

Functional Assessment of Pancreatic β -Cell Area in Humans

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OBJECTIVE— β -Cell mass declines progressively during the course of diabetes, and various antidiabetic treatment regimens have been suggested to modulate β -cell mass. However, imaging methods allowing the monitoring of changes in β -cell mass in vivo have not yet become available. We address whether pancreatic β -cell area can be assessed by functional test of insulin secretion in humans.

RESEARCH DESIGN AND METHODS—A total of 33 patients with chronic pancreatitis ($n = 17$), benign pancreatic adenomas ($n = 13$), and tumors of the ampulla of Vater ($n = 3$) at various stages of glucose tolerance were examined with an oral glucose load before undergoing pancreatic surgery. Indexes of insulin secretion were calculated and compared with the fractional β -cell area of the pancreas.

RESULTS— β -Cell area was related to fasting glucose concentrations in an inverse linear fashion ($r = -0.53$, $P = 0.0014$) and to 120-min postchallenge glycemia in an inverse exponential fashion ($r = -0.89$). β -Cell area was best predicted by a C-peptide-to-glucose ratio determined 15 min after the glucose drink ($r = 0.72$, $P < 0.0001$). However, a fasting C-peptide-to-glucose ratio already yielded a reasonably close correlation ($r = 0.63$, $P < 0.0001$). Homeostasis model assessment (HOMA) β -cell function was unrelated to β -cell area.

CONCLUSIONS—Glucose control is closely related to pancreatic β -cell area in humans. A C-peptide-to-glucose ratio after oral glucose ingestion appears to better predict β -cell area than fasting measures, such as the HOMA index. *Diabetes* 58: 1595–1603, 2009

Glucose homeostasis is tightly regulated by the secretion of insulin from pancreatic β -cells (1). Thus, hyperglycemia develops when insulin secretion is insufficient for a given degree of insulin resistance, and both type 1 and type 2 diabetes have been associated with a significant deficit in β -cell mass (~65% in type 2 diabetes, ~99% in type 1 diabetes) (2–4). Therapeutic approaches aiming to preserve or even restore β -cell mass have therefore gained widespread attention over the past years (5,6), and a number of agents have been proposed to prompt β -cell regeneration and thus augment β -cell mass in vivo (e.g., glucagon-like

peptide [GLP]-1 analogues and dipeptidyl peptidase-4 inhibitors) (7,8). However, because the majority of studies examining the effects of such compounds on β -cell mass have been carried out under in vitro conditions or in rodent models, it is difficult to directly translate these results to humans in vivo. Indeed, although in mice and rats the endocrine pancreas harbors an enormous capacity for regeneration (9–11), the overall regenerative potential of β -cells in adult humans appears to be substantially lower (12). In light of these obvious species differences, it is crucial to examine longitudinal changes in β -cell mass in humans in more detail. Unfortunately, as yet, all imaging techniques available have failed to determine β -cell mass in vivo with sufficient accuracy and specificity (13).

Given this technical inability to monitor changes in β -cell mass in humans, functional tests of insulin secretion may provide a feasible alternative. However, to be useful for clinical purposes, such a test would need to 1) be sufficiently practicable to allow for the repeated examination of large patient numbers and 2) predict β -cell mass with high accuracy.

The oral glucose tolerance test (OGTT) has commonly been applied to detect disturbances in glucose homeostasis in patients at risk of or with overt diabetes, and different indexes of insulin secretion have been derived from the OGTT (14). An even less complicated assessment of β -cell function may be derived from indexes based on fasting glucose and insulin measurements, such as the homeostasis model assessment (HOMA) index (15). However, because of the lack of accessibility of the human pancreas for routine biopsy sampling, the accuracy of these indexes for the prediction of β -cell mass has not yet been determined in humans. This question is of great clinical relevance for the design and interpretation of future clinical trials about the natural course of β -cell loss in type 1 and type 2 diabetes, the impact of various treatments on β -cell mass and turnover, and the time course of β -cell loss after pancreas or islet transplantation. Therefore, we examined patients with an oral glucose load before they underwent pancreatic surgery. Different measures of insulin secretion were determined and related to the fractional β -cell area in the pancreatic tissue that was collected at surgery. By these means, we addressed the following questions: 1) Does pancreatic β -cell area predict glycemic control in humans? 2) Do established indexes of β -cell function predict pancreatic β -cell area in humans? and 3) Which measure of insulin secretion and glucose control derived from a prolonged OGTT shows the closest association with β -cell area?

RESEARCH DESIGN AND METHODS

A total of 33 patients undergoing pancreatic surgery for chronic pancreatitis, benign pancreatic adenomas, or papillary tumors necessitating partial pancreatectomy were studied preoperatively with a 240-min oral glucose challenge.

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TABLE 1
Clinical characteristics of patients with NGT, IGT, and/or IFG and patients with diabetes

Parameter	NGT	IGT/IFG	Diabetes	P
Age (years)	55.9 \pm 15.9	63.2 \pm 13.9	59.0 \pm 10.1	0.30
Sex (female/male)	5/3	8/6	3/8	0.22
Clinical diagnoses				
Chronic pancreatitis	3 (38)	7 (50)	7 (64)	0.52
Benign adenoma	5 (63)	6 (43)	3 (27)	0.31
Ampullary tumor	0 (0)	1 (7)	1 (9)	0.70
BMI (kg/m ²)	23.6 \pm 2.7	23.4 \pm 3.2	24.1 \pm 4.7	0.69
Waist-to-hip ratio	0.89 \pm 0.07	0.89 \pm 0.08	0.90 \pm 0.06	0.89
RR systolic (mmHg)	116 \pm 18	119 \pm 35	125 \pm 20	0.77
RR diastolic (mmHg)	72 \pm 9	73 \pm 13	78 \pm 10	0.44
A1C (%)	5.7 \pm 0.5	5.7 \pm 0.5	6.9 \pm 1.4*	0.0086
White blood count (n/ μ l)	8,024 \pm 1,338	6,717 \pm 2,100	6,236 \pm 1,845	0.13
Hemoglobin (g/dl)	13.8 \pm 1.1	13.2 \pm 1.5	13.8 \pm 1.5	0.45
Serum amylase (units/l)	41.6 \pm 41.6	71.5 \pm 74.3	13.8 \pm 1.5	0.45
Serum urea (mg/dl)	31.9 \pm 4.2	31.2 \pm 4.1	31.5 \pm 2.9	0.99
Serum creatinine (mg/dl)	0.97 \pm 0.11	0.99 \pm 0.08	1.0 \pm 0.06	0.98
Triglycerides (mg/dl)	100.3 \pm 34.9	121.9 \pm 49.8	155.2 \pm 72.1	0.13
Cholesterol (mg/dl)	209.9 \pm 37.0	207.2 \pm 45.5	204.5 \pm 56.0	0.97
HDL cholesterol (mg/dl)	64.0 \pm 22.6	52.4 \pm 24.0	44.7 \pm 14.5	0.20
LDL cholesterol (mg/dl)	136.2 \pm 36.8	143.5 \pm 28.0	137.6 \pm 39.3	0.87

Data are means \pm SD, *n*, or *n* (%). Statistical analysis used ANOVA or χ^2 test. *Significantly different vs. control subjects (Duncan's post hoc test).

Different measures of glucose control and insulin secretion were determined and compared with the fractional β -cell area of the partially resected pancreas to establish potential predictors of β -cell area in humans. The study protocol was approved by the ethics committee of the Ruhr-University Bochum (registration no. 2528). All patients provided written informed consent before study enrollment.

Patients. We included 33 patients (17 male, 16 female) undergoing pancreatic resections in the Department of Surgery, St. Josef-Hospital, Ruhr-University Bochum, between the years 2004 and 2007. Of these patients, 17 had been diagnosed with chronic pancreatitis, 13 underwent surgery for the removal of benign pancreatic adenomas, and 3 underwent partial pancreatectomy because of tumors of the ampulla of Vater. The clinical diagnoses of chronic pancreatitis, pancreatic carcinoma, pancreatic adenoma, or ampullary cancer were confirmed by an independent pathologist in all cases. In patients with chronic pancreatitis, surgery was performed if conservative treatment approaches had failed to provide sufficient analgesia. In 9 patients distal pancreatectomies (pancreas tail resection) were performed, whereas 24 patients were treated with a proximal pancreatectomy (pancreas head resection). The latter group comprised 17 patients undergoing pancreaticoduodenectomy with pylorus preservation, 4 patients undergoing duodenum-preserving pancreatic head resections according to Beger, and 3 patients undergoing classic partial pancreaticoduodenectomy (Whipple's operation). Diabetes was previously known in six patients (treated with insulin in four patients, glimepiride in one, and diet in one), whereas the other patients had no history of known diabetes. Renal function was normal (serum creatinine <1.2 mg/dl) in 27 patients, whereas 6 patients had mild impairments in renal function (serum creatinine 1.2–2.0 mg/dl). None of the patients had a severe impairment in renal function. There also were no differences in the concentrations of creatinine and urea between the groups with normal glucose tolerance (NGT), impaired glucose tolerance (IGT)/impaired fasting glucose (IFG), and diabetes (Table 1).

Experimental procedures. The experiments were performed in the morning after an overnight fast with subjects in a supine position throughout the experiments. All other concomitant medications had been withdrawn since the evening of the preceding day. All antidiabetic treatment was withheld at least 24 h before the experiments. In insulin-treated patients, the last injection of short-acting insulin was performed on the evening before the tests, whereas all long-acting insulins were withheld for at least 24 h to avoid carryover effects.

No restrictions were made regarding the intake of water until the morning of the experiments. Both ear lobes were made hyperemic using Finalgon (4 mg/g Nonivamid, 25 mg/g Nicoboxil). The experiments were started by the ingestion of the oral glucose load (75 g glucose in 300 ml) over 5 min, and capillary and venous blood samples were drawn at *t* = -5, 0, 15, 30, 60, 90, 120, 150, 180, 210, and 240 min. Capillary blood samples (~100 μ l) were added to NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the immediate

measurement of glucose. Venous blood was drawn into chilled tubes containing EDTA and aprotinin (20,000 kIU/ml Trasylol, 200 μ l per 10 ml blood; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at -28°C.

Measurements. Glucose was measured as previously described (16) using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich). To adjust for the glucose concentration differences between capillary plasma and whole blood, glucose measurements were divided by the correction factor 1.11 (17).

Insulin was measured as previously described (16), using an insulin microparticle enzyme immunoassay (IMx Insulin; Abbott Laboratories, Wiesbaden, Germany). Cross-reactivity with proinsulin was <0.005%. The intra-assay CV was 4%. C-peptide was measured as previously described (16), using an enzyme-linked immunosorbent assay from Dakop (Cambridgeshire, U.K.). The intra-assay CV was 3.3–5.7%, and the interassay CV was 4.6–5.7%. Human insulin and C-peptide were used as standards.

Pancreatic tissue processing. Pancreatic resections were fixed in formaldehyde and embedded in paraffin for subsequent analysis as previously described (12). Sequential 5- μ m sections were stained for insulin using a guinea pig anti-insulin antibody (no. A 0564, lot no. 00001500; Dako) at 1:400 dilution and an Alkaline Phosphatase/RED detection system (REAL Envision detection system, no. K 5007 and K 5005, lot no. 00025382 and 00025812, respectively; Dako).

Morphometric analysis. For determination of the fractional β -cell areas, entire pancreatic sections stained for insulin were imaged at \times 100 magnification (\times 10 objective) using a Zeiss Axioplan microscope equipped with a motorized stage. A tile image of the tissue section was generated using the Mosaix tool of Zeiss Axiovision version 4.5 software. The fractional areas of the pancreas stained positive for insulin were digitally quantified using a color-based threshold using Axiovision software as previously described (12).

Calculations and statistical analysis. The HOMA index for β -cell function was calculated as previously described (15). Insulin-to-glucose ratios and C-peptide-to-glucose ratios were calculated at all time points before and after oral glucose ingestion. Subject characteristics are reported as the means \pm SD, and results are presented as the means \pm SE.

To address whether pancreatic β -cell area predicts glycemic control, the fractional β -cell area was first correlated to the fasting and 120-min postchallenge glucose concentrations and the A1C levels because these parameters have previously been validated as markers of glucose control. To address whether pancreatic β -cell area can be predicted by established indexes of β -cell function, the following parameters were correlated with β -cell area: 1) the HOMA β -cell function index, 2) the fasting insulin-to-glucose ratio, 3) the fasting C-peptide-to-glucose ratio, 4) the insulin-to-glucose ratio 30 min after oral glucose ingestion, 5) the C-peptide-to-glucose ratio 30 min after oral glucose ingestion, 6) the plasma insulin levels 30 min after oral glucose ingestion, and 7) the plasma C-peptide levels 30 min after oral glucose

ingestion. These parameters were chosen based on previous studies (15,18–21).

Finally, to identify the strongest predictor of β -cell area, the plasma concentrations of glucose, insulin, and C-peptide as well as the C-peptide-to-glucose ratios and insulin-to-glucose ratios at all time points during the prolonged OGTT were correlated with pancreatic β -cell area. The parameter yielding the greatest r value for the correlation with β -cell area was considered to be the best predictor of β -cell area. By these means, a total of 50 parameters were tested.

Time course measurements were carried out by unpaired ANOVA, using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). By these means, three different P values were calculated 1) for the determination of overall differences between the different groups (i.e., NGT, IFG/IGT, and diabetes), independent of the respective time patterns; 2) for the determination of differences over the time course, independent of the respective groups; and 3) for the determination of differences between the groups over the time course. If a significant ($P < 0.05$) interaction between group and time was documented, values at single time points were compared by one-way ANOVA. All other parameters were compared by one-way ANOVA. $P < 0.05$ was taken to indicate significant differences. Correlation analyses were carried out using GraphPad Prism 4 using linear or nonlinear regression functions. Decision criteria were the respective regression coefficients (r).

RESULTS

Postchallenge excursions of glucose, insulin, and C-peptide. Oral glucose tolerance was normal in 8 subjects, 2 subjects exhibited impaired fasting glucose alone, impaired glucose tolerance alone was present in 10 patients, 2 patients presented with both IFG and IGT, and 11 patients had a diabetic glucose tolerance. Patients with IGT and IFG were collectively presented as one group for subsequent analyses.

As expected, postchallenge glucose excursions were significantly higher both in patients with overt diabetes as well as in patients with IGT/IFG compared with patients with normal glucose tolerance ($P < 0.0001$) (Fig. 1). This was accompanied by a marked reduction in both insulin and C-peptide concentrations after the oral glucose load in the patients with diabetes (Fig. 1). In contrast, insulin and C-peptide levels were not significantly lower in IGT/IFG patients compared with NGT subjects.

β -Cell area and function. The fractional β -cell area of the pancreas was 1.22 ± 0.14 , 1.14 ± 0.13 , and $0.43 \pm 0.12\%$ in subjects with NGT, IGT/IFG, and diabetes, respectively ($P = 0.0003$), corresponding to a 65% β -cell deficit in patients with diabetes (Fig. 2). When the two subjects with isolated IFG were excluded, the fractional β -cell area in the remaining individuals with IGT was $1.08 \pm 0.14\%$. β -Cell area in the two individuals with isolated IFG (fasting glucose levels 107 and 102 mg/dl, 120-min glucose levels 106 and 120 mg/dl) was 1.48 and 1.45%, respectively. HOMA β -cell function (including all patients examined) was 81.1 ± 13.0 , 37.5 ± 30.0 , and $135.1 \pm 76.9\%$ for those with NGT, IGT/IFG, and diabetes, respectively ($P = 0.36$). There also were no differences in HOMA β -cell function when two outliers (calculated HOMA β -cell function 876 and -336%) were excluded ($P = 0.69$) (Fig. 2). The fasting insulin-to-glucose ratio and the fasting C-peptide-to-glucose ratio were not different between the groups ($P = 0.76$ and $P = 0.067$, respectively) (Fig. 2). In contrast, the insulin-to-glucose ratio and the C-peptide-to-glucose ratio 30 min after oral glucose ingestion were significantly lower in the patients with diabetes ($P = 0.033$ and $P < 0.0001$, respectively) (Fig. 2).

Relationship between glucose control and measures of β -cell area and function. There was a significant inverse association between fasting glucose concentrations and the fractional β -cell area ($r = -0.53$, $P = 0.0014$)

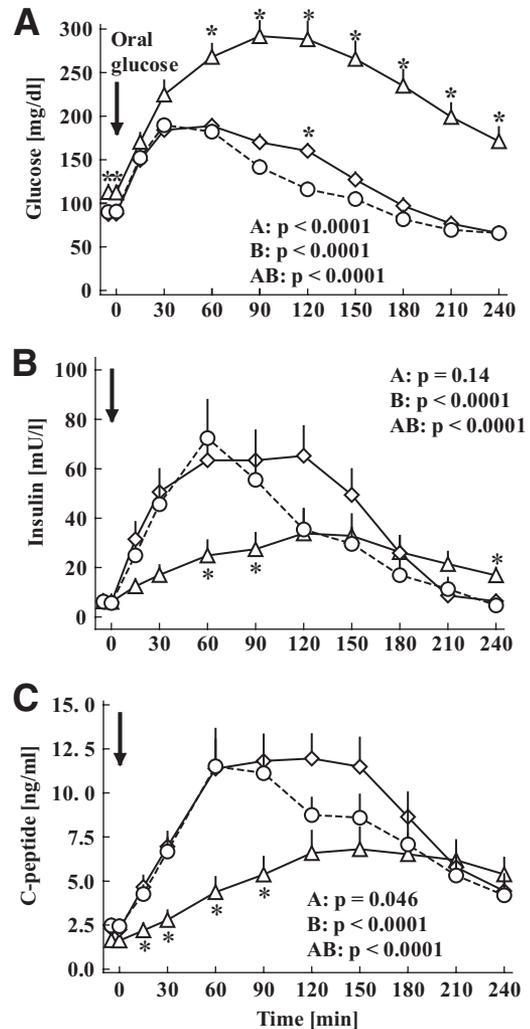


FIG. 1. Concentrations of glucose (A), insulin (B), and C-peptide (C) in eight individuals with NGT (\square), 14 individuals with IFG and/or IGT (\diamond), and 11 patients with diabetes (\triangle) after oral ingestion of 75 g glucose. Data are the means \pm SE. Statistics were carried out using repeated-measures ANOVA and denote differences between the experiments (A), differences over time (B), and differences due to the interaction of experiment and time (AB). *Significant ($P < 0.05$) differences vs. control subjects at individual time points (one-way ANOVA and Duncan's post hoc test).

(Fig. 3). An even closer association than in the fasting state was obtained when fractional β -cell area was expressed in relation to respective glucose excursions 120 min after an oral glucose load. However, although the correlation between β -cell area and fasting glucose levels tended to follow a linear relationship, the association with 120-min postchallenge glucose concentrations was best described by an exponential decay function using the following equation: $y = 358.6 \times \exp(3.21 \times x) + 134.9$ ($r = -0.89$) (Fig. 3). There also was a significant exponential relationship between fractional β -cell area and A1C levels ($r = -0.82$) (Fig. 3).

Because C-peptide levels 30 min after oral glucose ingestion yielded the closest association with postchallenge glucose excursions, this parameter was chosen for subsequent correlation analyses. There was no significant relationship between fasting glycemia and postchallenge C-peptide levels ($r = -0.14$, $P = 0.44$) (Fig. 3). In contrast, there was an inverse exponential relationship between

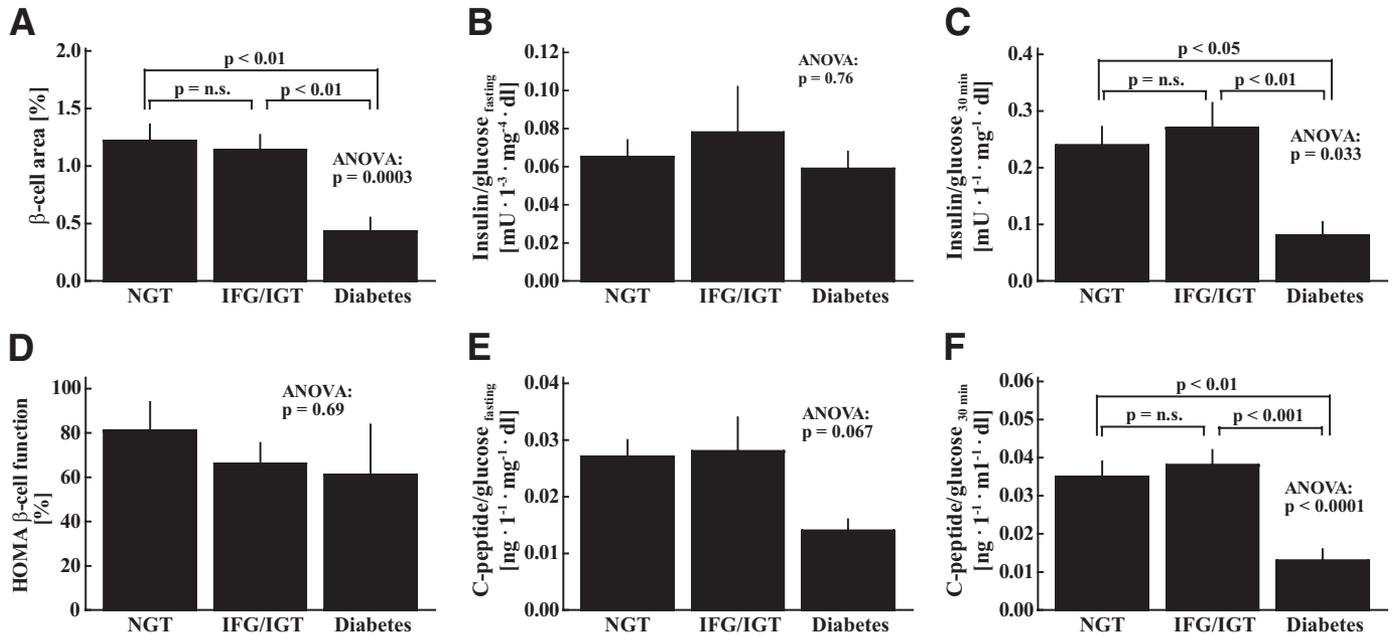


FIG. 2. Fractional β -cell area (A), fasting insulin-to-glucose ratio (B), insulin-to-glucose ratio 30 min after oral glucose ingestion (C), HOMA β -cell function (excluding two outlier patients) (D), fasting C-peptide-to-glucose ratio (E), and C-peptide-to-glucose ratio 30 min after oral glucose ingestion (F) in eight individuals with NGT, 14 individuals with IFG and/or IGT, and 11 patients with diabetes. Data are the means \pm SE. Statistics were carried out by ANOVA and Duncan's post hoc test.

C-peptide levels at $t = 30$ min after oral glucose ingestion and both the 120-min glucose levels ($r = -0.82$) (Fig. 3) and A1C concentrations ($r = -0.53$) (Fig. 3). As a rule, the associations between these parameters of glucose

control and C-peptide excursions were weaker than the respective associations with fractional β -cell area (Fig. 3). **Predictors of β -cell area.** Fractional β -cell area was compared with different established indexes of β -cell

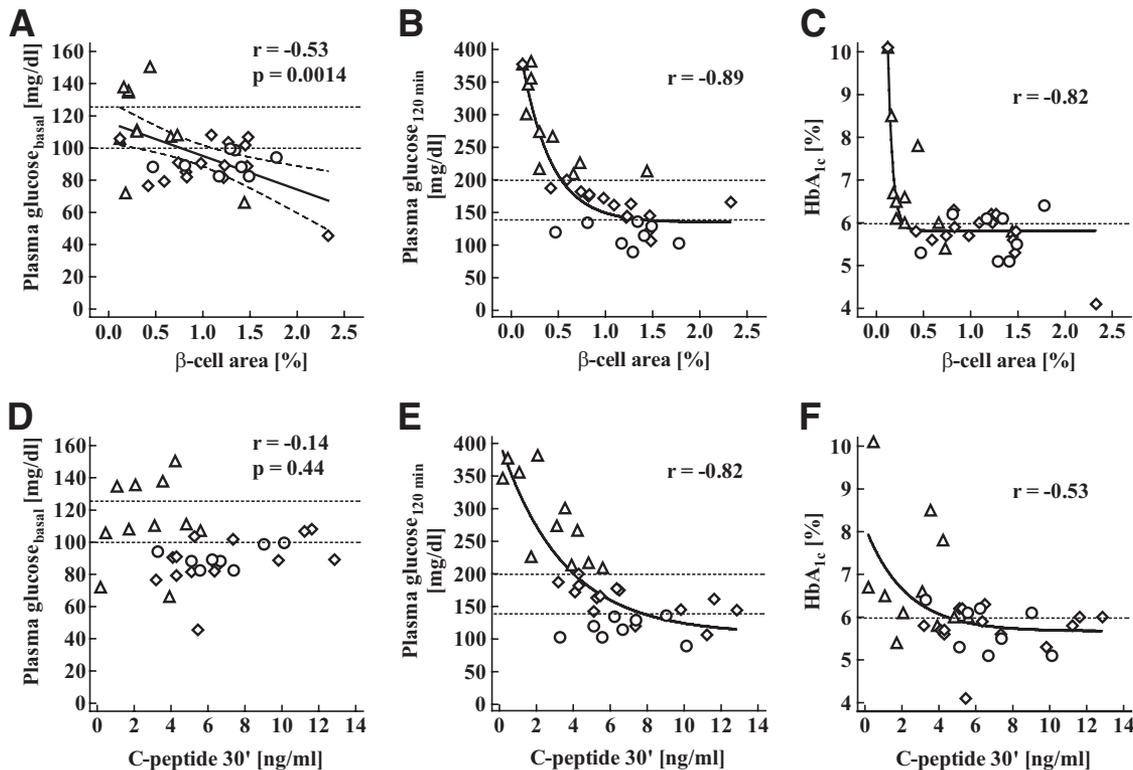


FIG. 3. Correlation analyses between fractional β -cell area (A, B, and C) or plasma C-peptide levels (D, E, and F) and basal blood glucose levels (A and D), blood glucose levels 120 min after oral glucose ingestion (B and E), and A1C levels (C and F) in eight individuals with NGT (\circ), 14 individuals with IFG and/or IGT (\diamond), and 11 patients with diabetes (\triangle). r = correlation coefficient. P values were calculated by linear regression analysis. r and P values in A and D were calculated by linear regression analyses. r values in panels B, C, E, and F were calculated by nonlinear regression analyses using an exponential decay function. Dotted lines in panels A, B, D, and E denote the respective margins of normal and impaired fasting glucose and normal and impaired glucose tolerance, respectively. C-peptide 30', C-peptide 30 min after oral ingestion.

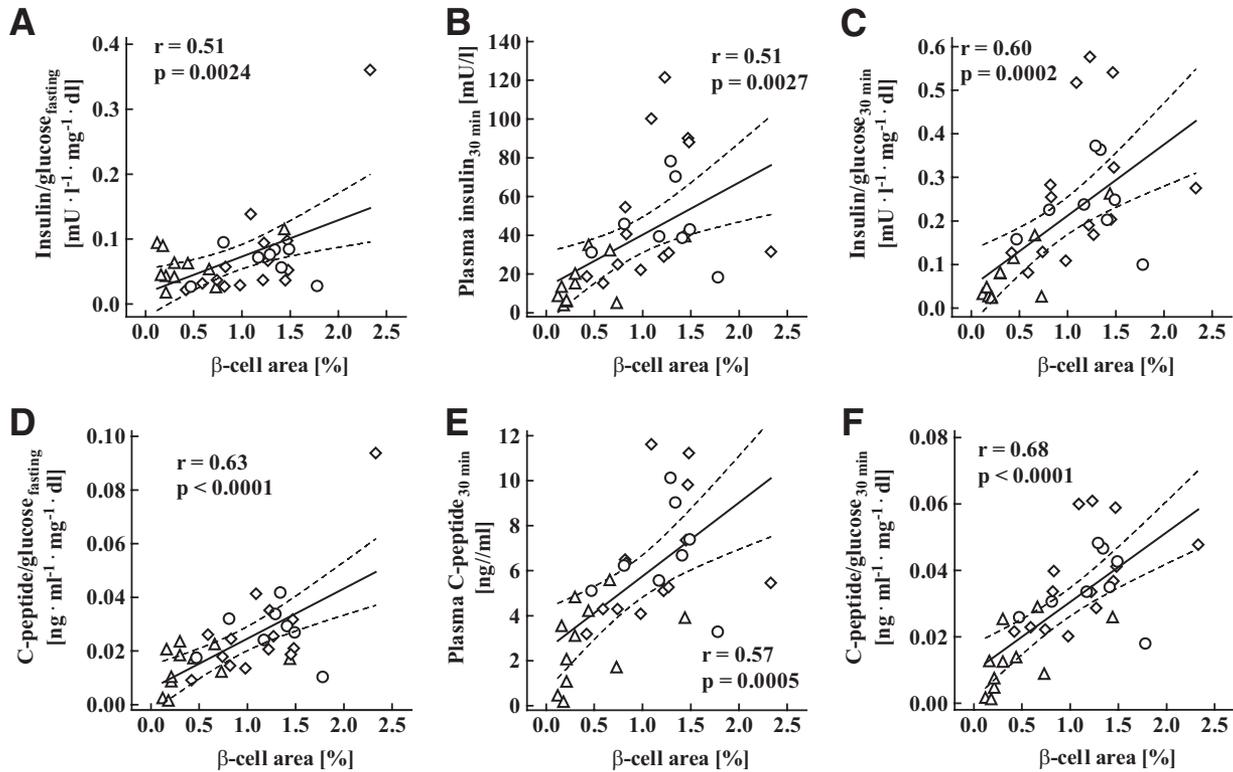


FIG. 4. Linear regression analyses between fractional β -cell area and established indexes of insulin secretion. *A–F*: Correlations with the fasting insulin-to-glucose ratio (*A*), plasma insulin levels 30 min after oral glucose administration (*B*), the insulin-to-glucose ratio 30 min after oral glucose administration (*C*), the fasting C-peptide-to-glucose ratio (*D*), plasma C-peptide levels 30 min after oral glucose administration (*E*), and the C-peptide-to-glucose ratio 30 min after oral glucose administration (*F*) in eight individuals with NGT (\circ), 14 individuals with IFG and/or IGT (\diamond), and 11 patients with diabetes (\triangle). Dashed lines denote the respective upper and lower 95% CIs. r = correlation coefficient.

function (Fig. 4). There was a significant linear relationship between fractional β -cell area and the fasting insulin-to-glucose ratio ($r = 0.51$, $P = 0.0024$), the fasting C-peptide-to-glucose ratio ($r = 0.64$, $P < 0.0001$), the insulin-to-glucose ratio 30 min after oral glucose ingestion ($r = 0.60$, $P = 0.0002$), the C-peptide-to-glucose ratio 30 min after oral glucose ingestion ($r = 0.68$, $P < 0.0001$), plasma insulin levels 30 min after oral glucose ingestion ($r = 0.51$, $P = 0.0027$), and C-peptide levels 30 min after

oral glucose ingestion ($r = 0.57$, $P = 0.0005$) (Fig. 4). In contrast, no significant relationship was found between fractional β -cell area and the HOMA index of β -cell function ($r = 0.03$, $P = 0.88$). Also, after excluding two outlier patients, this association failed to reach statistical significance ($r = 0.23$, $P = 0.21$) (Fig. 5).

To identify the strongest predictors of pancreatic β -cell area, fractional β -cell area was correlated with the glucose, insulin, and C-peptide levels as well as with the

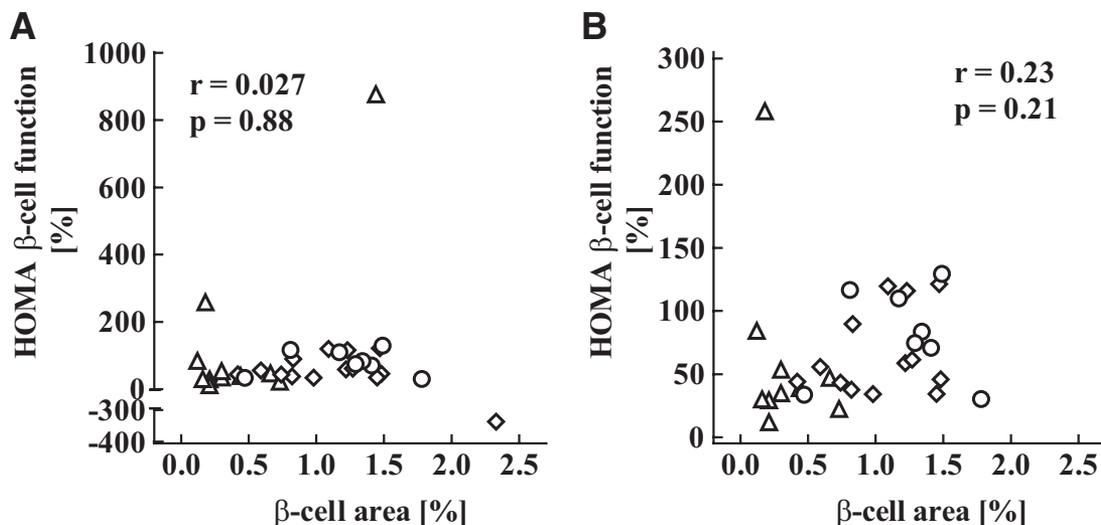


FIG. 5. Linear regression analyses between fractional β -cell area and the HOMA index of β -cell function in eight individuals with NGT (\circ), 14 individuals with IFG and/or IGT (\diamond), and 11 patients with diabetes (\triangle). *A*: The correlation including all patients examined. *B*: The correlation after excluding two outlier patients. r = correlation coefficient.

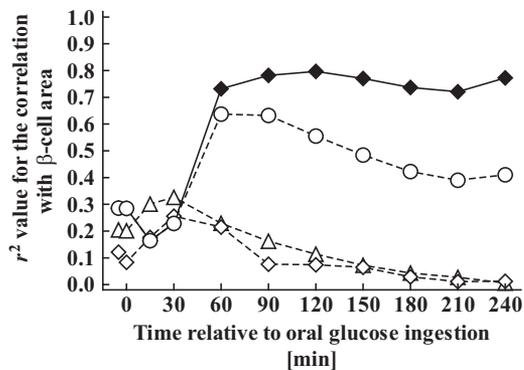


FIG. 6. Correlation coefficients (squared) for the relationship between fractional β -cell area and the respective insulin, C-peptide, and glucose levels obtained before and after oral glucose ingestion in eight individuals with NGT, 14 individuals with IFG and/or IGT, and 11 patients with diabetes determined by linear regression analysis (dashed lines) or by an exponential decay function (solid lines; shown for glucose only). \circ , glucose (linear); \diamond , insulin (linear); \triangle , C-peptide (linear); \blacklozenge , glucose (exponential).

respective insulin-to-glucose ratios and C-peptide-to-glucose ratios obtained before and after oral glucose ingestion. Based on these analyses, the plasma glucose levels measured 120 min after oral glucose ingestion yielded the closest association with β -cell area in an exponential model (Fig. 6). When a linear regression model was applied, the plasma glucose concentrations at 60 min after oral glucose ingestion showed the highest degree of correlation ($r = 0.80$, $P < 0.0001$) (Figs. 6 and 7).

The closest associations between β -cell area and individual insulin and C-peptide levels were found at $t = 30$ min after the oral glucose load ($r = 0.51$, $P = 0.0027$, and $r = 0.57$, $P = 0.0005$; respectively) (Fig. 4). An even greater degree of correlation was obtained when insulin and C-peptide levels were expressed in relation to the respective glucose concentrations. By these means, the closest correlations with fractional β -cell area were found for the C-peptide-to-glucose ratio 15 min after oral glucose ingestion ($r = 0.72$, $P < 0.0001$) (Fig. 7) and for the insulin-to-glucose ratio determined 30 min after the glucose load ($r = 0.60$, $P = 0.0002$) (Fig. 4).

DISCUSSION

The current study was designed to assess the validity of functional indexes of insulin secretion to predict β -cell area in humans in vivo. Using a combination of oral glucose tolerance tests carried out before pancreatic surgery and morphometric analyses of the respective pancreatic tissue samples, we report that 1) glucose control deteriorates with declining β -cell area in humans, 2) β -cell area is significantly related to different functional measures of insulin secretion, and 3) a C-peptide-to-glucose ratio determined 15 min after oral glucose ingestion appears to predict β -cell area better than fasting measures of insulin secretion, such as the HOMA index.

The lack of reliable imaging techniques suitable to determine β -cell mass in vivo has prompted great interest in the functional assessment of β -cell mass (22). Thus, a number of studies have been performed in different animal models of diabetes (rats, dogs, baboons, and minipigs) (23–27). The results of these studies have recently been elegantly reviewed by Robertson (22). As a rule, significant linear associations between the insulin secretory responses to arginine or glucagon administration and pancreatic β -cell mass have been reported in these animal studies (22). In humans, available information about the relationship between insulin secretion and β -cell mass is rather sparse, and two different studies have previously addressed this issue. Teuscher et al. (28) determined insulin secretory responses to intravenous glucose and arginine administration in eight subjects undergoing islet autotransplantation. In this study, all measures of insulin secretion were closely correlated with the transplanted islet cell mass. A similar association between the number of transplanted islet equivalents and insulin levels after arginine administration was found in patients with type 1 diabetes studied after islet allotransplantation (29). The current study extends these results by providing for the first time direct evidence for a linear correlation between intrapancreatic β -cell mass and insulin secretion in humans with and without diabetes in vivo. Of note, none of the imaging methods available so far has proven to predict β -cell mass with a comparable accuracy as the functional measures applied herein (13).

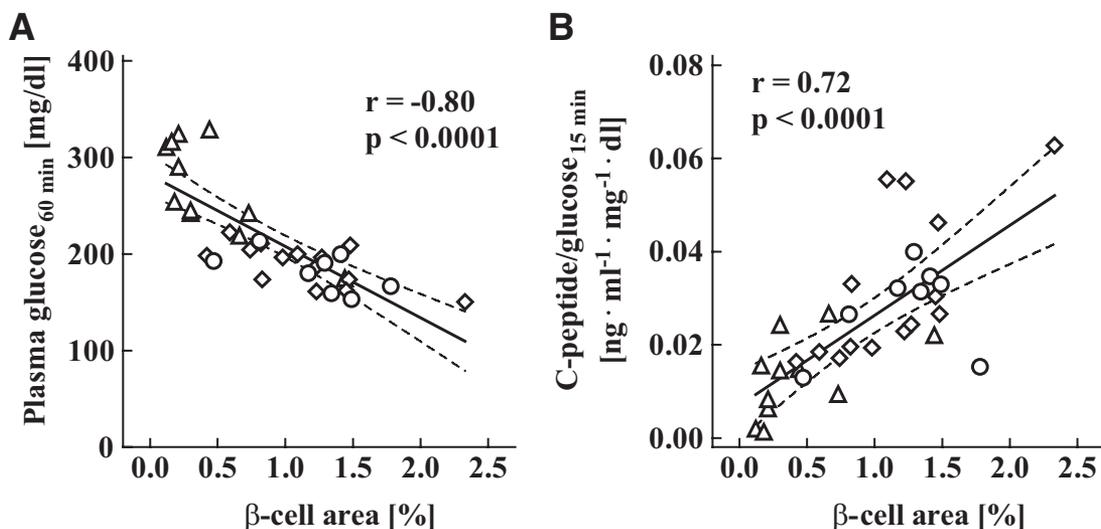


FIG. 7. Linear regression analyses between fractional β -cell area and glucose concentrations 60 min after oral glucose administration (A) and the C-peptide-to-glucose ratio 15 min after oral glucose administration (B) in eight individuals with NGT (\circ), 14 individuals with IFG and/or IGT (\diamond), and 11 patients with diabetes (\triangle). These parameters were identified as strongest predictors of β -cell area based on the respective r values. Dashed lines denote the respective upper and lower 95% CIs. r = correlation coefficient.

Although this study and the prior studies mentioned above lend strong support to the use of functional tests for the assessment of β -cell area in humans, it is important to bear in mind the potential limitations of such an approach. In fact, even though the overall association between β -cell area and insulin secretion in this study was rather close, any functional measure of insulin secretion can be confounded by a number of factors other than merely the amount of pancreatic β -cells. Along these lines, insulin secretion can be increased by as much as two- to threefold under conditions of obesity or insulin resistance (30), although the actual increase in β -cell mass in obese patients has been estimated to be only $\sim 30\%$ (2). Furthermore, the insulin responses to intravenous glucose administration in patients with type 2 diabetes can be enhanced acutely by $>300\%$ after the infusion of a GLP-1 analogue over 5 h (31), a time period during which any gain in β -cell mass is far from being realistic. Consistent with this, the current study has shown that despite the significant relationship between insulin secretion and β -cell area, there was still considerable variability in insulin secretion between different individuals with a similar extent of β -cell area. In this regard, it is also important to stress that any association between functional indexes of insulin secretion and β -cell area is only valid in the absence of concomitant antidiabetic treatment.

Moreover, because a large number of functional parameters were compared with β -cell area, there is a certain likelihood of significant results attributable to multiple testing. However, the overall number of significant associations identified in this study was far in excess of the expected random probability, and the relationships observed were in good agreement with the primary hypothesis.

The close inverse relationship between β -cell area and 120-min glucose levels during the OGTT is another intriguing finding from this study. As much as 80% of the variations in postchallenge glucose excursions could be attributed to differences in β -cell area. Interestingly, this relationship was much closer than the respective association with fasting glucose concentrations. Taken together, these results suggest that postprandial glucose control strictly depends on a sufficient extent of insulin-secreting β -cells, whereas fasting glucose levels may be affected by a number of additional factors as well. Collectively, these findings further emphasize the importance of β -cell mass for the development of diabetes. In this regard, it is noteworthy that the associations between the different parameters of glucose control and C-peptide secretion were even weaker than the respective associations with β -cell area, which may suggest that defects in β -cell mass rather than impairments in β -cell function primarily determine the development of diabetes.

Although β -cell area was significantly reduced in the patients with overt hyperglycemia, there was only a very small reduction in β -cell area in the pre-diabetic patients with IFG and IGT. This finding seems to be at variance with previous data by Butler et al. (2) showing an $\sim 40\%$ deficit in β -cell area in individuals with IFG. It is therefore important to bear in mind the differences between these studies. Thus, Butler et al. examined β -cell area in IFG subjects, whereas the majority of patients in this study were characterized by IGT. Given the marked differences in the pathogenesis of IFG and IGT (32), these findings suggest that the rise in postchallenge glucose levels is a

rather early phenomenon during the development of diabetes, whereas fasting hyperglycemia may be indicative of a more pronounced β -cell loss. Second, in the study by Butler et al., the diagnostic threshold for IFG was 110 mg/dl (33), whereas the revised ADA criteria with a glucose threshold of 100 mg/dl were used in this study (34). By these means, patients with relatively mild alterations in fasting glycemia (i.e., fasting glucose levels between 100 and 110 mg/dl) were included in the IFG/IGT group in this study as well, whereas the degree of fasting hyperglycemia was clearly more pronounced in the previous study. Finally, it is important to emphasize that all patients examined in this study underwent pancreatic surgery for underlying pancreatic disorders (especially chronic pancreatitis), whereas Butler et al. (2) studied a group of patients more typical of (pre-) type 2 diabetes. In line with this, the mean BMI of the patients in the IFG population studied previously was ~ 37 kg/m², whereas the current group of patients with IFG/IGT had a mean BMI of 23.4 kg/m². It is therefore likely that the mechanisms underlying the β -cell destruction in the patients studied herein were different from those typically found in patients with type 2 diabetes. Therefore, even though our current study provides novel information regarding the relationship between β -cell area and insulin secretion as well as glycemic control, the findings should not be generalized to the majority of patients with type 2 diabetes.

A number of different functional indexes have been proposed to estimate β -cell function in vivo. Among these, insulin responses to intravenous glucagon or arginine administration (with or without glucose potentiation) appear to provide the most reliable estimates of β -cell function (22). However, broad application of such tests is clearly limited by their laborious procedures, which prevent the routine examination of larger groups of individuals. In contrast, the oral glucose tolerance test can easily be performed under everyday conditions and is therefore commonly applied to detect disturbances in glucose control. The current data showing a close relationship between both insulin and C-peptide excursions during the OGTT and the fractional β -cell area of the pancreas lend further support to the broad use of this test and suggest that postchallenge glucose excursions may not only predict the risk of developing diabetes, but also allow for some conclusions regarding the residual β -cell area.

Because pancreatic weight cannot readily be measured at surgery in humans, the fractional β -cell area rather than the total β -cell mass has been determined in this study. Therefore, the actual β -cell mass of the patients may still vary to some extent, depending on the overall size of the pancreas. However, previous studies including $>1,800$ patients have demonstrated that pancreatic volume is not affected by the presence of type 2 diabetes and remains rather constant during adulthood in humans, suggesting that the overall impact of this factor on the current results should be rather minor (35). The close relationship between postchallenge glycemia and the fractional β -cell area observed in this study is consistent with the postulate that fractional β -cell area rather than pancreatic weight is the primary determinant of β -cell mass and suggests that in humans, β -cell mass may be readily estimated from the determination of the β -cell area in pancreatic tissue samples.

One important goal of this study was to identify simple and reliable predictors of β -cell area in humans. Judging from the respective *r* values alone, the best correlation

was obtained for the 120-min glucose levels during the OGTT. However, because this relationship followed an exponential rather than a linear function, the predictive value of such postchallenge glucose excursions may be low, especially in individuals with a relatively normal β -cell area (i.e., >50% of normal). A better estimation of β -cell area may be derived from a C-peptide-to-glucose ratio at $t = 15$ min after oral glucose ingestion, which in the current study explained as much as 51% of the variations in β -cell area. However, even a simple C-peptide-to-glucose ratio under fasting conditions already provides a reasonable estimate of β -cell area. In contrast, the HOMA β -cell function index, which is commonly applied as a measure of β -cell function in clinical studies (36,37), failed to predict pancreatic β -cell area in the setting of this study.

The results of this study may have important consequences for the design and interpretation of future longitudinal studies about the potential effects of different antidiabetic agents on pancreatic β -cell area and the progression of diabetes. Thus, although there is solid evidence for a progressive decline in β -cell mass and function with advancing type 2 diabetes from a number of cross-sectional studies (38,39), the only longitudinal studies that have examined the course of islet dysfunction in patients with type 2 diabetes (UKPDS [U.K. Prospective Diabetes Study] and ADOPT [A Diabetes Outcome Progression Trial]) have applied the HOMA index as a measure of β -cell function, which, according to the current results, does not necessarily predict changes in β -cell mass (36,40). In a similar fashion, a number of recent clinical trials have suggested improvements in β -cell function and presumably β -cell mass during treatment with dipeptidyl peptidase-4 inhibitors and GLP-1 analogues based on such calculations (37,41). The current study suggests that although the HOMA index may have some relevance to estimate the functional integrity of insulin secretion, it represents an odd surrogate of β -cell area in vivo. Future studies in this area should therefore consider the use of a C-peptide-to-glucose ratio after oral glucose ingestion as a functional estimate of β -cell area.

In conclusion, the current studies have revealed a close inverse relationship between postchallenge glycemia and β -cell area in humans in vivo. A C-peptide-to-glucose ratio after oral glucose ingestion or even in the fasting state can provide a reasonable estimate of pancreatic β -cell area, whereas the HOMA β -cell function index appears to be less suitable in this regard. Such indexes may be useful to assess the natural course of β -cell loss in longitudinal studies and to estimate the residual β -cell area in individual patients with diabetes.

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