

# Longitudinal examination of pancreatic $\beta$ -cell function in Japanese individuals

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## Keywords

$\beta$ -Cell function, Obesity, Oral glucose tolerance test

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## ABSTRACT

**Aims/Introduction:** We carried out a retrospective, longitudinal analysis of  $\beta$ -cell function between a diabetes mellitus group, including those that progressed to diabetes mellitus during the follow-up period, and a diabetic type with glycated hemoglobin (HbA1c) <6.5 group, including those that progressed to a diabetic type during the follow-up period.  $\beta$ -Cell function was assessed using homeostasis model of assessment of  $\beta$ -cell function.

**Materials and Methods:** The relationship between the duration of diabetes mellitus or the diabetic type and pancreatic  $\beta$ -cell function was compared between the diabetes mellitus group (1,817) and diabetic type with HbA1c <6.5 group (1,843) using results from an oral glucose tolerance test. Linear mixed effects models were used to analyze repeated measurements of oral glucose tolerance tests.

**Results:** The slope of the regression line of  $\beta$ -cell function for the duration of the diabetes mellitus group was  $-2.2\%/year$  before the diagnosis. The slope differed after the diagnosis, and the difference was 1.3. The slope of the diabetic type group was  $-1.2\%/year$ , and no significant difference was observed in the slope before and after the diagnosis.  $\beta$ -Cell function at the onset was 54.3% in the diabetic type group and 40.6% in the diabetes mellitus group, and the slope of the regression line was significantly higher in the diabetes mellitus group. We divided the diabetes mellitus and diabetic type with HbA1c <6.5 groups into obese and non-obese participants.  $\beta$ -Cell function declined more with obesity.

**Conclusions:** Subsequent declines in  $\beta$ -cell function were faster in the diabetes mellitus group than that in the diabetic type with HbA1c <6.5 group, and increased with obesity.

## INTRODUCTION

Diabetes is increasing worldwide, particularly in Asia<sup>1</sup>. Type 2 diabetes in East Asians is characterized by lower insulin secretion, higher insulin sensitivity and a smaller body mass index (BMI) than those in Caucasians<sup>2</sup>. Although the progression of pancreatic  $\beta$ -cell function in type 2 diabetes patients has been investigated in detail in Caucasians, limited information is currently available for Japanese people. Furthermore, the influence of obesity on pancreatic  $\beta$ -cell function in a Japanese population remains unclear. In order to clarify how obesity affects the long-term function of pancreatic  $\beta$ -cells, we herein carried out a retrospective, longitudinal analysis of pancreatic  $\beta$ -cell

function between a diabetes mellitus group, including those that progressed to diabetes mellitus during the follow-up period, and a diabetic type with glycated hemoglobin (HbA1c) <6.5 group, including those that progressed to the diabetic type during the follow-up period.

## METHODS

Participants were a fixed population of atomic bomb survivors in Hiroshima, Japan, who were born before May 1946. They received periodic health examinations based on medical law. Blood pressure was measured in a sitting position with the right arm. The inclusion criterion was that urine sugar in a fasting or a post-prandial state was positive. Exclusion criteria were pregnancy, post-gastrectomy and thyroid disease. We also registered individuals who received an oral glucose tolerance

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test (OGTT). We examined the OGTT of registered participants regularly between 1984 and 2012. Immunoreactive insulin was measured from 1971. There were 26,868 registered cases and 58,112 OGTT tests were carried out.

A diagnosis was made according to the 2010 diagnostic criteria of the Japan Diabetes Society<sup>3</sup>. The diabetic type was diagnosed if any of the following criteria were met: (i) fasting plasma glucose (FPG)  $\geq 126$  mg/dL; (ii) 2-h post-load plasma glucose  $\geq 200$  mg/dL; (iii) casual plasma glucose  $\geq 200$  mg/dL; or (iv) HbA1c  $\geq 6.5\%$ . A re-examination was carried out on a separate day, and if the diabetic type was reconfirmed, diabetes mellitus was diagnosed. If plasma glucose values – any of criteria (i), (ii) or (iii) – and (iv) HbA1c  $\geq 6.5\%$  in the same blood sample both indicated the diabetic type, diabetes mellitus was diagnosed based on the initial examination alone.

We classified participants with an FPG  $\geq 126$  mg/dL or 2-h post-load plasma glucose  $\geq 200$  mg/dL and HbA1c  $\geq 6.5\%$  as diabetes mellitus, and those with an FPG  $\geq 126$  mg/dL or 2-h post-load plasma glucose  $\geq 200$  mg/dL and HbA1c  $< 6.5\%$  as the diabetic type with HbA1c  $< 6.5$ . Participants with FPG  $\geq 126$  and 2-h-post-load plasma glucose levels  $\geq 200$  were included in the diabetes mellitus group.

Participants were included if they: (i) had undergone OGTT at least twice; and (ii) were normal type (FPG  $< 110$  mg/dL and 2-h post-load plasma glucose  $< 140$  mg/dL) at baseline. There were 1,817 participants who shifted from the normal type to diabetes mellitus. There were 3,714 participants who shifted from the normal to diabetic type with HbA1c  $< 6.5$ , 1,871 participants of those shifted to diabetes mellitus. We excluded 1,871 participants from normal to diabetes mellitus via the diabetic type, because they belonged to both groups. A total of 23,208 participants were subjected to OGTT once only or were diagnosed with diabetes mellitus or borderline type for the first time.

The diabetes mellitus group ( $n = 1,817$ ) comprised participants who progressed from normal to diabetes mellitus during the follow-up period, whereas the diabetic type with HbA1c  $< 6.5$  group ( $n = 1,843$ ) comprised participants who progressed from normal to the diabetic type with HbA1c  $< 6.5$  during the follow-up period (Table 1). The mean age of participants was 61.4 years (standard deviation 8.4 years). The average follow-up duration was 11 years. OGTT and immunoreactive insulin were measured 24,614 times each. Each

participant was subjected to an OGTT an average of 6.8 times. After the development of diabetes, they were recommended to visit a clinic. The diabetes mellitus group was treated with diet and exercise therapy after being diagnosed. The diabetic type with HbA1c  $< 6.5$  group is not targeted by medical services under the national health insurance system in Japan, and, thus, only received a lecture on diet and exercise at a Health Management Center. We examined OGTT in participants who had not received medication, but requested to be monitored.

The present study was approved by the ethics committee of the Health Management Center, Hiroshima Atomic Bomb Causality Council. It was carried out in accordance with the Declaration of Helsinki. All participants gave informed consent and signed the informed consent form.

HbA1c was assessed by high-performance liquid chromatography (Arkray Inc., Kyoto, Japan and Tosoh Corporation, Kyoto, Japan) and calibrated using a whole-blood standard from the Japan Diabetes Society. HbA1c at the time of OGTT was converted using the National Glycohemoglobin Standardization Program<sup>3</sup>. Plasma glucose was measured by the glucose oxidase method using venous blood. An immunoassay correction was made whenever a reagent was changed.

Pancreatic  $\beta$ -cell function was calculated using the equation of Turner *et al.* (i.e., homeostasis model assessment of  $\beta$ -cell function = fasting insulin concentration [ $\mu$ U/mL]  $\times 360$  / FPG [mg/dL] – 63)<sup>4</sup>. At the initial visit, participants with BMI  $\geq 25$  and  $< 25$  were classified as obese and non-obese, respectively.

Linear mixed effects models, which consider the correlation of measurements within each participant, were used to analyze the relationship between the repeated measurements of pancreatic  $\beta$ -cell function and covariates. Random effects were assumed for both the intercept and slope to model participant-specific regression lines for the time in the study or the duration of the follow-up period, adjusting for sex, BMI centered at 23 and age at the start of the follow-up period, which was centered at 60 years. The difference in slopes was tested by modeling both sexes together, and the relationship between pancreatic  $\beta$ -cell function and duration was evaluated separately for each sex. The intercept and slope of the regression lines were evaluated within the diabetes mellitus group and diabetic type with HbA1c  $< 6.5$  group. Furthermore, comparisons were carried out between obese and non-obese participants within the diabetes mellitus group and diabetic type with HbA1c  $< 6.5$  group. Regression slopes between the diagnosis of the diabetes mellitus and diabetic type with HbA1c  $< 6.5$  groups were compared in a linear mixed effects model assuming different slopes before and after diagnoses. Statistical analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R (version 3.5.1.4; The R Foundation for Statistical Computing, Vienna, Australia).

## RESULTS

The relationship between the duration of diabetes mellitus (1,817) or the diabetic type (1,843) and pancreatic  $\beta$ -cell

**Table 1** | Baseline characteristics

	Diabetic type with HbA1c $< 6.5\%$ ( $n = 1,843$ )	Diabetes mellitus (HbA1c $\geq 6.5\%$ ) ( $n = 1,817$ )	Total ( $n = 3,660$ )
Male (%)	60.6	68.2	60.6
Age (years)	60.3 (8.5)	62.5 (8.1)	61.4 (8.4)
BMI (kg/m <sup>2</sup> )	24.3 (3.1)	23.7 (3.1)	24.0 (3.1)

The mean of each variable is shown, and the numbers in the parentheses are the standard deviations of each variable. BMI, body mass index; HbA1c, glycated hemoglobin.

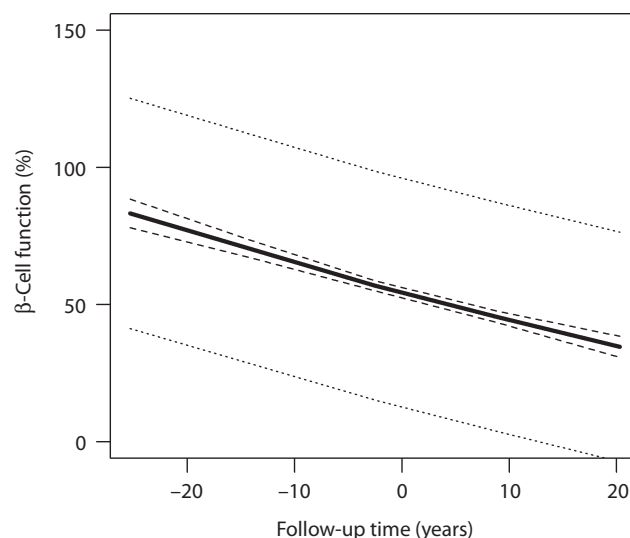
function was evaluated using linear mixed effects models. The slope of the regression line of  $\beta$ -cell function for the duration of the diabetes mellitus group was  $-2.16\%/year$  with a 95% confidence interval (CI) of  $-2.33$  to  $-1.99$  before the diagnosis. The slope differed after the diagnosis, and the difference was  $1.28$  with a 95% CI of  $1.04$ – $1.53$  (Table 2). The slope of the diabetic type with HbA1c  $<6.5$  group was  $-1.15\%/year$  with a 95% CI of  $-1.35$  to  $-0.96$ , and the difference in the slope before and after the diagnosis was  $0.20$  with a 95% CI of  $-0.09$  to  $0.49$  (Table 2). Pancreatic  $\beta$ -cell function at the onset, which was defined as duration = 0 for participants aged 60 years and with BMI of 23 at baseline, was  $53.99\%$  in the diabetic type with HbA1c  $<6.5$  group (Figure 1), but was significantly reduced to  $38.62\%$  in the diabetes mellitus group (95% CI of the difference  $-17.58$  to  $-13.05$ ; Figure 2), and the slope of the regression line (coefficient year of follow-up time) was significantly larger in the diabetes mellitus group (the difference:  $-0.95$ , 95% CI  $-1.21$  to  $-0.69$ ).

We divided the diabetes mellitus group into obese and non-obese participants at the initial visit, and the regression line of  $\beta$ -cell function for the duration (follow-up year) of the diabetes mellitus group was  $y = -1.72x + 0.85\max(x,0) + 36.94$ , where  $\max(x,0)$  specified the slope after the diabetes mellitus diagnosis (Figure 3, solid line) in non-obese participants, and  $y = -2.45x + 52.0$  (Figure 3, dotted line) in obese participants. The intercept and slope of the regression line before the diabetes mellitus diagnosis were both significantly different between obese and non-obese participants, whereas the slope after the

**Table 2** | Multivariate regression for each participant who developed diabetes mellitus and those who became diabetic type with glycated hemoglobin  $<6.5\%$

	Estimate	95% CI
Participants who developed DM		
$\beta$ -Cell function at age 60 years with BMI of 23	38.62	[37.34, 39.91]
Follow-up time	-2.16	[-2.33, -1.99]
Age at baseline	0.26	[0.15, 0.36]
Years after diagnosis	1.28	[1.04, 1.53]
BMI	3.65	[3.30, 3.99]
Sex	-0.61	[-2.48, 1.25]
Sex $\times$ BMI	-1.30	[-1.79, -0.82]
Participants who became diabetic type		
$\beta$ -Cell function at age 60 years with BMI of 23	53.99	[52.16, 55.81]
Follow-up time	-1.15	[-1.35, -0.96]
Age at baseline	0.01	[-0.17, 0.18]
Years after diagnosis	0.20	[-0.09, 0.49]
BMI	4.65	[4.20, 5.1]
Sex	2.99	[0.06, 5.92]
Sex $\times$ BMI	-0.96	[-1.66, -0.26]

Estimated coefficients of fixed effects, or population mean of the regression models, of linear mixed effects models. BMI, body mass index; CI, confidence interval; DM, diabetes mellitus.



**Figure 1** | Follow-up examination of  $\beta$ -cell function in participants whose results shifted from the normal to diabetic type with glycated hemoglobin  $<6.5\%$ . The solid line shows the mean. The dashed line is the confidence bound, and the small dotted line shows the prediction bound. The follow-up time is 0 at the onset of the diabetic type with glycated hemoglobin  $<6.5\%$ .

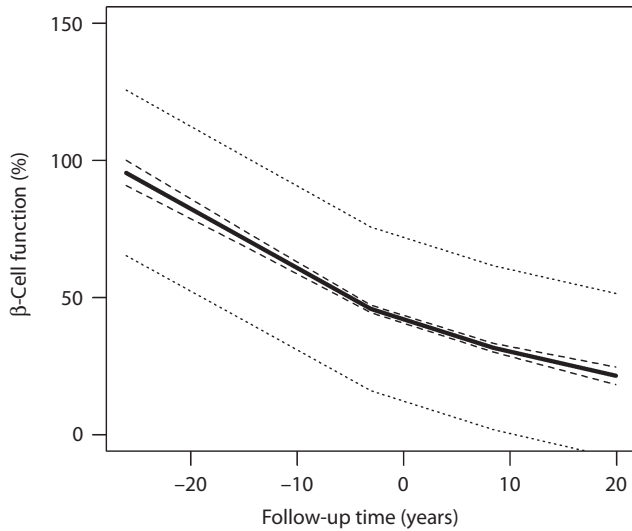
diabetes mellitus diagnosis was not. Thus,  $\beta$ -cell function at the onset was significantly higher in obese participants than in non-obese participants by  $15.1\%$ , whereas the slope of the regression line was significantly higher in obese participants before the diabetes mellitus diagnosis (Table 3).

We also evaluated obese and non-obese participants in the diabetic type with HbA1c  $<6.5$  group. The regression line of  $\beta$ -cell function for the duration of the diabetic type group was  $y = -0.894x + 49.4$  (Figure 4, solid line) in non-obese participants and  $y = -1.62x + 75.1$  (Figure 4, dotted line) in obese participants. The slope was not significantly different before and after the diabetes mellitus diagnosis, and the intercept and slope of the regression line were both significantly different between obese and non-obese participants.  $\beta$ -Cell function at the onset was significantly higher in obese participants than in non-obese participants by  $25.7\%$ , whereas the slope of the regression line was significantly larger in obese participants (Table 3).

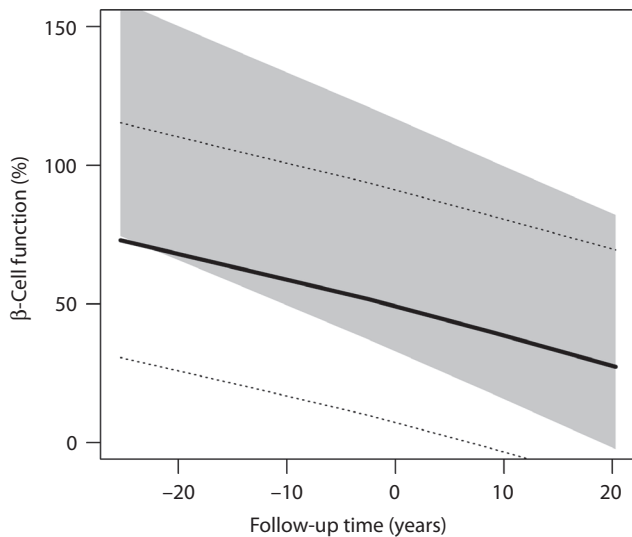
## DISCUSSION

We carried out a retrospective, longitudinal analysis of pancreatic  $\beta$ -cell function in Japanese individuals. The slope of the regression line of  $\beta$ -cell function was significantly larger in the diabetes mellitus group than in the diabetic type with HbA1c  $<6.5$  group. This result suggests that secretory insufficiency occurs earlier in the diabetes mellitus group than in the diabetic type with HbA1c  $<6.5$  group. Negative slopes indicated a decline in  $\beta$ -cell function in both groups.

Insulin secretion by pancreatic  $\beta$ -cells in type 2 diabetes is approximately  $50\%$  when patients are diagnosed<sup>5</sup>. In the



**Figure 2** | Follow-up examination of β-cell function in participants whose results shifted from the normal type to diabetes mellitus. The solid line shows the mean. The dashed line is the confidence bound, and the small dotted line shows the prediction bound. The duration is 0 at the onset of diabetes mellitus.



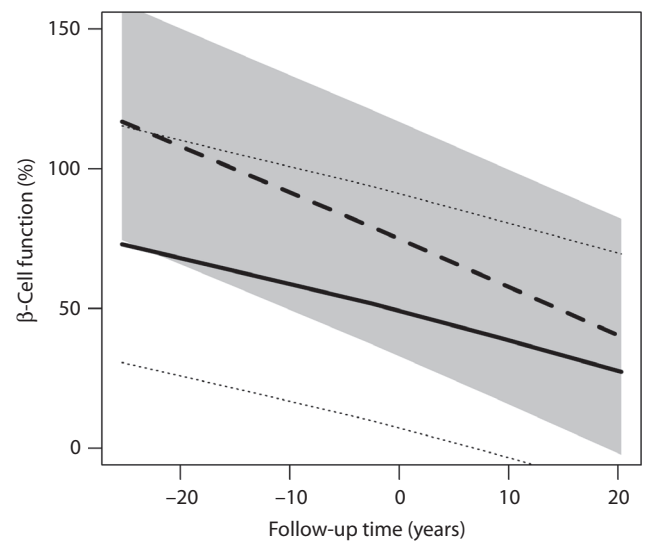
**Figure 3** | Follow-up examination of β-cell function in participants whose results shifted from the normal type to diabetes mellitus. The duration is 0 at the onset of diabetes mellitus (solid line: non-obese participants, the initial visit body mass index <25, dotted line: obese participants, the initial visit body mass index ≥25). The small dotted line shows the prediction bound of non-obese participants, and the shadow is the prediction bound of obese participants.

present study, this value was 54.3% in the diabetic type with HbA1c <6.5 group, but was significantly reduced to 40.6% in the diabetes mellitus group. Previous studies on insulin secretion mostly involved Western countries; however, a previous study showed reduced β-cell function and higher insulin

**Table 3** | Multivariate regression for each participant who developed diabetes mellitus and those who became diabetic type with glycated hemoglobin <6.5% with different slopes for obese and non-obese participants

	Estimate	95% CI
Participants who developed DM		
β-Cell function at age 60 years of non-obese	36.94	[35.40, 38.49]
Follow-up time	-1.72	[-1.87, -1.56]
Obesity	15.05	[12.98, 17.12]
Age at baseline	0.22	[0.10, 0.33]
Years after diagnosis	0.85	[0.60, 1.10]
Sex	-1.22	[-3.17, 0.74]
Follow-up time × obesity	-0.74	[-1.02, -0.46]
Obesity × years after diagnosis	-0.13	[-0.55, 0.29]
Participants who became diabetic type		
β-Cell function at age 60 years of non-obese	49.48	[47.37, 51.58]
Follow-up time	25.57	[22.29, 28.84]
Obesity	-0.93	[-1.16, -0.70]
Years after diagnosis	1.68	[-1.34, 4.70]
Sex	-0.16	[-0.52, 0.21]
Follow-up time × obesity	-0.72	[-1.20, -0.23]
Obesity × years after diagnosis	0.07	[-0.62, 0.77]

Estimated coefficients of fixed effects, or population mean of the regression models, of linear mixed effects models. CI, confidence interval; DM, diabetes mellitus.



**Figure 4** | Follow-up examination of β-cell function in participants whose results shifted from the normal to diabetic type with glycated hemoglobin <6.5%. The duration is 0 at the onset of the diabetic type with glycated hemoglobin <6.5% (solid line: non-obese participants, the initial visit body mass index <25, dotted line: obese participants, the initial visit body mass index ≥25). The small dotted line shows the prediction bound of non-obese participants, and the shadow is the prediction bound of obese participants.

sensitivity in East Asians than in Caucasians<sup>1</sup>. Therefore, low insulin secretion might be specific to East Asians.

In obese individuals, the proliferation and generation of pancreatic  $\beta$ -cells compensate for insulin resistance in addition to cell capacity and size increases<sup>6</sup>. However, the proliferation and generation of pancreatic  $\beta$ -cells in humans has not yet been firmly established. Regarding diabetes mellitus,  $\beta$ -cell capacity is reduced in obese and lean patients<sup>7</sup>.  $\beta$ -Cell impairments might involve apoptosis, hyperglycemia, endoplasmic reticulum stress<sup>8</sup> or oxidative stress<sup>9</sup>, which decrease  $\beta$ -cell capacity and size. Pancreatic  $\beta$ -cell function was better preserved in obese patients than in non-obese patients at the onset of diabetes, but declined more in obese patients. Although limited information is currently available on pancreatic  $\beta$ -cell function in obese patients in Japan, the present results suggest that pancreatic  $\beta$ -cell function was reduced by obesity, and the clinical correction of obesity might contribute to the preservation of pancreatic  $\beta$ -cell function.

The present study had some limitations. Pancreatic  $\beta$ -cell function was only assessed in homeostasis model assessment of  $\beta$ -cell function. Japanese diagnostic criteria differ from those in other countries, and obesity and insulin secretion also differ between the Japanese population and other countries.

In conclusion, the decline observed in pancreatic  $\beta$ -cell function in the diabetes mellitus group was faster than that in the diabetic type with HbA1c <6.5 group, and pancreatic  $\beta$ -cell function declined further with obesity. Therefore, the early detection and prevention of diabetes based on OGTT is beneficial for public health.

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#### DISCLOSURE

The authors declare no conflict of interest.

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