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【 ORIGINAL ARTICLE 】

Association of the Glycemic Fluctuation as well as Glycemic Control with the Pancreatic β-cell Function in Japanese Subjects with Type 2 Diabetes Mellitus

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Abstract:

Objective It is important to preserve the pancreatic β-cell function in order to maintain good glycemic control for a long period. The aim of this study was to examine which factors are associated with the β-cell function in subjects with type 2 diabetes mellitus.

Methods A total of 372 subjects with type 2 diabetes who had been hospitalized for the amelioration of their glycemic control and/or education about diabetes in Kawasaki Medical School Hospital were included in this study. We evaluated the remnant β-cell function as the HOMA-%β using the computer software program HOMA2 and estimated the glycemic fluctuation with the glycoalbumin (GA)/hemoglobin A1c (HbA1c) ratio. In addition, we divided the subjects into a relatively young group (<65 years old) (n=210) and an elderly group (≥65 years old) (n=162) and performed several analyses in each group.

Results The GA/HbA1c ratio, GA and HbA1c were independent determinant factors for the HOMA-%β regardless of age. We obtained almost the same results even after excluding those subjects using insulin secretagogues. These data suggest that the glycemic fluctuation and glycemic control are associated with the remnant β-cell function in Japanese subjects with type 2 diabetes.

Conclusion It is very important to reduce glycemic fluctuation as well as to maintain good glycemic control in order to preserve β-cell function in subjects with type 2 diabetes.

Key words: type 2 diabetes mellitus, fluctuation of the blood glucose levels, pancreatic β-cell function

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Introduction

Pancreatic β-cells promptly secrete insulin in response to the alteration of blood glucose levels in healthy subjects, which is very important for maintaining good glycemic control. Under diabetic conditions, however, when β-cells are chronically exposed to hyperglycemia, the β-cell function is gradually deteriorated, and β-cells fail to secrete sufficient amounts of insulin (1-6). This phenomenon is known as β-cell glucose toxicity in clinical practice and the field of islet biology research. Therefore, it is very important to know which factors are associated with the β-cell function and how we can preserve the β-cell function in subjects with type 2 diabetes mellitus. Such information will prove important for maintaining good glycemic control for a long period in subjects with type 2 diabetes.

However, fluctuating blood glucose levels and repeated hypoglycemia can lead to the exacerbation of various clinical findings. For example, it is known that the fluctuation of the blood glucose levels leads to the development of atherosclerosis or dementia, especially in elderly subjects (7-9). Furthermore, repeated hypoglycemia leads to the development of ischemic heart disease, fundus hemorrhaging, dementia and hypoglycemia unawareness (10, 11). However, whether or not such blood glucose fluctuations are associ-

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ated with the deterioration of the pancreatic β-cell function is unclear. In addition, the β-cell function appears to be more easily damaged by various stimuli, including blood glucose fluctuation, in elderly subjects with type 2 diabetes than in those without diabetes (12).

The glycoalbumin (GA)/hemoglobin A1c (HbA1c) ratio is reportedly a good marker for the fluctuation of the blood glucose levels, regardless of the degree of glycemic control (13-16). Albumin is known to be much more easily glycosylated than Hb (17). For example, after a meal, glucose binds more easily to albumin than Hb. Therefore, as the changes in the blood glucose level increase, the GA/HbA1c ratio increases as well. Indeed, it has been shown GA/HbA1 c ratio is closely correlated with the fluctuation of the blood glucose levels as assessed by continuous glucose monitoring (15).

The aim of this study was to examine which factors, including the fluctuation of the blood glucose levels, are associated with the pancreatic β-cell function in subjects with type 2 diabetes mellitus.

Materials and Methods

Study population

Subjects with type 2 diabetes mellitus who had been hospitalized for the amelioration of their glycemic control and/or education about diabetes in Kawasaki Medical School Hospital from April 2009 to March 2011 were included in this study. Patients with type 2 diabetes mellitus underwent laboratory tests on fasting. We excluded those with type 1 diabetes mellitus, some hepatic diseases (e.g., viral hepatitis or liver cirrhosis), renal failure [estimated glomerular filtration rate (eGFR) <50 mL/min/1.73 m^2], and/or using steroid drugs for the treatment of a disease. The study protocol was approved by the hospital ethics committee (No. 2519).

We used HOMA-%β as a marker of the remnant β-cell function and the GA/HbA1c ratio as a marker of the fluctuation of the blood glucose levels. HOMA-%β was calculated with the computer software program HOMA2 (Homeostasis Model Assessment calculator, released by Oxford University, UK, in 2004) utilizing the fasting plasma glucose (FPG) and insulin or C-peptide levels (18).

Blood glucose was measured by the oxygen electrode method with glucose oxidase (GOD) and serum C-reactive protein (CRP) measured by a chemiluminescent enzyme immunoassay (CELIA). Apolipoprotein B and apolipoprotein E were measured by a turbidimetric immunoassay (TIA).

Statistical analyses

All analyses were performed by using the JMP software program, version 9 (SAS Institute, Cary, USA). A Spearman's rank correlation analysis was performed to examine the association between the HOMA-%β and various clinical parameters. Furthermore, a multivariate regression method was used to determine which factors were independent determinants of the HOMA-%β. We performed multivariate regression analyses in various models as follows: (Model 1) including age, gender, the body mass index (BMI) and the GA/HbA1c ratio as independent variables; (Model 2) including the BMI, GA/HbA1c ratio and duration of diabetes as independent variables; (Model 3) including the age, gender, BMI, GA/HbA1c ratio and duration of diabetes as independent variables; (Model 4) including the age, gender, BMI, GA/HbA1c ratio, duration of diabetes, presence of hypertension and presence of dyslipidemia as independent variables.

In this study, we performed multivariate regression analyses by evaluating various statistical models as described above in order to obtain the same results even after adjusting for various factors that might influence the results. In the multivariate analyses, we basically included clinical parameters that were associated with the HOMA-%β in univariate analyses and/or basic adjustment factors, such as the age and gender as independent variables.

Results

Characteristics of the study subjects

In our hospital, we routinely measure various parameters in subjects who are hospitalized for the amelioration of glycemic control and/or education about diabetes. The clinical characteristics of all subjects (n=372) were as follows: men/women, 209/163; age, 60.2±14.5 years old [mean±standard deviation (SD)]; BMI, 25.7±5.2 kg/m^2; duration of diabetes, 11.4±9.2 years; HbA1c, 9.3±1.8%; GA, 26.5±7.5%; FPG, 173.3±67.2 mg/dL; GA/HbA1c, 2.82±0.52; HOMA-%β, 46.6±39.8; systolic blood pressure, 130.6±15.4 mmHg; diastolic blood pressure, 72.5±9.9 mmHg; Creatinine, 0.73±0.23 mg/dL; BUN, 17.1±7.3 mg/dL; Total cholesterol, 197.5±39.8 mg/dL; LDL cholesterol, 120.4±33.8 mg/dL; HDL cholesterol, 50.9±18.3 mg/dL; triglyceride, 135.3±71.8 mg/dL; apolipoprotein B, 99.4±24.9 mg/dL; apolipoprotein E, 4.46±2.97 mg/dL; CRP, 0.61±1.84 mg/dL; urinary albumin excretion, 31.4±46.7 mg/gCre (Supplementary material 1). In addition, the pancreatic β-cell function appeared to be more easily damaged by the fluctuation of the blood glucose levels in elderly subjects with type 2 diabetes than in relatively young subjects, although no conclusive evidence on this point was available. Therefore, we divided the subjects into 2 groups: a relatively young group (<65 years old) (n=210) and an elderly group (≥65 years old) (n=162). We then performed several analyses in each group as well as in all subjects. Supplementary material 1 shows the clinical characteristics of the subjects in the relatively young and elderly groups as well as in all subjects.

Glycemic fluctuation and glycemic control are associated with the pancreatic β-cell function in subjects with type 2 diabetes mellitus

To examine which factors were associated with the HOMA-%β, we performed a univariate analysis. As shown

Table 1. Univariate Analysis Evaluating the Association between HOMA-%β and Various Clinical Parameters in All Subjects in This Study.

Clinical parameter	All subjects		<65 years old		≥65 years old	
	ρ	p	ρ	p	ρ	p
Age	-0.099	n.s.	-0.058	n.s.	-0.046	n.s.
Duration of diabetes	-0.274	<0.0001	-0.190	0.0167	-0.307	0.001
BMI	0.359	<0.0001	0.347	<0.0001	0.341	<0.0001
GA/HbA1c ratio	-0.451	<0.0001	-0.468	<0.0001	-0.418	<0.0001
HbA1c	-0.341	<0.0001	-0.415	<0.0001	-0.309	<0.0001
Glycoalbumin	-0.560	<0.0001	-0.600	<0.0001	-0.500	<0.0001
Systolic blood pressure	0.004	n.s.	-0.044	n.s.	0.064	n.s.
Diastolic blood pressure	0.045	n.s.	0.022	n.s.	0.031	n.s.
Total cholesterol	-0.020	n.s.	-0.068	n.s.	0.018	n.s.
HDL cholesterol	-0.118	0.025	-0.073	n.s.	-0.140	n.s.
LDL cholesterol	0.152	n.s.	-0.049	n.s.	0.007	n.s.
Triglyceride	0.152	0.010	0.018	n.s.	0.280	0.002
Apolipoprotein B	0.055	n.s.	-0.006	n.s.	0.043	n.s.
Apolipoprotein E	0.124	0.040	0.074	n.s.	0.165	n.s.
Creatinine	0.026	n.s.	0.085	n.s.	0.035	n.s.
BUN	-0.003	n.s.	-0.005	n.s.	0.093	n.s.
CRP	0.092	n.s.	0.066	n.s.	0.100	n.s.
Urinary albumin excretion	-0.080	n.s.	-0.130	n.s.	-0.015	n.s.

BMI: body mass index, GA: glycoalbumin, LDL: low-density lipoprotein, HDL: high-density lipoprotein, CRP: C-reactive protein, n.s.: not significant

in Table 1, a very close association was noted between the HOMA-%β and various factors, including HbA1c, GA, duration of diabetes and the BMI (p<0.0001 in all 4 variables). Interestingly, a very close association was also noted between the HOMA-%β and the GA/HbA1c ratio (p<0.0001) (Table 1). In addition, we performed the same analyses in the relatively young and elderly groups. As shown in Table 1, in both groups, there were very close associations between the HOMA-%β and various factors, including HbA1c, GA and the BMI (p<0.0001 in all 3 variables). Furthermore, in both groups, there was a very close association between the HOMA-%β and the GA/HbA1c ratio (p<0.0001) (Table 1).

Next, to identify the independent determinant factors for the HOMA-%β, we performed multivariate regression analyses including the age, gender, BMI and GA/HbA1c ratio as independent variables (Table 2, Model 1). The results showed that the BMI and GA/HbA1c ratio were independent factors contributing to the HOMA-%β (p=0.004 and p< 0.0001, respectively). We performed the same analysis including the BMI, GA/HbA1c ratio and duration of diabetes as independent variables (Table 2, Model 2). Similar results were obtained, with the GA/HbA1c ratio and duration of diabetes found to be independent factors contributing to the HOMA-%β (p<0.0001 and p=0.014, respectively). In addition, we performed the same analysis including the age, gender, BMI, GA/HbA1c ratio and duration of diabetes as independent variables (Table 2, Model 3). Similar results were obtained again, with the GA/HbA1c ratio, duration of diabetes and age found to be independent factors contributing to HOMA-%β (p<0.0001, p=0.001 and p=0.008, respectively). Finally, we performed the same analysis including the age, gender, BMI, GA/HbA1c ratio, duration of diabetes, presence of hypertension and presence of dyslipidemia as independent variables (Table 2, Model 4). Similar results were obtained again, with the GA/HbA1c ratio, duration of diabetes and presence of hypertension found to be independent factors contributing to the HOMA-%β (p<0.0001, p= 0.001 and p=0.029, respectively). Taken together, these findings indicate that GA/HbA1c ratio was the strongest independent factor contributing to the HOMA-%β in subjects with type 2 diabetes mellitus.

Next, we performed the same analyses in the relatively young and elderly groups. As shown in Table 2, in both groups, the GA/HbA1c ratio was an independent factor contributing to the HOMA-%β in all models. There were no marked differences between the relatively young and elderly subjects. These data suggest that glycemic fluctuation is an independent factor contributing to the HOMA-%β, regardless of age, in subjects with type 2 diabetes mellitus.

Since GA and HbA1c were closely associated with the HOMA-%β (Table 1), we examined whether or not GA or HbA1c were also independent determinant factors for the HOMA-%β. We performed multivariate regression analyses including the same independent variables used in Table 2 except for GA/HbA1c being switched for GA (Supplementary material 2A) or HbA1c (Supplementary material 2B). In all models, both GA and HbA1c were independent determinant factors contributing to the HOMA-%β in subjects with type 2 diabetes (Supplementary material 2A and B). As the GA/HbA1c and GA are known to be markers of the glycemic control, we believe that glycemic control is an inde-

Table 2. Multivariate Analysis Evaluating the Association between HOMA-%β and Various Clinical Parameters Including GA/HbA1c Ratio in All Subjects.

Model 1	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.071	1.37	n.s.	0.043	0.64	n.s.	0.025	0.34	n.s.
Gender	-0.008	-0.16	n.s.	-0.015	-0.24	n.s.	0.009	0.12	n.s.
BMI	0.161	2.91	0.004	0.122	1.66	n.s.	0.191	2.44	0.016
GA/HbA1c ratio	-0.383	-7.04	<0.0001	-0.415	-5.89	<0.0001	-0.310	-3.91	0.0001
Model 2	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
BMI	0.047	0.76	n.s.	0.006	0.07	n.s.	0.137	1.56	n.s.
GA/HbA1c ratio	-0.347	-5.55	<0.0001	-0.421	-5.15	<0.0001	-0.237	-2.65	0.009
Duration of diabetes	-0.140	-2.47	0.014	-0.102	-1.38	n.s.	-0.240	-2.82	0.006
Model 3	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.171	2.69	0.008	0.167	2.10	0.037	0.089	1.04	n.s.
Gender	-0.014	-0.25	n.s.	-0.010	-0.41	n.s.	-0.002	-0.02	n.s.
BMI	0.096	1.52	n.s.	0.057	0.67	n.s.	0.141	1.60	n.s.
GA/HbA1c ratio	-0.368	-5.85	<0.0001	-0.419	-5.16	<0.0001	-0.240	-2.63	0.010
Duration of diabetes	-0.197	-3.26	0.001	-0.1496	-1.94	n.s.	-0.256	-2.93	0.004
Model 4	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.126	1.90	n.s.	0.148	1.79	n.s.	0.068	0.80	n.s.
Gender	-0.015	-0.27	n.s.	-0.013	-0.18	n.s.	0.017	0.20	n.s.
BMI	0.071	1.09	n.s.	0.047	0.54	n.s.	0.097	1.05	n.s.
GA/HbA1c ratio	-0.365	-5.74	<0.0001	-0.424	-5.08	<0.0001	-0.228	-2.51	0.013
Duration of diabetes	-0.200	-3.32	0.001	-0.143	-1.84	n.s.	-0.282	-3.27	0.001
Hypertension	-0.125	-2.19	0.029	-0.072	-0.96	n.s.	-0.213	-2.47	0.015
Dyslipidemia	0.021	0.37	n.s.	0.038	0.50	n.s.	0.017	0.19	n.s.

BMI: body mass index, GA: glycoalbumin, n.s.: not significant

pendent factor contributing to the HOMA-%β in subjects with type 2 diabetes mellitus.

In addition, we performed the same analyses in the relatively young and elderly groups. As shown in Supplementary material 2A, B, in both groups, GA and HbA1c were independent factors contributing to the HOMA-%β. No marked differences were noted between the relatively young and elderly subjects. These data suggest that glycemic control is an independent factor contributing to the HOMA-%β, regardless of age, in subjects with type 2 diabetes mellitus.

Glycemic fluctuation and glycemic control are associated with the pancreatic β-cell function only in subjects with type 2 diabetes mellitus not using insulin secretagogues

It is likely that the HOMA-%β is influenced by the usage of insulin secretagogues. Therefore, we performed the same analyses only in subjects not using insulin secretagogues (n=267). "Insulin secretagogues" in this manuscript refers to sulfonylurea and DPP-4 inhibitor but does not include glinide (Supplementary material 3). We also divided the subjects into two groups by age: a relatively young group (<65 years old) (n=158) and an elderly group (≥65 years old) (n=109), and performed several analyses in each group as well as in all subjects not using insulin secretagogues. Supplementary material 3 shows the clinical characteristics of the subjects in the relatively young and elderly groups as well as in all subjects.

As shown in Table 3, almost the same results were obtained in the subjects not using insulin secretagogues as in all subjects. In the univariate analyses, the GA/HbA1c, GA and HbA1c were closely associated with the HOMA-%β in all subjects not using insulin secretagogues (ρ=-0.442, p<0.0001, ρ=-0.530, p<0.0001, ρ=-0.353, p<0.0001, respectively) (Table 3). We also performed the same analyses in the relatively young and elderly groups. As shown in Table 3, in both groups, the GA/HbA1c ratio, GA and HbA1c were associated with the HOMA-%β in various models. There were no marked differences between the relatively young and elderly subjects. These data suggest that glycemic fluctuation and glycemic control are associated with the HOMA-%β, regardless of age, in subjects with type 2 diabetes mellitus not using insulin secretagogues.

Next, to identify the independent determinant factors for the HOMA-%β, we performed multivariate analyses in all subjects with type 2 diabetes mellitus not using insulin

Table 3. Univariate Analysis Evaluating the Association between HOMA-%β and Various Clinical Parameters in All Subjects without Using Insulin Secretagogues.

Clinical parameter	All subjects		<65 years old		≥65 years old	
	ρ	p	ρ	p	ρ	p
Age	-0.103	n.s.	-0.036	n.s.	-0.057	n.s.
Duration of diabetes	-0.2296	0.001	-0.193	0.038	-0.186	n.s.
BMI	0.386	<0.0001	0.365	<0.0001	0.371	<0.0001
GA/HbA1c ratio	-0.469	<0.0001	-0.461	<0.0001	-0.476	<0.0001
HbA1c	-0.368	<0.0001	-0.427	<0.0001	-0.363	0.0001
Glycoalbumin	-0.582	<0.0001	-0.579	<0.0001	-0.592	<0.0001
Systolic blood pressure	0.043	n.s.	-0.020	n.s.	0.014	n.s.
Diastolic blood pressure	0.016	n.s.	0.012	n.s.	-0.006	n.s.
Total cholesterol	-0.008	n.s.	-0.057	n.s.	0.018	n.s.
HDL cholesterol	-0.088	n.s.	-0.035	n.s.	-0.112	n.s.
LDL cholesterol	-0.054	n.s.	-0.097	n.s.	-0.064	n.s.
Triglyceride	0.166	0.019	0.071	n.s.	0.252	0.022
Apolipoprotein B	0.032	n.s.	-0.008	n.s.	-0.021	n.s.
Apolipoprotein E	0.129	n.s.	0.073	n.s.	0.178	n.s.
Creatinine	-0.018	n.s.	0.035	n.s.	0.001	n.s.
BUN	-0.040	n.s.	-0.055	n.s.	0.074	n.s.
CRP	0.040	n.s.	-0.023	n.s.	0.049	n.s.
Urinary albumin excretion	-0.037	n.s.	-0.055	n.s.	-0.011	n.s.

BMI: body mass index, GA: glycoalbumin, LDL: low-density lipoprotein, HDL: high-density lipoprotein, CRP: C-reactive protein, n.s.: not significant

secretagogues. As shown in Table 4 and Supplementary material 4A and B, the GA/HbA1c ratio, GA and HbA1c were determinant factors for the HOMA-%β in various models. We also performed the same analyses in the relatively young and elderly groups. As shown in Table 4 and Supplementary material 4A and B, in both groups, the GA/HbA1c ratio, GA and HbA1c were determinant factors for the HOMA-%β in various models. There were no marked differences between the relatively young and elderly subjects. These data suggest that glycemic fluctuation and glycemic control are associated with the HOMA-%β, regardless of age, in subjects with type 2 diabetes mellitus not using insulin secretagogues.

Even after excluding the subjects using insulin secretagogues, almost the same results were obtained for all analyses. These data strengthened the hypothesis that fluctuations in the blood glucose levels and glycemic control are associated with the pancreatic β-cell function in subjects with type 2 diabetes mellitus.

Discussion

In this study, we showed that the fluctuation of the blood glucose levels and average of blood glucose levels are associated with the HOMA-%β, a marker of the remnant β-cell function, in Japanese subjects with type 2 diabetes. The fluctuation of the blood glucose levels and subsequent repeated hypoglycemia can lead to the exacerbation of various clinical findings, including the development of atherosclerosis, ischemic heart disease, fundus hemorrhaging, dementia and hypoglycemia unawareness (7-11). Our current study showed that the fluctuation of the blood glucose levels and the average blood glucose levels likely lead to the deterioration of the β-cell function in addition to the exacerbation of the condition of various tissues, including large and small vessels.

Several reports have described the association between glycemic fluctuation and the β-cell function. For example, there was an excellent report showing a significant negative correlation between the GA/HbA1c ratio and the β-cell function (14). In the present study, we showed that the GA/HbA1c ratio was an independent predictor of the β-cell function in multivariate analyses of various models. In addition, we divided patients into two groups according to their age and showed that the GA/HbA1c ratio influenced the β-cell function regardless of age. Another important report showed a significant negative association between the GA/HbA1c ratio and the β-cell function as evaluated by the postprandial C-reactive protein immunoreactivity index (PCPRI) (15). In that study, when the subjects were divided into two groups according to the median PCPRI, subjects with a low PCPRI showed a higher GA/HbA1c than those with a high PCPRI, and a multiple regression analysis showed that the PCPRI was an independent predictor of the GA/HbA1c. In the present study, however, we used glycemic fluctuation as an independent variable and the β-cell function as a dependent variable. Therefore, we assume that the GA/HbA1c ratio influenced the β-cell function, although we cannot exclude the possibility that the β-cell function actually influenced the GA/HbA1c ratio. We also divided patients into two groups according to age here as well and showed that the GA/HbA1c ratio influenced the β-cell func-

Table 4. Multivariate Analysis Evaluating the Association between HOMA-%β and Various Clinical Parameters in All Subjects without Using Insulin Secretagogues.

Model 1	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.068	1.11	n.s.	0.015	0.19	n.s.	0.034	0.39	n.s.
Gender	0.009	0.16	n.s.	0.004	0.05	n.s.	0.011	0.12	n.s.
BMI	0.173	2.47	0.014	0.171	1.94	n.s.	0.136	1.30	n.s.
GA/HbA1c ratio	-0.362	-5.43	<0.0001	-0.349	-4.18	<0.0001	-0.356	-3.45	0.001
Model 2	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
BMI	0.054	0.69	n.s.	0.072	0.72	n.s.	0.071	0.60	n.s.
GA/HbA1c ratio	-0.357	-4.55	<0.0001	-0.358	-3.60	0.001	-0.352	-2.99	0.004
Duration of diabetes	-0.124	-1.87	n.s.	-0.136	-1.59	n.s.	-0.175	-1.70	n.s.
Model 3	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.205	2.62	0.010	0.177	1.88	n.s.	0.102	0.96	n.s.
Gender	-0.010	-0.16	n.s.	-0.003	-0.04	n.s.	-0.026	-0.24	n.s.
BMI	0.119	1.47	n.s.	0.132	1.25	n.s.	0.076	0.64	n.s.
GA/HbA1c ratio	-0.368	-4.75	<0.0001	-0.344	-3.47	0.001	-0.352	-2.97	0.004
Duration of diabetes	-0.206	-2.81	0.005	-0.192	-2.12	0.036	-0.188	-1.75	n.s.
Model 4	All subjects			<65 years old			≥65 years old		
Clinical parameter	β	t	p	β	t	p	β	t	p
Age	0.180	2.19	0.030	0.170	1.73	n.s.	0.100	0.95	n.s.
Gender	-0.014	-0.21	n.s.	-0.006	-0.06	n.s.	0.004	0.04	n.s.
BMI	0.098	1.16	n.s.	0.133	1.22	n.s.	0.008	0.06	n.s.
GA/HbA1c ratio	-0.359	-4.57	<0.0001	-0.350	-3.44	0.0008	-0.328	-2.76	0.007
Duration of diabetes	-0.211	-2.88	0.005	-0.188	-2.06	0.042	-0.214	-1.99	n.s.
Hypertension	-0.076	-1.10	n.s.	-0.030	-0.33	n.s.	-0.151	-1.35	n.s.
Dyslipidemia	-0.014	-0.21	n.s.	0.038	0.41	n.s.	-0.102	-0.94	n.s.

BMI: body mass index, GA: glycoalbumin, n.s.: not significant

tion, regardless of age. We believe that our study differs from previous reports in these points. Taken together, these present and previous findings suggest that there is very likely a close association between glycemic fluctuation and the pancreatic β-cell function.

As shown in the present study, the GA/HbA1c ratio was associated with the β-cell function. The data suggest that we should take care to reduce such blood glucose fluctuation in order to maintain the β-cell function for a long period of time. In addition, once the β-cell function has deteriorated to a certain extent, glycemic control worsens, which further augments the fluctuation of the blood glucose levels. Such phenomena form a vicious cycle, which should be avoided in order to maintain good glycemic control for a long period of time.

As shown in Table 2, in all models, the GA/HbA1c ratio was an independent factor for determining the pancreatic β-cell function. Therefore, we concluded that the GA/HbA1c ratio was associated with the deterioration of the β-cell function. In addition, in several models in Table 2, GA, HbA1c, the BMI and duration of diabetes were also independent factors for determining the β-cell function. Therefore, we believe that not only glycemic fluctuation but also glycemic control are associated with the deterioration of the β-cell function. In addition, since subjects with a higher BMI tend to have greater insulin resistance, their pancreatic β-cells often secrete more insulin to compensate for their insulin resistance. This may explain why there was a positive correlation between the HOMA-%b and BMI in this study.

Given that the fluctuation of the blood glucose levels seems to lead to the aggravation of various diseases, such as atherosclerosis and dementia, especially in elderly subjects (12, 19), we hypothesized that the fluctuation of the blood glucose levels was more closely associated with the remnant β-cell function in elderly subjects (≥65 years old) and examined this point in the present study. However, contrary to our expectations, no marked difference was noted in the results between relatively young subjects and elderly subjects. These data suggest that the fluctuation of the blood glucose levels worsens the pancreatic β-cell function in young and elderly subjects in a similar manner. Therefore, in order to preserve the β-cell function for a long period in subjects with type 2 diabetes, we should reduce the fluctuation of the blood glucose levels, regardless of age.

One limitation associated with this study is that it was performed in Japanese subjects with type 2 diabetes. The β-

cell function in Japanese patients is known to be more easily damaged by various stimuli than that in Caucasians due to differences in the genetic background. We therefore suspect that the results in this study cannot be directly applied to Caucasian patients. In this study, we excluded subjects with liver cirrhosis, as this disease is often accompanied by hypoalbuminemia. In addition, subjects with severe anemia were not included in our study population. This exclusion may have affected the albumin and/or hemoglobin values in our study, and we cannot exclude the possible influence of mild hypoalbuminemia and/or anemia on the present results.

Taken together, these findings suggest that the fluctuation of the blood glucose levels and average blood glucose levels are associated with the pancreatic β-cell function in Japanese subjects with type 2 diabetes. Therefore, it is very important to reduce glycemic fluctuation as well as to maintain good glycemic control in clinical practice in order to preserve the β-cell function in subjects with type 2 diabetes for a longer period of time.

Author's disclosure of potential Conflicts of Interest (COI).
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