Cross-sectional and Longitudinal Analyses of Factors Contributing to the Progressive Loss of the β-cell Function in Type 2 Diabetes

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Abstract

Objective Type 2 diabetes is a progressive disease characterized by insulin resistance and impaired insulin secretion. In this study, we assessed the factors contributing to an insulin secretory defect in type 2 diabetes patients.

Methods The subjects consisted of 382 patients with type 2 diabetes, aged 57±13 years. We estimated the β-cell function using 6-min post-glucagon increments in C-peptide (ΔCPR).

Results A significant inverse correlation was observed between the time since the diagnosis of diabetes and ΔCPR. A simple linear regression analysis showed that ΔCPR decreases at a rate of 0.056 ng/mL/year. According to a multiple regression model, body mass index (BMI) and log (triglyceride) were positively correlated with ΔCPR. Time since the diagnosis of diabetes, diabetes in 1st degree relatives, the presence of diabetic retinopathy, and HbA1c were inversely correlated with ΔCPR. In 50 patients who underwent the glucagon stimulation test twice, the ΔCPR decreased from 2.27±1.47 to 1.72±1.08 ng/mL over a period of 6.5±0.9 years. A multiple regression analysis revealed the BMI and fasting plasma glucose level to be significant contributing factors to the decline in ΔCPR.

Conclusion The duration of diabetes, a low BMI, genetic factors, and the presence of microangiopathy may be associated with β-cell dysfunction in diabetic patients. The observations in this study suggest that obese subjects showed a rapid decline in the β-cell function despite an initial high CPR response. Environmental factors causing insulin resistance and glucotoxicity may therefore be involved in progressive β-cell failure.

Key words: β-cell function, glucotoxicity, obesity, glycemic control

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Introduction

Type 2 diabetes is a progressive disease characterized by insulin resistance and impaired insulin secretion. The insulin secretory function is genetically determined, and impaired insulin secretion is observed in pre-diabetic stages (1-3). After the onset of type 2 diabetes, the β-cell function gradually declines over time (4). The progressive reduction in the insulin secretory capacity, which results from the shrinkage of the β-cell mass and abnormalities in the β-cell function (5, 6), is a major obstacle for preventing the development of chronic vascular complications. Although the trajectories of the insulin secretory capacity differ substantially between individuals, the mechanisms and factors affecting this process still remain obscure. Proposed hypotheses include amyloid accumulation in the islet (7, 8), the toxic effects of hyperglycemia (glucotoxicity) (9-12) and an increased degree of endoplasmic reticulum stress (13-15). In this study, we assessed the factors contributing to an insulin
secretory defect in patients with type 2 diabetes using cross-sectional and longitudinal analyses.

**Materials and Methods**

This study included 382 patients with type 2 diabetes, comprising 208 men and 174 women, aged 57±13 years, with a body mass index (BMI) of 24.3±5.1 (Table 1). The time since the diagnosis of type 2 diabetes was 9.8±8.1 years. The diagnosis of type 2 diabetes was established based on the Japan Diabetes Society (JDS) criteria for diabetes (16) and the absence of pancreatic autoimmunity markers, including glutamic acid decarboxylase (GAD) antibodies and insulinoma-associated protein 2 (IA-2) antibodies. We excluded any patients with renal failure, severe liver disease, or chronic pancreatitis. The HbAlc values were converted from JDS to the National Glycohemoglobin Standardization Program (NGSP) values (16, 17).

The plasma levels of glucose, creatinine, transaminases, and lipids were measured before breakfast. The glucagon stimulation test (GST) was performed to assess the pancreatic β-cell function. C-peptide (CPR) levels were measured before and 6 min after the intravenous injection of 1 mg of glucagon. We used 6-min post-glucagon increments in CPR (ΔCPR) as an estimate of β-cell function. In addition, the CPR index (CPI) was calculated by the formula 100×fasting CPR (ng/mL)/fasting plasma glucose (mg/dL) (18-20). Screening for diabetic retinopathy was performed by ophthalmologists using mydriatic retinal cameras. Albuminuria was defined as a urinary albumin excretion level of 30 mg/24 h or more.

Among these subjects, 22 men and 28 women, aged 55.8±9.7 years with a BMI of 24.2±4.5, repeated the GST after 4-9 years. The negative slope of ΔCPR over the range from JDS to the National Glycohemoglobin Standardization Program (NGSP) values (16, 17).

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between the two GSTs was calculated.

The data are expressed as the mean and SD. A statistical analysis was performed using SAS v.9.3 (SAS Institute, Cary, USA). Pearson’s correlations and a multiple regression analysis were used to evaluate the factors associated with ΔCPR or the decline rate of ΔCPR. The triglyceride, aspartate transaminase (AST), and alanine transaminase (ALT) values were transformed into logarithms to improve the skewed distribution. Results with p values <0.05 were considered to be statistically significant.

### Results

A cross-sectional study of 382 diabetic patients revealed a significantly inverse correlation between the time since the diagnosis of diabetes and ΔCPR in GST (Fig. 1). A simple linear regression analysis showed that ΔCPR decreases at a rate of 0.056 ng/mL/year. The ΔCPR significantly correlated with the CPI (r=0.51, p<0.0001). Although both the ΔCPR and the CPI were significantly associated with the time of the diagnosis of diabetes, the correlation was higher in the former (r=0.325, p<0.0001 vs. r=-0.263, p<0.0001). Therefore, we used the ΔCPR of GST in the following analysis. Pearson’s correlation analysis showed the BMI, log (triglyceride), log (AST), and log (ALT) to be positively associated with ΔCPR, and age, time since the diagnosis of diabetes, diabetes in 1st degree relatives, the presence of diabetic retinopathy, albuminuria, and high-density lipoprotein (HDL)-cholesterol were negatively associated with ΔCPR (Table 2). In a multiple regression model with ΔCPR as a dependent variable, BMI and log (triglyceride) were positively correlated with ΔCPR, and time since the diagnosis of diabetes, diabetes in 1st degree relatives, HbA1c were inversely associated with ΔCPR (Table 3). In the 50 patients who repeated the GST, ΔCPR decreased from 2.27±1.47 to 1.72±1.08 ng/mL over a period of 6.5±

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**Table 3.** Multiple Regression Analysis of Contributing Factors to ΔCPR in Glucagon Stimulation Tests.

| Label                                          | Parameter estimates | SD     | Pr>|t|  |
|------------------------------------------------|---------------------|--------|------|
| Gender (male)                                  | 0.01129             | 0.05543| 0.8388 |
| Age                                            | -0.01428            | 0.05763| 0.8045 |
| Time since diagnosis of diabetes               | -0.18487            | 0.05331| 0.0006 |
| BMI                                            | 0.26453             | 0.05137| <0.0001 |
| Diabetes in 1st degree relatives               | -0.12986            | 0.04514| 0.0043 |
| Habitual alcohol drink                         | -0.03303            | 0.05269| 0.5312 |
| Diabetic retinopathy                           | -0.11324            | 0.05266| 0.0322 |
| Albuminuria                                    | -0.01808            | 0.04935| 0.7143 |
| eGFR                                           | 0.04122             | 0.05391| 0.4450 |
| Fasting plasma glucose                         | 0.06741             | 0.04961| 0.1750 |
| HbA1c                                          | -0.11648            | 0.05179| 0.0251 |
| Total cholesterol                              | 0.03042             | 0.05823| 0.6017 |
| HDL-cholesterol                                | -0.00275            | 0.05070| 0.9568 |
| log(triglyceride)                              | 0.14992             | 0.05882| 0.0112 |
| log(AST)                                       | 0.00770             | 0.09491| 0.9354 |
| log(ALT)                                       | 0.06987             | 0.09803| 0.4765 |

**Table 4.** Pearson’s Correlation Coefficients with the Longitudinal Changes in ΔCPR in Glucagon Stimulation Tests.

| Label                                          | Coefficient | p value |
|------------------------------------------------|-------------|---------|
| Gender (male)                                  | 0.23093     | 0.1066  |
| Age                                            | -0.03168    | 0.8271  |
| Time since diagnosis of diabetes               | 0.13952     | 0.3339  |
| BMI                                            | -0.26324    | 0.0647  |
| Diabetes in 1st degree relatives               | 0.31983     | 0.0236  |
| Habitual alcohol drink                         | 0.25779     | 0.0707  |
| Diabetic retinopathy                           | 0.25846     | 0.0699  |
| Albuminuria                                    | 0.19534     | 0.1735  |
| eGFR                                           | 0.09317     | 0.5199  |
| Fasting plasma glucose                         | -0.18427    | 0.2002  |
| HbA1c                                          | 0.05772     | 0.6905  |
| Total cholesterol                              | 0.04939     | 0.7334  |
| HDL-cholesterol                                | -0.04661    | 0.7479  |
| log(triglyceride)                              | -0.05067    | 0.6989  |
| log(AST)                                       | 0.05365     | 0.7113  |
| log(ALT)                                       | 0.12027     | 0.4054  |

Figure 2. ΔCPR in the GST repeated at an interval of 4-9 years in 50 patients with type 2 diabetes.
In this study, we identified a significant association between the duration of type 2 diabetes and β-cell dysfunction using the ΔCPR of GST as an index of the insulin secretory capacity. Glucagon is a potent stimulus for islet β-cells, and an intravenous bolus injection of 1 mg of glucagon has been widely used to assess the β-cell function for clinical or research purposes (21-24). The decline rate of the insulin secretory capacity observed in the cross-sectional analysis was comparable to the findings of a previous cross-sectional study (25). The progressive loss of the insulin secretory capacity was also seen when the CPI was used as an estimate for the β-cell function. The decline in the β-cell function in type 2 diabetes was most likely caused by the progressive loss of β-cell mass. Histological studies of the pancreas from subjects with long-standing type 2 diabetes showed disruptions of islet structure and a marked reduction in β-cell numbers (5, 26, 27). The regulation of the β-cell mass involves a balance of β-cell replication and apoptosis. However, β-cell loss by apoptosis appears to play a significant role in the progression of the disease (28, 29). Although the molecular mechanism of the progressive loss of insulin secretory capacity has not yet been fully elucidated, glucose toxicity caused by elevated plasma glucose levels has been implicated as a primary cause of β-cell dysfunction and apoptosis due to increased oxidative stress (9-12).

A multiple regression analysis of the cross-sectional data revealed that the time since the diagnosis, the family history of diabetes, the presence of diabetic retinopathy, and HbA1c were inversely correlated with insulin secretory capacity. The association between retinopathy and β-cell dysfunction might be simply because patients with diabetic retinopathy had developed diabetes long before the diagnosis. However, intra-islet hypoperfusion could be involved in the β-cell dysfunction in patients with advanced microangiopathy, because signs of islet microangiopathy have been reported in rodent models of type 2 diabetes (30, 31). The inverse association between HbA1c and β-cell function suggests the role of glucose toxicity in the insulin secretory defect. On the other hand, the BMI and log (triglyceride) were found to be positive contributing factors to ΔCPR in that model. The association may be explained by a compensatory increase in insulin secretion because both obesity and hypertriglyceridemia are associated with insulin resistance. Among the variables significantly associated with ΔCPR in Pearson’s correlation analysis, age and albuminuria which correlated with the time since the diagnosis (r=0.32, p<0.0001, and r=0.28, p=0.0001, respectively), and HDL-cholesterol, log (AST), and log (ALT) which correlated with BMI (r=-0.16, p=0.0014, r=0.24, p<0.0001, and r=0.24, p<0.0001, respec-
tively) did not remain significant according to a multiple regression analysis.

The progressive loss of the insulin secretory capacity was also verified by sequential analyses of patients who repeated the GST. The Pearson correlation analysis showed that the presence of diabetic subjects in the 1st degree relatives was also significant. The multiple regression analysis, however, confirmed the contribution of BMI and the fasting plasma glucose level. Although the number of the patients was limited, these observations suggest that individuals genetically susceptible to diabetes have a low β-cell function, but the progressive β-cell dysfunction is determined by environmental factors causing insulin resistance and glucotoxicity.

In conclusion, the long duration of diabetes, a low BMI, genetic factors, and the presence of microangiopathy may be associated with β-cell dysfunction in diabetic patients. The positive association between BMI and ΔCPR in the cross-sectional study and the negative correlation between BMI and longitudinal changes in ΔCPR suggest that obese subjects showed a rapid decline in the β-cell function despite demonstrating an initial high CPR response. Elevated HbA1c and fasting plasma glucose levels were associated with β-cell dysfunction in cross-sectional and longitudinal studies, respectively. The adequate control of body weight and blood glucose may therefore be essential for preserving the residual insulin secretory capacity in type 2 diabetes.

A part of the study was presented at the 49th annual meeting of the European Association for the Study of Diabetes held in Barcelona, on 23-27 September 2013.

The authors state that they have no Conflict of Interest (COI).

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