0.005
Postprandial CPR index	1.29 (1.12–1.50)	1.40 (1.15–1.70)	0.001
Urinary CPR (μg/day)	1.00 (1.00–1.01)	1.12 (0.93–1.35)	0.096
Urinary CPR index	1.76 (1.05–2.96)	1.22 (1.02–1.46)	0.032

CPR, C-peptide immunoreactivity. A univariate logistic regression model was used for analysis. To compare the odds ratio of each index, the odds ratio of each index was estimated with standardized data.

Table 4 Multivariate logistic regression analysis for good glycemic control (HbA1c <6.9%) after 2 years

Variables	Model 1		Model 2	
	Adjusted odds ratio (95%CI)	*P* value	Adjusted odds ratio (95%CI)	*P* value
Sex (male = 1, female = 0)	1.82 (1.18–2.82)	0.007	1.77 (1.13–2.76)	0.012
Age (years)	1.01 (0.99–1.03)	0.298	1.01 (0.99–1.03)	0.525
Duration of diabetes (years)	0.96 (0.93–0.98)	0.001	0.98 (0.95–1.00)	0.102
Family history of diabetes(yes = 1, no = 0)	0.63 (0.42–0.94)	0.023	0.64 (0.43–0.97)	0.036
BMI	0.90 (0.85–0.95)	<0.001	0.91 (0.86–0.97)	0.004
HbA1c (%)	0.96 (0.85–1.07)	0.429	0.87 (0.76–0.98)	0.025
Postprandial CPR index	1.32 (1.12–1.56)	0.001	1.23 (1.03–1.48)	0.021
Number of OHA before admission	-	-	0.65 (0.51–0.83)	0.001
Insulin use before admission (yes = 1, no = 0)	-	-	0.19 (0.08–0.42)	<0.001
Number of OHA at discharge	-	-	0.90 (0.64–1.27)	0.544
Insulin use at discharge (yes = 1, no = 0)	-	-	1.23 (0.68–2.23)	0.501

CPR; C-peptide immunoreactivity, OHA; oral hypoglycemic agent.

years (r = -0.156, P <0.001). Postprandial CPR index was significantly related to good glycemic control after 2 years in both the subgroup of patients in whom insulin therapy had been introduced at discharge (OR 1.26 for 1 unit increase, 95%CI 1.01–1.57, P = 0.038) and the subgroup in whom it had not (OR 1.32, 95%CI 1.05–1.66, P = 0.019). Furthermore, the association between postprandial CPR index and good glycemic control after 2 years remained significant after adjustment for other covariates including medication which related to good glycemic control in Table 2 (OR 1.23 for 1 unit increase, 95%CI 1.03–1.48, P = 0.021, Table 4). However, receiver-operating characteristic (ROC) analysis of postprandial CPR index for predicting good glycemic control revealed a significant but small area under the curve (AUC) (0.597, 95%CI 0.542–0.651, P <0.001, cut-off value: 1.99 with 47.9% sensitivity and 69.4% specificity).

Discussion

In this study, we showed that postprandial CPR index was associated with future glycemic control in patients with type 2 diabetes. Logistic regression analysis estimated that a 1 unit increase in postprandial CPR index was associated with an ~30% increase in the odds of achieving good glycemic control after 2 years. The association between deterioration of beta cell function and worsening of glycemic control has been shown in prospective studies such as UKPDS and ADOPT [2, 5]. However, a correlation between baseline beta cell function and future glycemic control was not reported in those trials. Although several studies have examined the correlation between CPR level and treatment outcome [14, 15], the correlation remains to be established and there are few available data, especially in the Japanese population. The present study showed an association between baseline CPR and future glycemic control in the Japanese population, using our cohort with a relatively large sample size and long duration of follow-up. Although this study had a retrospective design, our results reflect the "real world" situation.

In this study, we used CPR to plasma glucose ratio *i.e.*, CPR index, as a marker of beta cell function. It is of note that when CPR and plasma glucose levels were separately included in the multivariate logistic regression model, the association between CPR itself and future glycemic control remained significant (data not shown). Since an advantage of CPR measurement is that it reflects endogenous insulin secretary capacity even under insulin treatment, CPR measurement can be used widely in clinical settings.

In this study, we further compared the predictive value among CPR indices. As a result, postprandial CPR index showed the greatest odds for predicting future good glycemic control compared with fasting CPR index or urinary CPR index, while 95%CI of OR for CPR indices mostly overlapped each other. This result is consistent with our previous finding that postprandial CPR index showed the greatest odds for predicting future insulin therapy compared with other CPR indices [8, 16]. The reason for this may be that CPR in a postprandial state more closely reflects the maximal functional capacity of beta cells compared with a