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Association of glucose tolerance status with pancreatic β- and α-cell mass in community-based autopsy samples of Japanese individuals: The Hisayama Study

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ABSTRACT

Aims/Introduction: Changes in histologically quantified β- and α-cell mass during the development of glucose intolerance have not been fully elucidated. The aim of the present study was to explore differences in β- and α-cell mass according to the glucose tolerance status.

Materials and Methods: Autopsy samples from a total of 103 individuals (40 with normal glucose tolerance, 31 with prediabetes and 32 with type 2 diabetes mellitus) who underwent a 75-g oral glucose tolerance test within 5 years before death were selected from 643 community-based autopsy samples collected from 2002 to 2016. Fractional β-cell area (BCA) and α-cell area were quantified with Image Pro Plus software. Associations of BCA and α-cell area with glucose tolerance status were assessed using a linear regression analysis, and Spearman's correlation coefficients between glycemic markers and β-cell function were estimated.

Results: The mean values of BCA decreased significantly with worsening glucose tolerance status (mean ± standard error 1.85 ± 0.10% in normal glucose tolerance, 1.59 ± 0.11% in prediabetes and 1.17 ± 0.11% in type 2 diabetes mellitus, *P* for trend < 0.001), whereas there was no significant association between α-cell area and glucose tolerance status. BCA was inversely correlated with fasting and 2-h plasma glucose levels during oral glucose tolerance test and glycated hemoglobin measurement, and positively correlated with disposition index (all $P < 0.01$).

Conclusions: β-Cell mass decreased significantly with worsening glucose tolerance, from the stage of prediabetes, in the Japanese population. Prevention of declining β-cell mass before the onset of glucose intolerance is important to reduce the burden of type 2 diabetes mellitus.

INTRODUCTION

The number of people with diabetes mellitus worldwide continues to increase, and is projected to rise from 425 million in 2017 to 629 million in 2045[1]. To counter this pandemic, effective strategies for preventing diabetes mellitus based on its disease pathophysiology during the development stage are urgently required.

Type 2 diabetes mellitus is characterized by progressive loss of β-cell function[2,3]. Recent studies have consistently shown that β-cell mass is reduced by 30–65% in people with type 2 diabetes mellitus[4–9], showing that a deficit of β-cells is a common feature of both type 1 diabetes mellitus and type 2 diabetes mellitus[2]. In contrast, some studies carried out in white

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European populations have reported a compensatory increase in β-cell mass in obese non-diabetic individuals[5,10,11]. To clarify the changes in β-cell mass during the process of development of glucose intolerance, studies using both clinical data of glucose tolerance status and histological data of β-cell mass in pancreas tissue samples obtained from autopsy, pancreas surgery or organ donation are required. To date, however, there have been few studies investigating this topic from a histological point-of-view.

The Hisayama Study is a prospective, population-based study of cardiovascular disease and lifestyle-related disease in Japanese people[12]. This study is characterized by accurate diagnosis of type 2 diabetes mellitus based on the 75-g oral glucose tolerance test (OGTT) at an annual health checkup for the residents of the town of Hisayama, and autopsy verification of the cause of death in approximately 75% of the deceased people in this community[13,14]. Therefore, the aim of the present study was to explore differences in β-and α-cell mass according to glucose tolerance status assessed by OGTT in community-based autopsy samples of Japanese individuals.

METHODS

Study participants

In the present study, we sought to obtain community-based autopsy samples with information on the glucose tolerance status determined by OGTT among decedents who had participated in the Hisayama Study. The Hisayama Study was carried out in the town of Hisayama, a suburb of the Fukuoka prefecture on Japan's Kyushu Island. The design of the Hisayama Study has been described in detail elsewhere[12,15]. Annual health checkups of the Hisayama residents and autopsy examinations of deceased people have been repeated since 1961. In this town, the participants received OGTT in an annual health checkup since 1988. OGTT data including both plasma glucose and serum insulin levels were available in the health checkups carried out in 2002, 2007 and 2012. In addition, to date, approximately 75% of the decedents enrolled in the Hisayama study have undergone autopsy examinations[13,14].

For the present study, we used autopsy specimens obtained from deceased people in the town of Hisayama from June 2002 to August 2016. During this period, a total of 643 residents of Hisayama were autopsied. Among them, 181 individuals had undergone OGTT including serum insulin level within 5 years before death. After excluding 25 individuals with pancreatic cancer or pancreatitis, one with the use of insulin, 15 who did not undergo an autopsy within 36 h of death, and 37 without pancreatic tissue stored that was of adequate size and quality, the remaining 103 individuals were enrolled in the present study (Figure S1). In this study, no individuals were determined to have type 1 diabetes, as patients who received insulin therapy were excluded. The study was carried out with the approval of the Kyushu University and Keio University Institutional Review Board for Clinical Research, and informed written consent was obtained from all participants.

Definition of glucose tolerance status and glycemic indices

In the health checkups, clinical evaluations and laboratory measurements were carried out in a similar manner across years, as previously described[12,15]. The study participants underwent the OGTT in each survey between 08.00 and 10.30 hours after an overnight fast of at least 12 h. During OGTT, plasma glucose and serum insulin were measured at 0 and 120 min after glucose load in the 2002, 2007 and 2012 surveys. In the 2007 and 2012 surveys, 30-min postload plasma glucose and serum insulin were also measured. Plasma glucose levels were measured by the glucose hexokinase method. Serum insulin was measured by a chemiluminescent enzyme immunoassay in 2002 and 2012, and by an electrochemiluminescence immunoassay in 2007. Glucose tolerance status was defined based on the 2006 WHO criteria; namely, for normal glucose tolerance (NGT): fasting plasma glucose (FPG) <6.1 mmol/L and 2-h postload glucose (2hPG) <7.8 mmol/L; for impaired fasting glycemia: FPG 6.1–6.9 mmol/L and 2hPG <7.8 mmol/L; for impaired glucose tolerance (IGT): FPG <7.0 mmol/L and 2hPG 7.8–11.0 mmol/L; and for diabetes mellitus: FPG ≥7.0 mmol/L or 2hPG ≥11.1 mmol/L or both, or the use of antidiabetic medications. Prediabetes (PDM) was defined as either impaired fasting glycemia or IGT. Glycated hemoglobin (HbA1c) levels were measured by latex aggregation immunoassay in 2002 and 2007, and high-performance liquid chromatography in 2012. The value for HbA1c was expressed as a National Glycohemoglobin Standardization Program equivalent value[16]. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: FPG (mmol/L) × fasting serum insulin (mU/L) / 22.48155. For the 52 individuals who underwent the OGTT in the 2007 or 2012 surveys among the total 103 participants, the insulinogenic index and disposition index were calculated using the following equations: insulinogenic index = (serum insulin at 30 min [mU/L] − fasting serum insulin [mU/L]) / (plasma glucose at 30 min [mmol/L] − FPG [mmol/L]) and disposition index = insulinogenic index / HOMA-IR[17].

Other risk factor measurements

A self-administered questionnaire regarding alcohol intake, smoking habits, current use of antihypertensive agents, oral glucose-lowering agents and insulin was checked by trained interviewers at the health examination. These variables were classified as being either habitual or not. The participants engaging in sports or other forms of exertion three or more times a week during their leisure time made up a regular exercise group. Diabetes mellitus in first- or second-degree relatives was taken to show a family history of diabetes. Hypertension was defined as blood pressure ≥140/90 mmHg and/or current use of antihypertensive agents. The body mass index (BMI) was calculated, and overweight was defined as a BMI ≥23.0 kg/m^2 based on the Asian-specific BMI cut-off point[18]. Underlying cause of death was determined by several physicians of the study team and coded according to the International Classification of Diseases, 10th Revision (ICD-10). Causes of death were classified

into the following categories: cancer (ICD-10 code of C00–C97), cardiovascular diseases (ICD-10 code of I00–I99), respiratory disease (ICD-10 code of J00–J99.8) and death from other causes.

Morphological examination of the pancreas

Autopsy examination was carried out at the Departments of Pathology, Kyushu University. All autopsies were carried out using the standard methods[14], and the autopsy procedures were uniform throughout the study period. The pancreatic body or tail was fixed in formaldehyde at autopsy and embedded in paraffin. The sections were stained for light microscopy as follows: (i) with hematoxylin–eosin; (ii) for insulin (peroxidase staining) with hematoxylin; (iii) for glucagon with hematoxylin; (iv) for insulin and Ki67 for assessment of β-cell replication; and (v) with direct fast scarlet 4BS to detect amyloid deposits in islets. For immunohistochemical staining, guinea pig polyclonal antibodies against porcine insulin (Dako, Tokyo, Japan), mouse monoclonal antibodies against human glucagon (Sigma Aldrich, St. Louis, MO, USA) and murine monoclonal antibodies against human Ki67 (Dako) were used.

The entire pancreatic section (mean ± standard deviation, 167 ± 70 mm^2 per case) was imaged at an original magnification of ×200 (×20 objective) using a NanoZoomer-XR slide scanner and viewing software of NanoZoomer Digital Pathology (Hamamatsu Photonics K.K., Shizuoka, Japan) to quantify the fractional β-cell area (BCA). As previously reported[9,19], the ratio of BCA to total pancreas area as indices of β-cell mass was digitally measured using Image Pro Plus software (Media Cybernetics, Silver Springs, MD, USA). The ratio of α-cell area (ACA) to total pancreas area as indices of α-cell mass was also digitally measured, and the ratio of ACA to BCA (ACA/BCA) in each case was determined. All measurements were carried out twice by a single investigator (JI), and the mean of the two measurements was used. The size and density of islets, density of scattered β-cells, insulin-positive duct cells, β-cell replication based on evaluation of double-positive cells for insulin and Ki67, and islet amyloid deposits were quantified in randomly selected areas of the pancreas that contained approximately 100 islets in each case using a software of NanoZoomer Digital Pathology (mean ± standard deviation, 109 ± 6 islets per case). Using this software, the size of each islet was directly measured along the edge of the islet in the section stained for insulin with hematoxylin. Scattered β-cells were defined as a cluster of three or fewer β-cells in acinar tissue. The density of scattered β-cells and density of insulin-positive duct cells are assessed as surrogate markers of β-cell neogenesis.

Statistical analysis

Natural log-transformed values were used to improve the skewed distributions of the values for serum triglycerides, HOMA-IR, insulinogenic index and disposition index. The linear trends in the characteristics of participants in the three glucose tolerance categories were assessed using a linear regression model for mean values and logistic regression model for frequencies. The multivariable-adjusted mean values of morphometric parameters, including BCA, ACA and the ACA/BCA ratio, were compared according to the glucose tolerance status by using linear regression analysis, with adjustments made for potential confounding factors at baseline: namely, age at the time of 75-g OGTT, sex, family history of diabetes, BMI, hypertension, serum triglycerides, serum high-density lipoprotein cholesterol, smoking habits, alcohol intake, regular exercise, time from death to autopsy and causes of death. In a subgroup analysis, we repeated the analysis according to status of overweight. Spearman's correlation coefficient was used to assess the correlation between BCA and ACA according to glucose tolerance status, as well as to estimate the correlation of glycemic parameters with BCA, ACA and the ACA/BCA ratio. To investigate whether there exists an effect modification among the glucose tolerance groups, the heterogeneity in the association of ACA with BCA among the glucose tolerance groups was tested by adding an interaction term to the linear regression model. The SAS software package version 9.4 (SAS Institute, Cary, NC, USA) was used to carry out all statistical analyses. A value of $P < 0.05$ was considered statistically significant in all analyses.

RESULTS

Participant characteristics

The characteristics of participants according to the glucose tolerance status are shown in Table 1. The mean or geometric mean values of BMI, FPG, 2hPG, HbA1c, HOMA-IR and serum triglycerides increased with the deterioration of glucose tolerance status, whereas the insulinogenic index, disposition index and serum high-density lipoprotein cholesterol all decreased (all P for trend < 0.05). There were no significant differences in other factors among the three glucose tolerance groups. Among the 31 participants with PDM, most ($n = 29$) were classified into the IGT group. Among the 32 participants with type 2 diabetes mellitus, 13 were newly diagnosed. Among the 19 participants already diagnosed with type 2 diabetes mellitus (duration of type 2 diabetes mellitus: median 12 years [range 2–57 years]), 15 participants were treated with oral hypoglycemic agents.

Glucose tolerance status and b- and a-cell mass

Figure 1 shows representative photomicrographs of the pancreas immunostained for insulin or glucagon with hematoxylin for each glucose tolerance status. The mean values of BCA decreased significantly with worsening glucose tolerance status (mean ± standard error: 1.85 ± 0.10% in NGT, 1.59 ± 0.11% in PDM and 1.17 ± 0.11% in type 2 diabetes mellitus, P for trend < 0.001; Figure 2a). In contrast, there was no significant difference in ACA among the three groups (0.37 ± 0.05% in NGT, 0.30 ± 0.06% in PDM and 0.36 ± 0.05% in type 2 diabetes mellitus, P for trend = 0.80; Figure 2b). The ratio of ACA/BCA was then significantly increased with deterioration

Table 1 | Characteristics of participants according to the glucose tolerance status

	NGT (n = 40)	PDM (n = 31)	Type 2 diabetes mellitus (n = 32)	P for trend
Male (%)	60.0	80.6	59.3	0.94
Age at the time of 75-g OGTT (years)	77 ± 10	75 ± 6	73 ± 10	0.08
Age at death (years)	80 ± 10	78 ± 7	76 ± 10	0.11
Family history of diabetes mellitus (%)	17.5	6.5	15.6	0.75
FPG (mmol/L)	5.3 ± 0.4	5.7 ± 0.5	7.0 ± 1.7*	<0.001
2hPG (mmol/L)	6.0 ± 1.2	8.8 ± 1.4*	14.8 ± 3.8*	<0.001
HbA1c (%)	5.2 ± 0.5	5.4 ± 0.5	6.2 ± 1.0*	<0.001
HOMA-IR	0.9 (0.7–1.1)	1.3 (1.0–1.7)	2.4 (1.9–3.1)*	<0.001
Insulinogenic index†	0.74 (0.47–1.18)	0.38 (0.26–0.55)	0.25 (0.15–0.42)*	0.003
Disposition index†	0.96 (0.65–1.40)	0.29 (0.23–0.38)*	0.12 (0.09–0.15)*	<0.001
Body mass index (kg/m^2)	20.4 ± 2.3	22.1 ± 3.6*	23.6 ± 3.1*	<0.001
Overweight (%)	15.0	32.2	53.1*	0.001
Hypertension (%)	52.5	64.5	71.9*	0.09
Serum triglycerides (mmol/L)	1.00 (0.85–1.16)	1.25 (1.04–1.50)	1.46 (1.23–1.74)*	0.002
Serum HDL cholesterol (mmol/L)	1.64 ± 0.42	1.54 ± 0.48	1.40 ± 0.41*	0.02
Smoking habits (%)	52.5	81.0	59.3	0.46
Alcohol intake (%)	35.0	41.9	28.1	0.74
Regular exercise (%)	12.5	12.9	0	0.09
Time from death to autopsy (h)	15.3 ± 8.0	14.3 ± 6.6	13.7 ± 7.5	0.35
Causes of death (%)				
Cancer	30.0	35.5	50.0	0.09
Cardiovascular diseases	22.5	29.0	21.9	0.99
Respiratory diseases	27.5	9.7	12.5	0.09
Other causes	20.0	25.8	15.6	0.69

Homeostasis model assessment of insulin resistance (HOMA-IR), insulinogenic index, disposition index and serum triglycerides are presented as the median (interquartile range). All other values are presented as the mean ± standard deviations or percentages. *P < 0.05 versus normal glucose tolerance (NGT). †Insulinogenic index and disposition index were calculated in 52 individuals with measurement of plasma glucose and serum insulin at 30 min: NGT (n = 22), prediabetes (PDM; n = 20) and type 2 diabetes mellitus (n = 10). 2hPG, 2-h postload glucose; FPG, fasting plasma glucose; HDL, high-density lipoprotein.

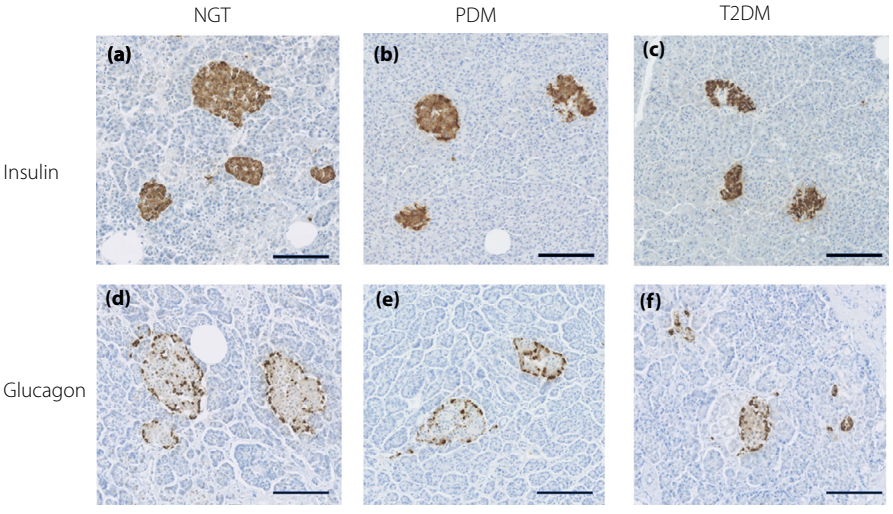

Figure 1 | Representative photomicrographs of the pancreas immunostained for (a–c) insulin (a) in normal glucose tolerance (NGT), (b) in prediabetes (PDM) and (c) in type 2 diabetes mellitus (T2DM) or (d–f) glucagon (d) in NGT, (e) in PDM and (f) in type 2 diabetes mellitus with hematoxylin in each glucose tolerance status. Scale bar, 200 μm.

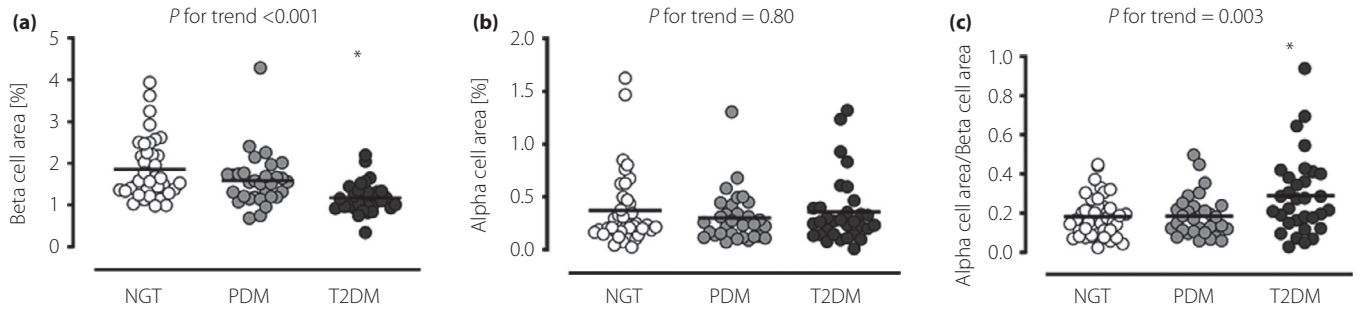

Figure 2 | Comparisons of (a) β-cell area, (b) α-cell area and (c) the ratio of the α-cell area to β-cell area among the individuals with different glucose tolerance status. NGT, normal glucose tolerance; PDM, prediabetes; T2DM, type 2 diabetes mellitus. *$P < 0.05$ vs NGT.

of glucose tolerance status (0.18 ± 0.02, 0.19 ± 0.03 and 0.29 ± 0.03 in NGT, PDM and type 2 diabetes mellitus, P for trend = 0.003; Figure 2c). These associations remained unchanged after adjusting for potential confounding factors; that is, age at the time of 75-g OGTT, sex, family history of diabetes, BMI, hypertension, serum triglycerides, serum high-density lipoprotein cholesterol, smoking habits, alcohol intake, regular exercise, time from death to autopsy and causes of death (Table 2). In addition, these associations remained unchanged after separating PDM into impaired fasting glycemia and IGT (Figure S2). Significant negative associations between glucose tolerance status and the mean values of BCA were observed in both subgroups with and without overweight (Figure S3a,d).

Mean islet size and islet density tended to decrease with the deterioration of glucose tolerance status (5,504 ± 280, 5,347 ± 318 and 4,805 ± 313 μm^2 for mean islet size, and 5.68 ± 0.49, 5.20 ± 0.55 and 4.47 ± 0.54/mm^2 for islet density in NGT, PDM and type 2 diabetes mellitus, respectively, both P for trend = 0.10; Figures S4a,b). There were no differences in the density of scattered β-cells, density of insulin-positive duct cells or β-cell replication among the glucose tolerance groups (Figures S4c–e). The mean values of islet amyloid deposits increased significantly with worsening glucose tolerance status (P for trend = 0.03; Figure S4f). No significant associations between islet amyloid deposits and BCA or ACA were observed (R = –0.11 and 0.09, respectively; Figure S5a,b).

Next, we analyzed the correlation between BCA and ACA according to the glucose tolerance status (Figure 3a–c). Although there was a positive correlation between BCA and ACA in overall participants (R = 0.44, $P < 0.001$), the correlation became weakened with worsening glucose tolerance status (R = 0.59, 0.51 and 0.35, $P < 0.001$, 0.004 and 0.049, in NGT, PDM and type 2 diabetes mellitus, respectively). In the analysis for heterogeneity among the glucose tolerance groups, the magnitude of the association was weaker in the participants with type 2 diabetes mellitus than in those with NGT or PDM (β-coefficient 1.63, 95% confidence interval 1.26–2.00 for NGT; 2.05 95% confidence interval 1.46–2.65 for PDM; 0.56, 95% confidence interval 0.15–1.00 for type 2 diabetes mellitus; P for heterogeneity <0.001).

Glycemic indices and b- and a-cell mass

BCA was significantly inversely correlated with all the glycemic parameters; that is, FPG, 2hPG and HbA1c among the total participants (R = –0.26, –0.46 and –0.30, respectively, all $P < 0.01$; Figures 4a–c). ACA was significantly positively correlated only with HbA1c (R = 0.32, P = 0.001; Figure 4f). The

Table 2 | Multivariable-adjusted mean values of β-cell area, α-cell area and the ratio of α-cell area to β-cell area according to the glucose tolerance status

Glucose tolerance status	No. participants	Multivariable-adjusted mean of β-cell area, % (95% CI)	P for trend	Multivariable-adjusted mean of α-cell area, % (95% CI)	P for trend	Multivariable-adjusted mean of the ratio of α-cell area to β-cell area (95% CI)	P for trend
NGT	40	1.82 (1.61, 2.04)	0.01	0.37 (0.26, 0.47)	0.31	0.18 (0.13, 0.23)	0.02
PDM	31	1.59 (1.36, 1.83)		0.31 (0.19, 0.42)		0.19 (0.14, 0.25)	
Type 2 diabetes mellitus	32	1.20 (0.97, 1.44)*		0.36 (0.25, 0.48)		0.29 (0.23, 0.34)*	

*$P < 0.05$ versus normal glucose tolerance (NGT). The values were adjusted for age at the time of 75-g oral glucose tolerance test, sex, family history of diabetes, body mass index, hypertension, serum triglycerides, serum high-density lipoprotein cholesterol, smoking habits, alcohol intake, regular exercise, time from death to autopsy and causes of death. CI, confidence interval; PDM, prediabetes.

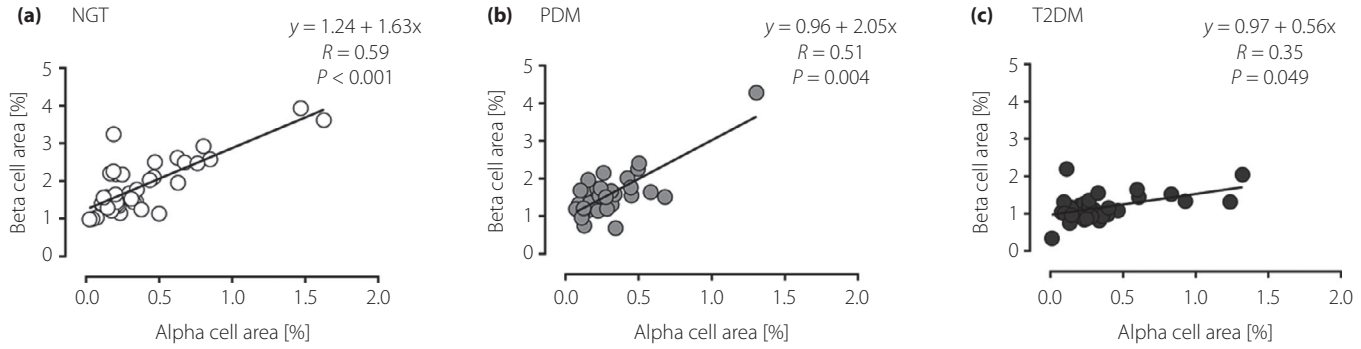

Figure 3 | Correlations between α- and β-cell area in individuals with (a) normal glucose tolerance (NGT), (b) prediabetes (PDM) and (c) type 2 diabetes mellitus (T2DM).

ACA/BCA ratio was significantly correlated with FPG, 2hPG and HbA1c (R = 0.22, 0.25 and 0.45, all $P < 0.05$; Figures 4g–i). In contrast, in the subgroup analyses according to each glucose tolerance status, correlations between BCA and glycemic parameters were not observed, whereas significant positive correlations between ACA or the ACA/BCA ratio and glycemic parameters were found only in participants with type 2 diabetes mellitus, not in those with NGT or PDM (Table S1).

There was a significant correlation between the insulinogenic index and ACA/BCA ratio (R = –0.39, P = 0.004; Figure 5i), whereas the disposition index, an index of true β-cell function, was significantly correlated with both BCA and the ACA/BCA ratio (R = 0.38 and –0.30, both $P < 0.05$; Figures 5b,j). HOMA-IR was correlated with neither BCA, nor ACA nor the ACA/BCA ratio (all $P > 0.1$; Figure 5c,g,k).

There was a significant negative correlation between BMI and BCA (R = –0.29, P = 0.003; Figure 5d), but not ACA and the ACA/BCA ratio. In the subgroup analyses according to each glucose tolerance status, a correlation between BMI and BCA was not observed (Table S1).

DISCUSSION

The present study showed that BCA decreased with worsening glucose tolerance status, being approximately 14 and 37% lower in participants with PDM and type 2 diabetes mellitus compared with those with NGT, respectively, based on the analysis of pancreas tissues from Japanese community-based autopsy samples. Previous studies have consistently shown a reduction in β-cell mass ranging from 30 to 65% in individuals with type 2 diabetes mellitus[4,7–9]. The examinations of surgically resected pancreas samples have also shown ~30% reduction in β-cell mass in individuals with PDM, but they could not exclude the effects of pancreatic diseases, operative procedures or preoperative anticancer agents[9,20,21]. Studies carried out in obese white Europeans with BMI >27 kg/m^2 have reported a 40–50% reduction in β-cell mass in individuals with PDM[4,22]. The extent of reduction in β-cell mass in individuals with PDM has varied among these reports because of their different study designs and the different backgrounds of the study samples. Therefore, a community-based histological study is required to elucidate the difference in β-cell mass according to the glucose tolerance status. In this regard, this is the first study using community-based autopsy samples to provide the valuable finding that the β-cell mass gradually declined, without a compensatory increase, with worsening glucose tolerance in a Japanese population.

The mechanisms underlying the β-cell loss in type 2 diabetes mellitus remain controversial. In the present study, there were no decreases of β-cell neogenesis and replication with worsening glucose tolerance status. An increase in amyloid deposits, which enhances β- and α-cell loss[23,24], was observed in individuals with type 2 diabetes mellitus. However, there were no significant associations between amyloid deposits and BCA or ACA. Further studies, including detailed analyses of a larger number of individuals with amyloid deposits, will be required to clarify the underlying mechanism of islet remodeling with worsening glucose tolerance status.

We did not find any significant changes in ACA among the different glucose tolerance groups, in line with our previous studies and those of others[8,9,25], although there are also some reports describing an increase in ACA in individuals with type 2 diabetes mellitus[6,26]. In the present study, the significant negative correlations between BCA and glycemic parameters disappeared in the subgroup analysis according to the glucose tolerance status, suggesting these correlations might reflect the difference in the glucose tolerance status, whereas the significant positive correlations between ACA or the ACA/BCA ratio and glycemic parameters were significant only in individuals with type 2 diabetes mellitus, not in those with NGT or PDM. Furthermore, the association between BCA and ACA was weaker in those with type 2 diabetes mellitus compared with those with NGT or PDM. The results in a previous rodent study proposed that β-cell dedifferentiation and transdifferentiation to α-cells occurs in hyperglycemia[27]. Our present and these previous findings raise the possibility that both increased ACA and decreased BCA contribute to poor glycemic control in individuals with type 2 diabetes mellitus, whereas the role of α-cells on the glycemic control in NGT and PDM remains unclear.

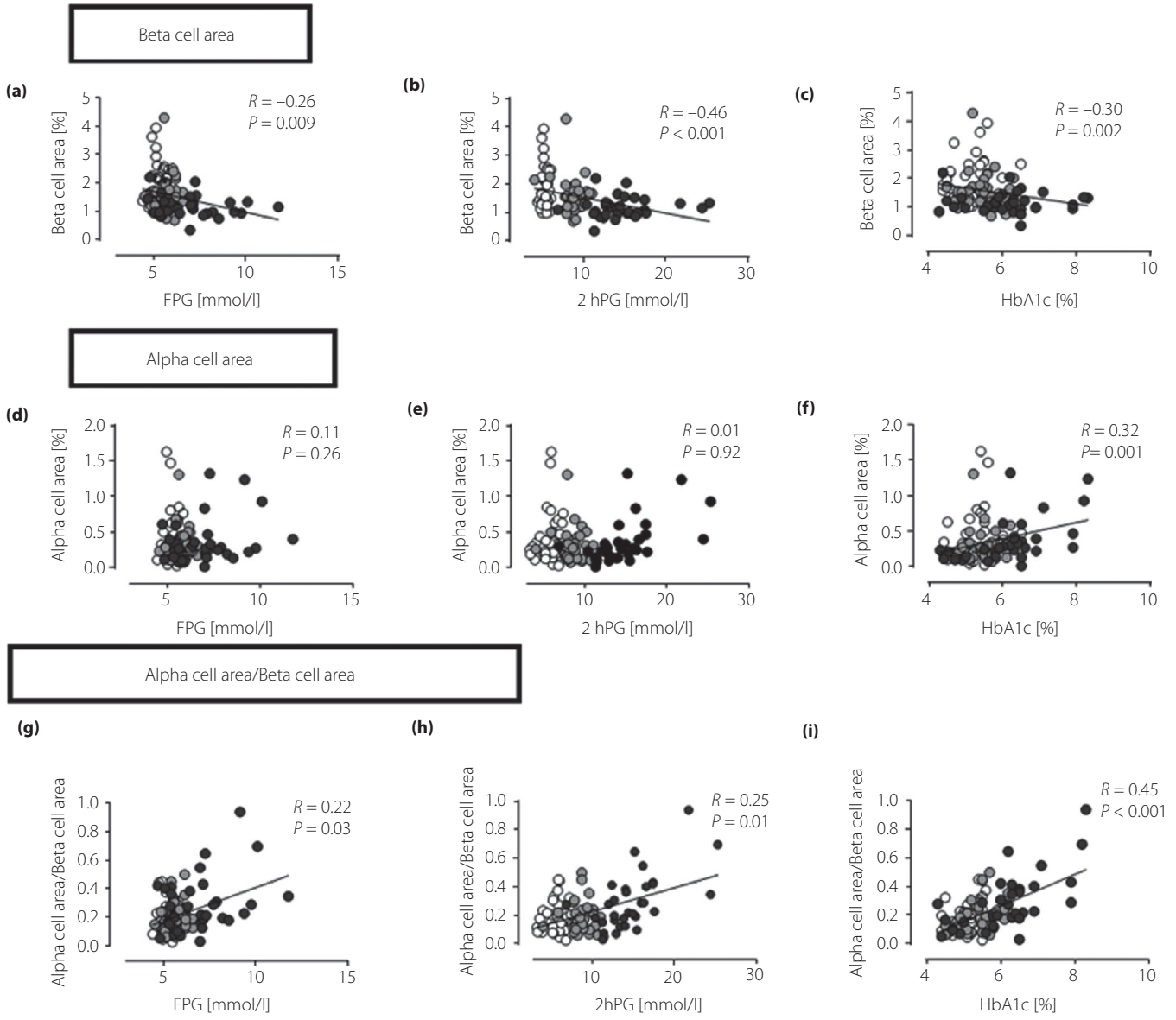

Figure 4 | Correlations of fasting plasma glucose (FPG), 2-h postload glucose (2hPG) and glycated hemoglobin (HbA1c) with (a–c) β-cell area, (d–f) α-cell area and (g–i) the ratio of α-cell area to β-cell area. White, light gray and dark gray circles show individuals with normal glucose tolerance, prediabetes and type 2 diabetes mellitus, respectively.

BCA was positively correlated with the disposition index, which represents the true β-cell function considered for insulin sensitivity in the present study. Some of the previous reports carried out using the surgically resected pancreases of Japanese patients showed correlations between BCA and indices of β-cell function, such as the C-peptide immunoreactivity index[9,28], consistent with our present findings. These results indicate that both β-cell function and mass in Japanese simultaneously and continuously decline during the deterioration of glucose tolerance. Notably, the ACA/BCA ratio was inversely correlated with not only the disposition index, but also the insulinogenic index. In addition, the correlation of the ACA/BCA ratio with HbA1c was stronger than that of BCA. These results suggest that the ACA/BCA ratio might better reflect glycemic status than BCA itself.

Previous studies in Japanese populations observed no significant increase in β-cell mass in obese non-diabetic adults[9,19,26]. The significant correlations between BMI and BCA disappeared in the subgroup analysis according to the glucose tolerance status. These results suggest that the change of β-cell mass in the face of obesity might be limited in Japanese individuals.

The strengths of the present study include: (i) the examined cases were selected from a community-based autopsy sample with a high autopsy rate; and (ii) the glucose tolerance status

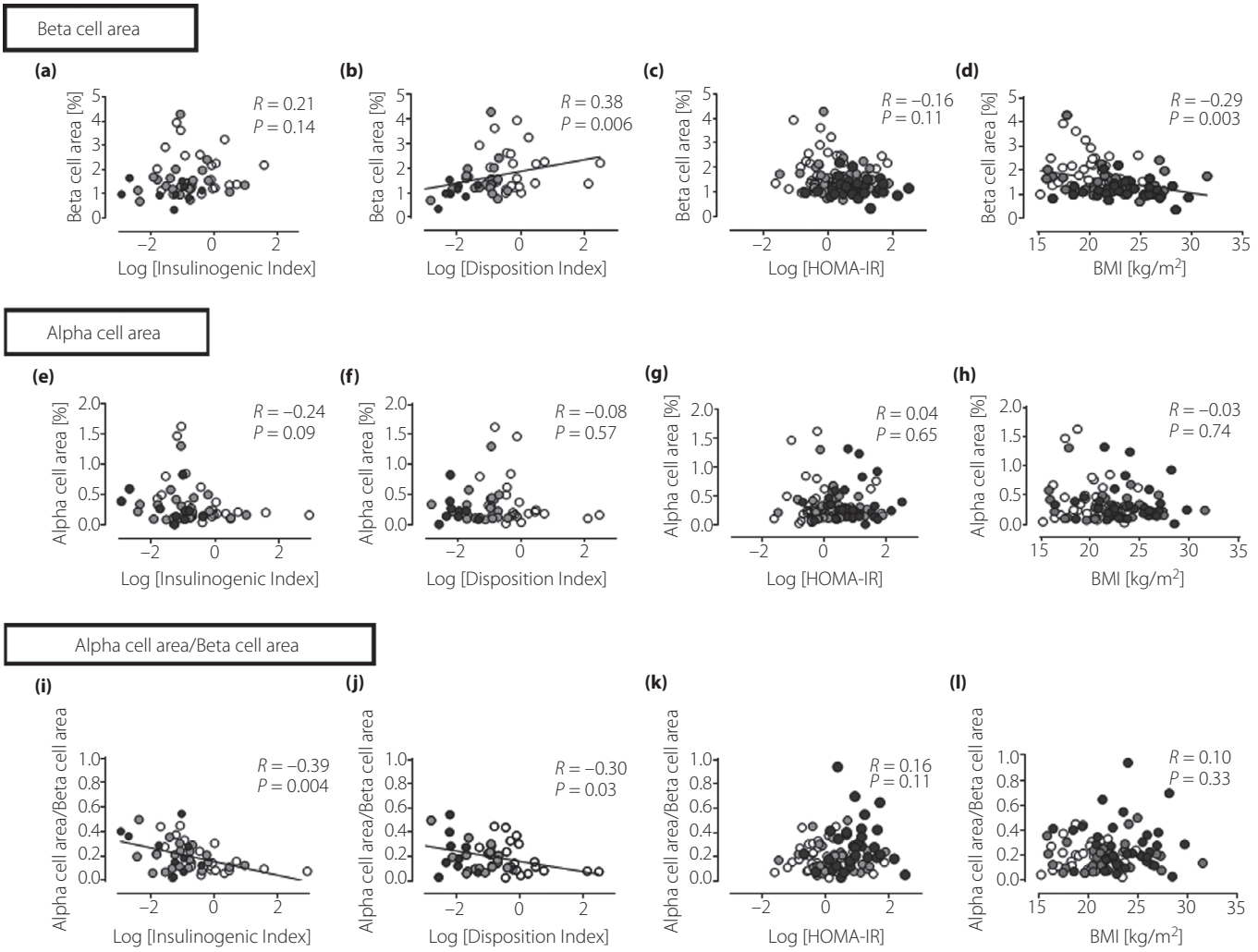

Figure 5 | Correlations of (a–d) insulinogenic index, disposition index, homeostasis model assessment of insulin resistance (HOMA-IR) and body mass index (BMI) with β-cell area, (e–h) α-cell area and (i–l) the ratio of α-cell area to β-cell area. White, light gray and dark gray circles show individuals with normal glucose tolerance, prediabetes and type 2 diabetes mellitus, respectively. Insulinogenic index and disposition index were calculated in 52 subjects with measurement of plasma glucose and serum insulin at 30 min: normal glucose tolerance (n = 22), prediabetes (n = 20), type 2 diabetes mellitus (n = 10).

was diagnosed accurately based on OGTT and clinical information before death. However, there were also several limitations of the study. First, the causality of the findings remains to be determined, because of the cross-sectional nature of autopsy studies. Second, the mean interval from OGTT to death in this study was approximately 3 years, although the glucose tolerance status of the participants was closely examined by OGTT within 5 years before death. The glucose tolerance status might have changed over time after OGTT. Third, the generalizability of the findings would be limited, because the mean age at death was ≥75 years in this group of individuals, and the major causes of death were cancer, cardiovascular diseases or respiratory diseases. Thus, aging and its related comorbidities might have affected glucose metabolism. Fourth, we assessed β- and α-cell mass by measuring BCA and ACA, because we lacked data on the pancreas weight in the present study. Any difference in pancreas volume might have affected our findings. A fifth potential limitation regards the methods used for the morphological examinations. We cannot rule out the possibility of an underestimation of islet size, because the size of each islet was measured in the section stained for insulin. In addition, BCA and ACA were individually evaluated with sections for each hormone. Unfortunately, we could not estimate β- and α-cells in identical sections using double or more immunofluorescence or immunohistochemistry. Thus, the change in the proportions of each cell in the islets between the different sections might have affected the association between each cell area and the diabetes status. Finally, we might not have had

sufficient statistical power to detect differences in some analyses, especially in the subgroup analyses, because of the limited sample size of this study. Despite these limitations, however, we believe that the present findings enhance our understanding of the natural history of β-cell mass during the development of type 2 diabetes mellitus.

In conclusion, β-cell mass decreased significantly with worsening glucose tolerance, from the stage of PDM, in the Japanese population. The present findings highlight that the prevention of declining β-cell mass before the onset of glucose intolerance is important to reduce the burden of type 2 diabetes mellitus.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 8th edn. Brussels: International Diabetes Federation, 2017. https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/134-idf-diabetesatlas-8th-edition.html. Accessed February 23 2020.
2. Saisho Y. Beta cell dysfunction: its critical role in prevention and management of type 2 diabetes. *World J Diabetes* 2015; 6: 109–124.
3. Halban PA, Polonsky KS, Bowden DW, *et al*. Beta-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *Diabetes Care* 2014; 37: 1751–1758.
4. Butler AE, Janson J, Bonner-Weir S, *et al*. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; 52: 102–110.
5. Rahier J, Guiot Y, Goebbels RM, *et al*. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab* 2008; 10(Suppl 4): 32–42.
6. Yoon KH, Ko SH, Cho JH, *et al*. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 2003; 88: 2300–2308.
7. Sakuraba H, Mizukami H, Yagihashi N, *et al*. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese type 2 diabetic patients. *Diabetologia* 2002; 45: 85–96.
8. Sato S, Saisho Y, Inaishi J, *et al*. Effects of glucocorticoid treatment on beta- and alpha-cell mass in Japanese adults with and without diabetes. *Diabetes* 2015; 64: 2915–2927.
9. Inaishi J, Saisho Y, Sato S, *et al*. Effects of obesity and diabetes on alpha- and beta-cell mass in surgically resected human pancreas. *J Clin Endocrinol Metab* 2016; 101: 2874–2882.
10. Saisho Y, Butler AE, Manesso E, *et al*. B-cell mass and turnover in humans: effects of obesity and aging. *Diabetes Care* 2013; 36: 111–117.
11. Mezza T, Muscogiuri G, Sorice GP, *et al*. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes* 2014; 63: 994–1007.
12. Hata J, Ninomiya T, Hirakawa Y, *et al*. Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). *Circulation* 2013; 128: 1198–1205.
13. Kubo M, Kiyohara Y, Kato I, *et al*. Risk factors for renal glomerular and vascular changes in an autopsy-based population survey: the Hisayama study. *Kidney Int* 2003; 63: 1508–1515.
14. Nagata M, Ninomiya T, Doi Y, *et al*. Temporal trends in sudden unexpected death in a general population: the Hisayama study. *Am Heart J* 2013; 165; 932–938.e1.
15. Mukai N, Doi Y, Ninomiya T, *et al*. Trends in the prevalence of type 2 diabetes and prediabetes in community-dwelling Japanese subjects: the Hisayama Study. *J Diabetes Investig* 2014; 5: 162–169.
16. Committee on the Standardization of Diabetes Mellitus-Related Laboratory Testing of Japan Diabetes Society. International clinical harmonization of glycated hemoglobin in Japan: from Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *Diabetol Int* 2012; 3: 8–10.
17. Utzschneider KM, Prigeon RL, Faulenbach MV, *et al*. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care* 2009; 32: 335–341.
18. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; 363: 157–163.
19. Kou K, Saisho Y, Satoh S, *et al*. Change in beta-cell mass in Japanese nondiabetic obese individuals. *J Clin Endocrinol Metab* 2013; 98: 3724–3730.
20. Meier JJ, Menge BA, Breuer TG, *et al*. Functional assessment of pancreatic beta-cell area in humans. *Diabetes* 2009; 58: 1595–1603.

21. Yoneda S, Uno S, Iwahashi H, *et al*. Predominance of beta-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *J Clin Endocrinol Metab* 2013; 98: 2053–2061.
22. Ritzel RA, Butler AE, Rizza RA, *et al*. Relationship between beta-cell mass and fasting blood glucose concentration in humans. *Diabetes Care* 2006; 29: 717–718.
23. Jurgens CA, Toukatly MN, Fligner CL, *et al*. Beta-cell loss and beta-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol* 2011; 178: 2632–40.
24. Kamata K, Mizukami H, Inaba W, *et al*. Islet amyloid with macrophage migration correlates with augmented beta-cell deficits in type 2 diabetic patients. *Amyloid* 2014; 21: 191–201.
25. Henquin JC, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. *Diabetologia* 2011; 54: 1720–1725.
26. Mizukami H, Takahashi K, Inaba W, *et al*. Involvement of oxidative stress-induced DNA damage, endoplasmic reticulum stress, and autophagy deficits in the decline of beta-cell mass in Japanese type 2 diabetic patients. *Diabetes Care* 2014; 37: 1966–1974.
27. Talchai C, Xuan S, Lin HV, *et al*. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell* 2012; 150: 1223–1234.
28. Fujita Y, Kozawa J, Iwahashi H, *et al*. Increment of serum C-peptide measured by glucagon test closely correlates with human relative beta-cell area. *Endocrine J* 2015; 62: 329–337.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Correlations of fasting plasma glucose (FPG), 2-h postload glucose (2hPG), HbA1c, body mass index (BMI) and islet amyloid deposits (Amyloid) with β-cell area (BCA), α-cell area (ACA) and the ratio of α-cell area to β-cell area in individuals with (a) normal glucose tolerance (NGT), (b) prediabetes (PDM) and (c) type 2 diabetes mellitus (T2DM).
Figure S1 | Flowchart of participants in this study.
Figure S2 | Comparisons of (a) β-cell area, (b) α-cell area and (c) the ratio of the α-cell area to β-cell area among the participants with different glucose tolerance status.
Figure S3 | Comparisons of β-cell area, α-cell area and the ratio of α-cell area to β-cell area among the participants with different glucose tolerance status and (a–c) with (n=34) or (d–f) without overweight (n=69).
Figure S4 | Mean (a) islet size, (b) islet density, (c) scattered β-cells, (d) insulin-positive duct cells, (e) β-cell replication and (f) islet amyloid deposits according to the glucose tolerance status.
Figure S5 | Correlations of islet amyloid deposits with (a) β-cell area, (b) α-cell area and (c) the ratio of α-cell area to β-cell area.