Increment of serum C-peptide measured by glucagon test closely correlates with human relative beta-cell area

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Abstract. Pancreatic beta-cell mass contributes to glucose tolerance. The aim of this study was to evaluate the relationships between human beta-cell mass and various clinical parameters, including insulin secretory capacity. The study included 32 Japanese patients who underwent pancreatectomy and were naive to oral hypoglycemic agents and insulin. They were classified into those with normal glucose tolerance (n=13), impaired glucose tolerance (n=9) and diabetes (n=10), and their insulin secretory capacity and insulin resistance were evaluated. Immunohistochemistry was used to determine relative beta-cell area (%) which represented the proportion of insulin-positive cell area to whole pancreatic section. Increment of C-peptide immunoreactivity level by glucagon test (ΔC-peptide, increment of serum C-peptide [nmol/L] at 6 min after intravenous injection of 1-mg glucagon; r=0.64, p=0.002), homeostasis model assessment of beta-cell function (HOMA-beta, fasting immunoreactive insulin [µIU/mL] x 20 / (fasting plasma glucose [mmol/L] – 3.5); r=0.50, p=0.003), C-peptide index (CPI, fasting C-peptide [nmol/L] / fasting plasma glucose [mmol/L]; r=0.36, p=0.042), and fasting immunoreactive insulin (F-IRI [µmol/L]; r=0.36, p=0.044) correlated significantly and positively with the relative beta-cell area. The area under the curve of plasma glucose level from 0 to 120 min by 75 g-OGTT (AUC0-120) also correlated significantly and inversely with the relative beta-cell area (r=-0.36, p=0.045). Stepwise multiple regression analysis identified ΔC-peptide as the only independent and significant determinant of the relative beta-cell area. We conclude that ΔC-peptide, HOMA-beta, CPI, F-IRI and AUC0-120 correlated closely with the relative beta-cell area, and ΔC-peptide was the most valuable index for the prediction of the area.

Key words: Beta-cell area, Immunohistochemistry, Insulin secretory capacity, Human islet

PANCREATIC beta-cell mass contributes to glucose tolerance in several clinical situations. At the onset of type 1 diabetes mellitus (T1DM), over 80% of beta-cells are lost [1, 2], and there are scattered or very few beta-cells after prolonged duration of diabetes [3]. Appropriate insulin therapy is required to avoid life-threatening hyperglycemia and ketosis in T1DM [4]. In other types of diabetes characterized by impaired insulin secretion, such as mitochondrial diabetes [5, 6] and Wolfram’s syndrome [7], beta-cell mass is also markedly reduced. Furthermore, diabetes mellitus develops in 20-50% of the patients who undergo pancreatic resection [8]. In type 2 diabetes mellitus (T2DM), the relationship between beta-cell mass and glucose intolerance is not as simple as in T1DM because both insulin resistance and insulin secretory capacity are associated with glucose intolerance [4]. However, there is general agreement that reduced beta-cell mass also

F-CPR, fasting C-peptide immunoreactivity; F-IRI, fasting immunoreactive insulin; FPG, fasting plasma glucose; HOMA-beta, homeostasis model assessment of beta-cell function; HOMA-R, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; II, insulinogenic index; IRI120, immunoreactive insulin at 120 min by 75 g-OGTT; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG120, plasma glucose level at 120 min by 75 g-OGTT; PG30, plasma glucose level at 30 min by 75 g-OGTT; PG60, plasma glucose level at 60 min by 75 g-OGTT
worsens glucose intolerance in T2DM [9-13], and overt diabetes manifests when beta cell area diminishes by approximately 40-65% [13, 14]. Beta-cell apoptosis [12], autophagic deficit through oxidative stress in pancreatic islets [15] and dedifferentiation of beta-cell [16] are some of the proposed mechanisms of beta-cell reduction in T2DM.

Beta-cell mass can be measured directly in rodent models of diabetes after resection of the pancreas and staining with dithizon [17, 18]. But in human, it is not easy to measure beta-cell mass noninvasively in clinical situation. However, parameters representing insulin secretory capacity based on results of fasting blood tests or certain loading tests are candidate noninvasive indicators of beta-cell mass. In clinical setting, we also use homeostasis model assessment of beta-cell function (HOMA-beta) [19], insulinogenic index (II) calculated from 75 g-oral glucose tolerance test (75 g-OGTT) [20, 21] and increment of C-peptide immunoreactivity level in the glucagon test (ΔC-peptide) [22-24], to select the most appropriate treatment for a given patient by reference to these parameters. Beta-cell area was reported to correlate with plasma C-peptide or insulin levels and C-peptide-to-glucose or insulin-to-glucose ratios [25]. However, the relationships between these parameters and beta-cell mass have not been fully established.

Because insulin secretion is affected by insulin resistance and glucose-lowering agents, a simple relationship between beta-cell mass and insulin secretory capacity is not expected in long-standing T2DM. Therefore, indices that can be used to assess beta-cell mass should be established only in patients who have not been treated with glucose-lowering agents. In this study, we analyzed human pancreatic tissues obtained from pancreatectomy using an immunohistochemical approach, and evaluated the relationships between beta-cell mass and various clinical parameters, including insulin secretory capacity, in drug-naive patients who were classified into three different stages of glucose tolerance: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and diabetes mellitus (DM).

Patients and Methods

Patients

Thirty-two Japanese patients (19 males and 13 females) who had undergone pancreatic resection between 2008 and 2012 in the Department of Gastroenterological Surgery, Osaka University Hospital, and who had agreed to participate in this study were enrolled. The study protocol was approved by the Ethics Committee of Osaka University. Diabetic patients treated by oral hypoglycemic agents or insulin, patients with renal failure (estimated glomerular filtration rate <30 ml/min/1.73 m²) and patients with pancreatic endocrine tumors were excluded from this study. The mean age was 64±11 years (±SD), and the mean BMI was 21.6±2.8 kg/m². Among the 32 patients, one patient was diagnosed with DM based on fasting plasma glucose (FPG) level, and another patient had been already diagnosed with DM before visiting our hospital. Thirty-one patients, with the exception of one who was diagnosed with DM based on FPG level, underwent 75 g-OGTT 1-60 days before pancreatic resection, and the results of the test were used to classify the patients into three groups [26]: the NGT group included 13 patients, IGT 9, and DM 10 patients. Glucagon test was carried out in 19 patients (10 NGT, 4 IGT and 5 DM).

Laboratory tests

Preoperative insulin secretory capacity was evaluated by HOMA-beta [19], C-peptide index (CPI) [27], II [20] and ΔC-peptide [23]. Insulin resistance was evaluated by HOMA-insulin resistance (HOMA-R) [19], and insulin sensitivity was evaluated by Matsuda index [28, 29]. HOMA-beta was calculated by F-IRI (µIU/mL) x 20 / (FPG (mmol/L) – 3.5), where F-IRI represents fasting immunoreactive insulin. CPI was calculated by F-CPR (nmol/L) / FPG (mmol/L), where F-CPR represents fasting C-peptide immunoreactivity. II was defined as the ratio of increment in plasma insulin level to that of plasma glucose level at 30 min during 75 g-OGTT [Δ serum insulin 0-30 min (pmol/L) / Δ plasma glucose 0-30 min (mmol/L)]. The value of ΔC-peptide was defined as increment in serum C-peptide level (nmol/L) at 6 min after intravenous injection of 1-mg glucagon after an overnight fast. HOMA-R was calculated by the following formula: FPG (mmol/L) x F-IRI (µIU/mL) / 22.5. Matsuda index was calculated by the following formula: 10000 / (FPG (mg/dL) x F-IRI (µIU/mL) x PG_{120} (mg/dL) x IRI_120 (µIU/mL))^{1/2} [29], where IRI_{120} is immunoreactive insulin at 120 min by 75 g-OGTT and PG_{120} is plasma glucose level at 120 min by 75 g-OGTT. Serum immunoreactive insulin and C-peptide immunoreactivity were measured by chemiluminescent enzyme immunoassay. HbA1c values were converted to
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National Glycohemoglobin Standardization Program (NGSP) equivalent values (%) in accordance with the official equation [30] and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)- recommended units (mmol/mol).

Pancreatic tissue processing

We obtained pancreas head tissue samples from patients who underwent pancreaticoduodenectomy and pancreas body or tail tissue samples from patients who underwent distal pancreatectomy. The region of the available pancreatic tissue sample could not be determined in one patient who underwent total pancreatectomy. Pancreatic samples at normal region were collected during operation. The tissues were cut off from near the resected margins after intraoperative consultation, fixed immediately in formaldehyde and embedded in paraffin for subsequent analysis. The tissues were cut from these paraffin blocks into 5-μm thick sections and then stained with hematoxylin and eosin and confirmed to contain no cancer elements. Sections with more than 30% fibrous area estimated by Azan staining were excluded from this study.

Immunohistochemistry

We adopted “relative beta-cell area” as a parameter representing beta-cell mass, which was determined by the proportion of insulin-positive cell area to whole pancreatic section (%). We examined one section per patient, and the mean area of the section was 71.4±44.5-mm² (±SD). Pancreatic sections were stained by the indirect immunoperoxidase method to measure the relative beta-cell area [31]. Guinea pig anti-insulin immunoglobulins (Dako Japan, Kyoto, Japan) were used as the primary antibodies, and biotinylated goat anti-guinea pig immunoglobulins (Vector Laboratories, Burlingame, CA) were used as the secondary antibodies. The reactions were developed with an avidin-biotin complex (Vector Laboratories) and a 3,3-diaminobenzidine tetrahydrochloride substrate kit (Molecular Probes, Eugene, OR), followed by methyl green counterstaining. The area of insulin-positive cells in the entire pancreatic section was quantified digitally with the WinROOF software program (Mitani Corporation, Fukui, Japan).

Statistical analysis

Data are presented as mean±SD. Statistical significance was determined by one-way ANOVA followed by post-hoc analysis of Games-Howell, Pearson product-moment correlation coefficient and stepwise multiple regression analysis was conducted to identify explanatory variable for the relative beta-cell area. P value less than 0.05 denoted the presence a statistically significant difference. All statistical analyses were performed with the StatView software program (Statistical Analysis System Inc., Cary, NC).

Results

Clinical characteristics and laboratory data including results of 75 g-OGTT for NGT, IGT and DM subjects

Table 1 lists the clinical characteristics of the patients. Primary diseases were mainly pancreatic cancer (n=12) and cystic lesions of the pancreas (n=10), including intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, and simple cyst. Other diseases included cholangiocarcinoma (n=3), tumor of the ampulla of Vater (n=3), hepatocellular carcinoma (n=1), cholangitis (n=1), chronic pancreatitis (n=1), and pancreatic metastasis from renal cell carcinoma (RCC) (n=1). The operative procedures were pancreaticoduodenectomy (n=22), distal pancreatectomy (n=9) and total pancreatectomy (n=1). Ten patients had been treated with anticancer agents before surgery. There was no significant difference among the three groups with regard to age and BMI. HbA1c was significantly higher in DM than NGT (p<0.01) and IGT (p<0.05). There was no significant difference in FPG level among the three groups. Plasma glucose level was higher in DM than IGT at 30 min (p<0.05), higher in DM than NGT (p<0.05) and IGT (p=0.01) at 60 min, and higher in DM than NGT and IGT at 120 min (p<0.01, each). The area under the curve of glucose level from 0 to 120 min (AUC0-120) was higher in DM than NGT and IGT (p<0.01, each). II was lower in DM than NGT (p<0.01) and IGT (p<0.05). There were no significant differences in F-IRI level, F-CPR level, HOMA-beta, CPI, ΔC-peptide and HOMA-R among the three groups. Matsuda index was higher in NGT than IGT and DM (p<0.01, each). The mean relative beta-cell area was 1.072±0.424, 0.998±0.419 and 0.762±0.441 in NGT, IGT and DM, respectively, and tended to decrease with worsening of glucose intolerance.

The relative beta-cell area was not different between patients with malignant diseases (n=20) and benign diseases (n=12) (0.984±0.468% vs 0.904±0.388%, p=0.620), between samples obtained by pancre-
Table 1 Clinical characteristics of patients and laboratory data.

<table>
<thead>
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<th>Clinical diagnosis (n)</th>
<th>NGT</th>
<th>IGT</th>
<th>DM</th>
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<tr>
<td>n (M/F)</td>
<td>13 (8/5)</td>
<td>9 (4/5)</td>
<td>10 (7/3)</td>
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<tr>
<td>Pancreas cancer</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Cystic lesions of the pancreas</td>
<td>5</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Cholangiocarcinoma</td>
<td>1</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Tumor of the ampulla of Vater</td>
<td>1</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholangitis</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic metastasis of RCC</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<th>Operative procedure (n)</th>
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<tr>
<td>Pancreatoduodenectomy</td>
<td>11</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Distal pancreatectomy</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<th>Preoperative anticancer agents</th>
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<tr>
<td>Yes/No</td>
<td>4/9</td>
<td>4/5</td>
<td>2/8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64±11</td>
<td>61±15</td>
<td>68±6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5±3.1</td>
<td>20.8±1.8</td>
<td>22.4±3.1</td>
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<tr>
<td>HbA1c (%; mmol/mol)</td>
<td>5.3±0.6, 35±6</td>
<td>5.6±0.6, 37±6</td>
<td>6.3±0.4, 45±5↓</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.3±0.4</td>
<td>5.2±0.5</td>
<td>5.6±0.6</td>
</tr>
<tr>
<td>PG30 (mmol/L)</td>
<td>8.9±2.3</td>
<td>8.3±1.2</td>
<td>10.4±1.7↑(n=9)</td>
</tr>
<tr>
<td>PG60 (mmol/L)</td>
<td>9.9±4.1</td>
<td>9.5±2.2</td>
<td>13.3±1.9↑(n=9)</td>
</tr>
<tr>
<td>PG120 (mmol/L)</td>
<td>6.1±1.1</td>
<td>9.4±1.0↓</td>
<td>13.6±2.2↑(n=9)</td>
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<tr>
<td>AU_{C_{0-120}} (mmol·hr/L)</td>
<td>16.3±4.5</td>
<td>17.3±2.5</td>
<td>23.3±3.2↑(n=9)</td>
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<tr>
<td>F-IRI (pmol/L)</td>
<td>44±37</td>
<td>38±14</td>
<td>35±22</td>
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<td>F-CPR (nmol/L)</td>
<td>0.55±0.23</td>
<td>0.48±0.16</td>
<td>0.49±0.23</td>
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<td>HOMA-beta (%)</td>
<td>66.9±42.0</td>
<td>65.1±22.9</td>
<td>51.8±36.6</td>
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<tr>
<td>CPI (mmol/mmol)</td>
<td>0.099±0.033</td>
<td>0.093±0.026</td>
<td>0.090±0.044</td>
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<tr>
<td>II (pmol/mmol)</td>
<td>112±51</td>
<td>82±42</td>
<td>31±20↓↓(n=8)</td>
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<td>ΔC-peptide (nmol/L)</td>
<td>1.09±0.40 (n=10)</td>
<td>0.98±0.35 (n=4)</td>
<td>0.81±0.37 (n=5)</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>1.56±1.44</td>
<td>1.28±0.57</td>
<td>1.25±0.74</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>9.05±4.99</td>
<td>4.71±1.86↑</td>
<td>4.85±2.01↑(n=9)</td>
</tr>
<tr>
<td>Relative beta-cell area (%)</td>
<td>1.072±0.424</td>
<td>0.998±0.419</td>
<td>0.762±0.441</td>
</tr>
</tbody>
</table>

Data are mean±SD. Statistical analyses were carried out by one-way ANOVA followed by post-hoc analysis of Games-Howell. *p<0.05 vs NGT, †p<0.01 vs NGT, ‡p<0.05 vs IGT, §p<0.01 vs IGT.

RCC, renal cell carcinoma; FPK, fasting plasma glucose; PG30; plasma glucose level at 30 min by 75 g-OGTT; PG60, plasma glucose level at 60 min by 75 g-OGTT; PG120, plasma glucose level at 120 min by 75 g-OGTT; AU_{C_{0-120}} area under the curve of plasma glucose level from 0 to 120 min by 75 g-OGTT; F-IRI, fasting immunoreactive insulin; F-CPR, fasting C-peptide immunoreactivity; HOMA-beta, homeostasis model assessment of beta-cell function; CPI, C-peptide index; II, insulinogenic index; ΔC-peptide, increment of C-peptide immunoreactivity level by glucagon test; HOMA-R, homeostasis model assessment of insulin resistance.

Relationship between relative beta-cell area and clinical parameters

Fig. 1 shows the correlations between various clinical parameters and the relative beta-cell area. Fig. 1A is a scattergram of age and the relative beta-cell area. Age tended to correlate inversely but not significantly with the relative beta-cell area (r=-0.33, p=0.066). Neither BMI nor HbA1c showed a significant correlation with the relative beta-cell area (r=0.26, p=0.147; r=-0.16, p=0.378, respectively) (Fig. 1B, C).

Fig. 2 shows scattergrams and linear regression analyses of plasma glucose levels in 75 g-OGTT and the relative beta-cell area. Plasma glucose levels at 0 and 30 min did not correlate significantly with the relative beta-cell area (r=-0.26, p=0.145; r=-0.30, p=0.015, respectively) (Fig. 2A, B). Plasma glucose levels at 60 and
Beta-cell area vs insulin secretion

120 min tended to correlate inversely with the relative beta-cell area ($r=-0.31, p=0.087, r=-0.34, p=0.064$, respectively) (Fig. 2C, D). AUC$_{0-120}$ correlated significantly and inversely with the relative beta-cell area ($r=-0.36, p=0.045$) (Fig. 2E).

Fig. 3 shows scattergrams and linear regression analyses of indices of insulin secretory capacity or insulin resistance and the relative beta-cell area. F-IIRI, HOMA-beta, CPI and ΔC-peptide correlated significantly and positively with the relative beta-cell area ($r=0.36, p=0.044; r=0.50, p=0.003; r=0.36, p=0.042; r=0.64, p=0.002$, respectively) (Fig. 3A, C, D and F). Among these four parameters, ΔC-peptide showed the closest association with the relative beta-cell area. II tended to correlate positively but not significantly with the relative beta-cell area ($r=0.33, p=0.078$) (Fig. 3E). ΔC-peptide correlated significantly and positively with the relative beta-cell area in NGT patients ($r=0.67, p=0.034$), and
also tended to correlate positively with the relative beta-cell area in IGT and DM patients ($r=0.64, p=0.066$). ΔC-peptide also correlated positively and significantly with the relative beta-cell area in patients with malignant diseases ($n=20$, $r=0.59, p=0.019$). HOMA-R (an index of insulin resistance) and Matsuda index (an index of insulin sensitivity), did not correlate significantly with the relative beta-cell area ($r=0.31, p=0.090; r=0.01, p=0.967$, respectively) (Fig. 3G, H).

Stepwise multiple regression analysis performed with the variables that correlated significantly with the relative beta-cell area identified ΔC-peptide as the only independent and significant determinant of the relative beta-cell area (Table 2).

### Discussion

In this study, we demonstrated significant positive correlations between the relative beta-cell area and various parameters of insulin secretory capacity
We also demonstrated that among the above variables, ΔC-peptide by glucagon test was the most valuable parameter for predicting the relative beta-cell area. Because glucagon directly stimulates beta-cells [32], ΔC-peptide by glucagon test is a logical index of insulin secretory capacity, reflecting beta-cell area. On the other hand, F-IRI, HOMA-beta, CPI and II might be affected by certain factors including beta-cell insulin secretory function and insulin resistance, other than pancreatic beta-cell mass. II is also affected by glucose-stimulated incretin and glucagon secretion, which varies among NGT, IGT and DM [33].

The results demonstrated that ΔC-peptide by glucagon test can be used to estimate beta-cell area even in subjects with glucose intolerance or patients with malignancies. In our study, ΔC-peptide by glucagon test correlated positively and significantly with the relative beta-cell area in NGT subjects as well as the entire group, and tended to correlate with the area even in IGT and DM patients whose beta-cell function should be worse by glucotoxicity [34]. ΔC-peptide by glucagon test also correlated positively and significantly with the relative beta-cell area in patients with malignant diseases, although inflammation associated with malignancies should affect peripheral insulin action and insulin secretion [35, 36]. These results highlight the usefulness of ΔC-peptide in estimating beta cell area even in subjects with glucose intolerance or with malignant diseases.

Meier et al. [25] reported the correlation of beta-cell area with C-peptide-to-glucose ratio calculated from the results of 75 g-OGTT but not with HOMA-beta. On the other hand, our results showed a significant correlation between HOMA-beta and beta-cell area. This might be because our subjects were only drug-naive patients, whereas the above study included patients using anti-diabetic drugs such as sulfonylurea or insulin, which could affect basal insulin concentration and HOMA-beta.

The results of the present study also showed that the relative beta-cell area correlated inversely and significantly with AUC\textsubscript{0-120} and tended to decrease with worsening of glucose intolerance. Previous studies showed significant correlation between beta-cell mass and plasma glucose levels [37]. Indeed, beta-cell mass could be one of the most important determinants of plasma glucose level. The present study showed a tendency for an inverse correlation between age and the relative beta-cell area, a finding consistent with previous report [9].

This study has certain limitations that should be considered when interpreting the results. First, we could not confirm that each sample obtained from the head or body-tail actually represented the total pancreatic beta-cell area. Relative beta-cell areas were not different between in samples obtained from pancreatic head and from pancreatic body-tail in this study. In organ donor samples, no significant differences of relative beta-cell area were found among the regions of pancreas (head, body and tail) [11]. Thus, we assumed that the relative beta-cell area in each pancreatic part also represents the total pancreatic beta-cell area. Second, in this hospital-based study, HbA1c might be unstable in association with the primary disease, anemia and rapid improvement of glycemic control. In addition, BMI also could be easily affected by the existence of the primary (mainly malignant) disease. These could be the reasons for the lack of correlations between the relative beta-cell area and HbA1c and BMI, compared to previous reports [9, 25]. Third, some of our patients pre-operatively received anticancer agents for malignant diseases. Although the relative beta-cell area was not different between these patients and those who had not been treated with anticancer agents, we could not completely exclude the effect of chemotherapy on pancreatic histology or glucose tolerance [38]. While we admit the above limitations using operative pancreatic samples, the most important merit of this study is the thorough evaluation of the pathophysiological condition together with detailed histopathological analysis of the pancreas.

In conclusion, we demonstrated in the present study that ΔC-peptide by glucagon test, HOMA-beta, CPI,

![Table 2 Results of stepwise multiple regression analysis with relative beta-cell area as the dependent variable](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>Standardized regression coefficient</th>
<th>F value</th>
<th>p value</th>
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<tbody>
<tr>
<td>ΔC-peptide</td>
<td>0.255</td>
<td>0.074</td>
<td>0.641</td>
<td>11.86</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

Coefficient of determination ($\bar{R}^2$): 0.411.

Variables not accepted in this analysis (F value <4.0) included area under the curve of plasma glucose level from 0 to 120 min by 75 g-OGTT, fasting immunoreactive insulin, homeostasis model assessment of beta-cell function and C-peptide index. ΔC-peptide, increment of C-peptide immunoreactivity level by glucagon test.

(F-IRI, HOMA-beta, CPI and ΔC-peptide by glucagon test).
F-IRI and AUC$_{0-120}$ correlated closely with beta-cell area and that ΔC-peptide was the most valuable index for the estimation of the relative beta-cell area.

**Author Contributions**

Y.F. analyzed the data and wrote the manuscript. J.K. analyzed the data and reviewed/edited the manuscript. S.Y., S.U., A.Y., and K.O. contributed to the discussion. H.E. and H.N. examined the patients and obtained pancreatic tissue samples. H.I., A.I. and I.S. contributed to the discussion and reviewed/edited the manuscript. A.I. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of data analysis.

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**Conflict of Interest**

Yukari Fujita, Junji Kozawa, Hiromi Iwahashi, Syo Yoneda, Sae Uno, Atsushi Yoshikawa, Kohei Okita, Hidetoshi Eguchi, Hiroaki Nagano, Akihisa Imagawa and Iichiro Shimomura declare no conflict of interest.

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**Human and Animal Rights**

All procedures followed in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institution and national) and with the Helsinki Declaration of 1975, as revised in 2008.

**Informed Consent**

Informed consent was obtained from all subjects who participated in the study.

**References**


