Functional assessment of pancreatic beta-cell area in humans

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Objective: Beta-cell mass declines progressively during the course of diabetes, and various antidiabetic treatment regimens have been suggested to modulate beta-cell mass. However, imaging methods allowing to monitor changes in beta-cell mass in vivo have not yet become available. We addressed whether pancreatic beta-cell area can be assessed by functional test of insulin secretion in humans.

Research design and methods: 33 patients with chronic pancreatitis (n = 17), benign pancreatic adenomas (n = 13), and tumours of the ampulla of Vater (n = 3) at various stages of glucose tolerance were examined with an oral glucose load prior to undergoing pancreatic surgery. Indices of insulin secretion were calculated and compared to the fractional beta-cell area of the pancreas.

Results: Beta-cell area was related to fasting glucose concentrations in an inverse linear fashion (r = -0.53, p = 0.0014), and to 120 min post-challenge glycaemia in an inverse exponential fashion (r = -0.89). Beta-cell area was best predicted by a C-peptide/glucose ratio determined 15 min after the glucose drink (r = 0.72, p < 0.0001). However, a fasting C-peptide/glucose ratio already yielded a reasonably close correlation (r = 0.63, p < 0.0001). HOMA beta-cell function was unrelated to beta-cell area.

Conclusions: Glucose control is closely related to pancreatic beta-cell area in humans. A C-peptide/glucose ratio after oral glucose ingestion appears to better predict beta-cell area than fasting measures, such as the HOMA index.
Glucose homoeostasis is tightly regulated by the secretion of insulin from pancreatic beta-cells (1). Thus, hyperglycaemia 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 beta-cell mass (~65% in type 2 diabetes, ~99% in type 1 diabetes) (2-4). Therapeutic approaches aiming to preserve or even restore beta-cell mass have therefore gained widespread attention over the past years (5; 6), and a number of agents have been proposed to prompt beta-cell regeneration and thus augment beta-cell mass in vivo (e.g. GLP-1 analogues, DPP-4 inhibitors) (7; 8). However, since the majority of studies examining the effects of such compounds on beta-cell mass have been carried-out under in vitro conditions or in rodent models, it is difficult to directly translate these results to the situation in humans in vivo. Indeed, while in mice and rats the endocrine pancreas harbours an enormous capacity for regeneration (9-11), the overall regenerative potential of beta-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 beta-cell mass in humans in more detail. Unfortunately, as yet all imaging techniques available have failed to determine beta-cell mass in vivo with sufficient accuracy and specificity (13).

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

The oral glucose tolerance test (OGTT) has commonly been applied to detect disturbances in glucose homoeostasis in patients at risk of or with overt diabetes, and different indices of insulin secretion have been derived from the OGTT (14). An even less complicated assessment of beta-cell function may be derived from indices based on fasting glucose and insulin measurements, such as the HOMA index (15). However, owing to the lack of accessibility of the human pancreas for routine biopsy sampling, the accuracy of these indices for the prediction of beta-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 beta-cell loss in type 1 and type 2 diabetes, the impact of various treatments on beta-cell mass and turnover, and the time course of beta-cell loss following pancreas or islet transplantation. Therefore, we examined patients prior to undergoing pancreatic surgery with an oral glucose load. Various measures of insulin secretion were determined and related to the fractional beta-cell area in the pancreatic tissue that was collected at surgery. By these means, we addressed the following questions: (1) Does pancreatic beta-cell area predict glycaemic control in humans? (2) Do established indices of beta-cell function predict pancreatic beta-cell area in humans? (3) Which measure of insulin secretion and glucose control derived from a prolonged OGTT shows the closest association with beta-cell area?

**PATIENTS AND METHODS**

**Study design:** 33 patients undergoing pancreatic surgery for chronic pancreatitis, benign pancreatic adenomas, or papillary tumours necessitating partial pancreatectomy were studied pre-operatively with a 240 min oral glucose challenge. Various measures of glucose control and insulin secretion were determined and compared to the fractional beta-cell area of the partially resected pancreas to establish potential predictors of
beta-cell area in humans. The study protocol was approved by the ethics committee of the Ruhr-University Bochum (registration number 2528). All patients provided written informed consent prior to study enrolment.

Patients: A total of 33 patients (17 males, 16 females) undergoing pancreatic resections in the Department of Surgery, St. Josef-Hospital, Ruhr-University Bochum, between the years 2004 and 2007 were included. Amongst those, 17 patients had been diagnosed with chronic pancreatitis, 13 underwent surgery for the removal of benign pancreatic adenomas, and 3 patients underwent partial pancreatectomy because of tumours of the ampulla of Vater. The clinical diagnoses 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 nine 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, four patients undergoing duodenum-preserving pancreatic head resections according to Beger, and three patient undergoing classic partial pancreaticoduodenectomy (Whipple’s operation). Diabetes was previously known in six patients (treated with insulin in 4 cases, glimepiride in one case, and diet in one case), whereas the other patients had no history of known diabetes. Renal function was normal (serum creatinine < 1.2 mg/dl) in 27 patients, whereas six 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 NGT, IGT/IFG und 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 medication was withdrawn since the evening of the preceding day. All antidiabetic treatment was withheld at least 24 hours prior to 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 hours in order to avoid carry-over effects.

No restrictions were made regarding the intake of water until the morning of the experiments. Both ear lobes were made hyperemic using Finalgon® (Nonivamid 4 mg/g, Nicoboxil 25 mg/g). 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 (approximately 100 µl) 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 (Trasylo®; 20000 KIU/ml, 200 µl per 10 ml blood; Bayer AG, 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 described (16) using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany). In order 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 described (16) using an insulin microparticle enzyme immunoassay (MEIA), IMx Insulin, Abbott
Laboratories, Wiesbaden, Germany. Cross-reactivity with proinsulin was < 0.005%. The intra-assay coefficient of variation was 4 %.

C-peptide was measured as described (16) using an enzyme-linked immunoabsorbent assay (ELISA) from DAKOP Ltd., Cambrigshire, UK. Intra-assay coefficient of variation was 3.3 to 5.7 %, inter-assay variation was 4.6 to 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 µm sections were stained for insulin using a guinea pig anti-insulin antibody (DAKO # A 0564 ; Lot-no. 00001500) at 1:400 dilution and an Alkaline Phosphatase/RED (DAKO Real Envision Detection System, # K 5007 and # K 5005; Lot-no. 00025382 and lot-no. 00025812, respectively) detection system.

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

**Calculations and statistical analysis:** The HOMA index for beta-cell function was calculated as described (15). Insulin over glucose ratios and C-peptide over glucose ratios were calculated at all time points before and after oral glucose ingestion. Subject characteristics are reported as mean ± SD, results are presented as mean ± SEM.

In order to address, whether pancreatic beta-cell area predicts glycaemic control, the fractional beta-cell area was first correlated to the fasting glucose and 120 min concentrations and the HbA1c levels, because these parameters have previously been validated as markers of glucose control. To address, whether pancreatic beta-cell area can be predicted by established indices of beta-cell function, the following parameters were correlated with beta-cell area: (1) The HOMA beta-cell function index, (2) the fasting insulin/glucose ratio, (3) the fasting C-peptide/glucose ratio, (4) the insulin/glucose ratio 30min after oral glucose ingestion, (5) the C-peptide/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, in order to identify the strongest predictor of beta-cell area, the plasma concentrations of glucose, insulin, C-peptide as well as the C-peptide/glucose ratios and insulin/glucose ratios at all time points during the prolonged OGTT were correlated with pancreatic beta-cell area. The parameter yielding the greatest r-value for the correlation with beta-cell area was considered to be the best predictor of beta-cell area. By these means, a total of 50 parameters were tested.

Time course measurements were carried out by unpaired analysis of variance (ANOVA), using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). By these means, three different p-values were calculated: (A) For the determination of overall differences between the different groups (i.e. NGT, IFG/IGT, diabetes), independent of the respective time patterns. (B) For the determination of differences over the time course, independent of the respective groups, and (AB) 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. A p-value < 0.05 was taken to indicate significant differences. Correlation analyses were carried-out using GraphPad Prism 4 using linear or non-linear regression functions. Decision criteria were the respective regression coefficients (r).

RESULTS

Post-challenge excursions of glucose, insulin and C-peptide: Oral glucose tolerance was normal in eight subjects, two subjects exhibited impaired fasting glucose alone, impaired glucose tolerance alone was present in ten patients, two patients presented with both IFG and IGT, and eleven patients had a diabetic glucose tolerance. Patients with IGT and IFG were collectively presented as one group for subsequent analyses. As expected, post-challenge glucose excursions were significantly higher in both patients with overt diabetes as well as in patients with IGT/IFG compared to 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 to NGT subjects.

Beta-cell area and function: The fractional beta-cell area of the pancreas was 1.22 ± 0.14 %, 1.14 ± 0.13 %, and 0.43 ± 0.12 % in individuals with NGT, patients with IGT/IFG, and patients with diabetes, respectively (p = 0.0003), corresponding to a 65% beta-cell deficit in patients with diabetes (Fig. 1). When the two subjects with isolated IFG were excluded, the fractional beta-cell area in the remaining individuals with IGT was 1.08 ± 0.14 %. Beta-cell area in the two individuals with isolated IFG (fasting glucose levels 107 and 102 mg/dl, 120 min glucose levels 106 and 120 min) was 1.48 % and 1.45 %.

HOMA beta-cell function (including all patients examined) was 81.1 ± 13.0 %, 37.5 ± 30.0 %, and 135.1 ± 76.9 %, respectively (p = 0.36). There also were no differences in HOMA beta-cell function when two outliers (calculated HOMA beta-cell function 876 % and -336 %) were excluded (p = 0.69, Fig. 2). The fasting insulin/glucose ratio and the fasting C-peptide/glucose ratio were not different between the groups (p = 0.76 and p = 0.067, respectively; Fig. 2). In contrast, the insulin/glucose ratio and the C-peptide/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 beta-cell area and function: There was a significant inverse association between the fasting glucose concentrations and the fractional beta-cell area (r = -0.53, p = 0.0014; Fig. 3). An even closer association than in the fasting state was obtained when fractional beta-cell area was expressed in relation to the respective glucose excursions 120 min after the oral glucose load. However, while the correlation between beta-cell area and fasting glucose levels tended to follow a linear relationship, the association with the 120 min post-challenge glucose concentrations was best described by an exponential decay function using the equation: y = 358.6 * exp(3.21 * x) + 134.9 (r = -0.89; Fig. 3). There also was a significant exponential relationship between fractional beta-cell area and the HbA1c levels (r = -0.82; Fig. 3).

Since C-peptide levels 30 min after oral glucose ingestion yielded the closest association with post-challenge glucose excursions, this parameter was chosen for subsequent correlation analyses. There was no significant relationship between fasting glycaemia and post-challenge C-peptide levels (r = -0.14, p = 0.44; Fig. 3). In contrast, there was an inverse exponential relationship
between C-peptide levels at $t = 30\text{ min}$ after oral glucose ingestion and the 120 min glucose levels ($r = -0.82$; Fig. 3) and the HbA1c 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 beta-cell area (Fig. 3).

**Predictors of beta-cell area:** Fractional beta-cell area was compared to different established indices of beta-cell function (Fig. 4). There was a significant linear relationship between fractional beta-cell area and the fasting insulin/glucose ratio ($r = 0.51$, $p = 0.0024$), the fasting C-peptide/glucose ratio ($r = 0.64$, $p < 0.0001$), the insulin/glucose ratio 30 min after oral glucose ingestion ($r = 0.60$, $p = 0.0002$), the C-peptide/glucose ratio 30 min after oral glucose ingestion ($r = 0.68$, $p < 0.0001$), the plasma insulin levels 30 min after oral glucose ingestion ($r = 0.51$, $p = 0.0027$), and the 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 beta-cell area and the HOMA index of beta-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).

In order to identify the strongest predictors of pancreatic beta-cell area, fractional beta-cell area was correlated with the glucose, insulin, and C-peptide levels as well as with the respective insulin/glucose ratios and C-peptide/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 beta-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, 7).

The closest associations between beta-cell area and individual insulin and C-peptide levels were found at $t = 30\text{ min}$ 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 beta-cell area were found for the C-peptide/glucose ratio 15 min after oral glucose ingestion ($r = 0.72$, $p < 0.0001$; Fig. 7) and for the insulin/glucose ratio determined 30 min after the glucose load ($r = 0.60$, $p = 0.0002$; Fig. 4).

**DISCUSSION**

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

The lack of reliable imaging techniques suitable to determine beta-cell mass in vivo has prompted great interest in the functional assessment of beta-cell mass (22). Thus, a number of studies have been performed in different animal models of diabetes (rats, dogs, baboons, minipigs) (23-27). The results of these studies have recently been elegantly reviewed by R.P. Robertson (22). As a rule, significant linear associations between the insulin secretory responses to arginine or glucagon administration and pancreatic beta-cell mass have been reported in these animal
studies (22). In humans, the available information about the relationship between insulin secretion and beta-cell mass is rather sparse, and two different studies have previously addressed this issue: Teuscher and colleagues determined insulin secretory responses to intravenous glucose and arginine administration in eight subjects undergoing islet autotransplantation (28). 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 the insulin levels after arginine administration was found in patients with type 1 diabetes studied after islet allotransplantation (29). The present study extends these results by for the first time providing direct evidence for a linear correlation between intrapancreatic beta-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 beta-cell mass with a comparable accuracy as the functional measures applied herein (13).

While this and the aforementioned prior studies therefore lend strong support to the use of functional tests for the assessment of beta-cell area in humans, it is important to still bear in mind the potential limitations of such approach. In fact, even though the overall association between beta-cell area and insulin secretion in this study was rather close, any functional measure of insulin secretion can be confounded by a number of other factors than merely the amount of pancreatic beta-cells. Along these lines, insulin secretion can be increased by as much as 2-3-fold under conditions of obesity or insulin resistance (30), although the actual increase in beta-cell mass in obese patients has been estimated to be only ~30 % (2). Furthermore, the insulin responses to intravenous glucose administration in patients with type 2 diabetes can be enhanced acutely by more than 300 % following the prior infusion of a GLP-1 analogue over five hours (31), a time period during which any gain in beta-cell mass is far from being realistic. Consistent with this, the present study has shown that despite the significant relationship between insulin secretion and beta-cell area, there was still considerable variability in insulin secretion between different individuals with a similar extent of beta-cell area. In this regard, it is also important to stress that any association between functional indices of insulin secretion and beta-cell area is only valid in the absence of any concomitant antidiabetic treatment.

Moreover, since a large number of functional parameters were compared to beta-cell area, there is a certain likelihood of significant results due 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 beta-cell area and the 120 min glucose levels during the OGTT is another intriguing finding from this study. Thus, as much as 80 % of the variations in post-challenge glucose excursions could be attributed to the differences in beta-cell area. Interestingly, this relationship was much closer that 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 beta-cells, whereas fasting glucose levels may be affected by a number of additional factors as well. Collectively, these findings further emphasize the importance of beta-cell mass for the development of diabetes. In this regard, it is noteworthy that the associations between the various parameters of glucose control and C-peptide secretion was even weaker than the respective associations with beta-cell area, which may suggest that defects in beta-cell
mass rather than impairments in beta-cell function primarily determine the development of diabetes.

While beta-cell area was significantly reduced in the patients with overt hyperglycaemia, there was only a very small reduction in beta-cell area in the pre-diabetic patients with IFG and IGT. This finding seems to be at variance with previous data by Butler and colleagues showing an ~40% deficit in beta-cell area in individuals with IFG (2). It is therefore important to bear in mind the differences between these studies. Thus, Butler and colleagues examined beta-cell area in IFG subjects, whereas the majority of patients in this study were characterised by IGT. Given the marked differences in the pathogenesis of IFG and IGT (32), these findings suggest that the rise in post-challenge glucose levels is a rather early phenomenon during the development of diabetes, whereas fasting hyperglycaemia may be indicative of a more pronounced beta-cell loss. Second, the diagnostic threshold for IFG was 110 mg/dl in the study by Butler et al. (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 glycaemia (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 hyperglycaemia 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 and colleagues studied a group of patients more typical of (pre-) type 2 diabetes (2). In line with this, the mean BMI of the patients in the IFG population studied previously was ~37 kg/m², whereas the present group of patients with IFG/IGT had a mean BMI of 23.4 kg/m². It is therefore likely that the mechanisms underlying the beta-cell destruction in the patients studied herein were different from those typically found in patients with type 2 diabetes. Therefore, even though our present study provides novel information regarding the relationship between beta-cell area and insulin secretion as well as glycaemic control, the findings should not be generalized to the majority of patients with type 2 diabetes.

A number of different functional indices have been proposed to estimate beta-cell function in vivo. Amongst those, the insulin responses to intravenous glucagon or arginine administration (with or without glucose potentiation) appear to provide the most reliable estimates of beta-cell function (22). However, the broad application of such tests is clearly limited by their laborious procedures preventing the routine examination of a 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 present data showing a close relationship between the insulin and C-peptide excursions during the OGTT and the fractional beta-cell area of the pancreas lend further support to the broad use of this test and suggest that post-challenge glucose excursions may not only predict the risk of developing diabetes, but also allow for some conclusions regarding the residual beta-cell area.

Since pancreatic weight cannot readily be measured at surgery in humans, the fractional beta-cell area rather than the total beta-cell mass has been determined in this study. Therefore, the actual beta-cell mass of the patients may still vary to some extent depending on the overall size of the pancreas. However, previous studies including more than 1800 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 present results should be rather minor (35). The close relationship between post-challenge glycaemia and the fractional beta-cell area observed in this study is consistent with the postulate that fractional beta-cell area rather than pancreatic weight is the primary determinant of beta-cell mass and suggests that in humans beta-cell mass may readily be estimated from the determination of the beta-cell area in pancreatic tissue samples.

One important goal of this study was to identify simple and reliable predictors of beta-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, since this relationship followed an exponential rather than a linear function, the predictive value of such post-challenge glucose excursions may be low especially in individuals with a relatively normal beta-cell area (i.e. greater than 50% of normal). A better estimation of beta-cell area may be derived from a C-peptide/glucose ratio at t = 15 min after oral glucose ingestion, which in the present study explained as much as 51% of the variations in beta-cell area. However, even a simple C-peptide/glucose ratio under fasting conditions already provides a reasonable estimate of beta-cell area. In contrast, the HOMA beta-cell function index, which is commonly applied as measure of beta-cell function in clinical studies (36; 37), completely failed to predict pancreatic beta-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 various antidiabetic agents on pancreatic beta-cell area and the progression of diabetes. Thus, while there is solid evidence for a progressive decline in beta-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, ADOPT) have applied the HOMA index as a measure of beta-cell function, which according to the present results does not necessarily predict changes in beta-cell mass (36; 40). In a similar fashion, a number of recent clinical trials have suggested improvements in beta-cell function and presumably beta-cell mass during treatment with DPP-4 inhibitors and GLP-1 analogues based on such calculations (37; 41). The present studies suggest that while the HOMA index may have some relevance to estimate the functional integrity of insulin secretion, it represents an odd surrogate of beta-cell area in vivo. Future studies in this area should therefore consider the use of a C-peptide/glucose ratio after oral glucose ingestion as a functional estimate of beta-cell area.

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

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Table 1: Clinical characteristics of patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) and patients with diabetes

<table>
<thead>
<tr>
<th>Parameter [unit]</th>
<th>NGT</th>
<th>IGT/IFG</th>
<th>Diabetes</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age [years]</td>
<td>55.9 ± 15.9</td>
<td>63.2 ± 13.9</td>
<td>59.0 ± 10.1</td>
<td>0.30</td>
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<tr>
<td>Sex [female/male]</td>
<td>5/3</td>
<td>8/6</td>
<td>3/8</td>
<td>0.22</td>
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<tr>
<td>BMI [kg/m²]</td>
<td>23.6 ± 2.7</td>
<td>23.4 ± 3.2</td>
<td>24.1 ± 4.7</td>
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<tr>
<td>Waist-hip-ratio</td>
<td>0.89 ± 0.07</td>
<td>0.89 ± 0.08</td>
<td>0.90 ± 0.06</td>
<td>0.89</td>
</tr>
<tr>
<td>RR systolic [mmHg]</td>
<td>116 ± 18</td>
<td>119 ± 35</td>
<td>125 ± 20</td>
<td>0.77</td>
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<tr>
<td>RR diastolic [mmHg]</td>
<td>72 ± 9</td>
<td>73 ± 13</td>
<td>78 ± 10</td>
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<tr>
<td>HbA1c [%]</td>
<td>5.7 ± 0.5</td>
<td>5.7 ± 0.5</td>
<td>6.9 ± 1.4*</td>
<td>0.0086</td>
</tr>
<tr>
<td>White blood count [n/µl]</td>
<td>8024 ± 1338</td>
<td>6717 ± 2100</td>
<td>6236 ± 1845</td>
<td>0.13</td>
</tr>
<tr>
<td>Haemoglobin [g/dl]</td>
<td>13.8 ± 1.1</td>
<td>13.2 ± 1.5</td>
<td>13.8 ± 1.5</td>
<td>0.45</td>
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<tr>
<td>Serum amylase [U/l]</td>
<td>41.6 ± 41.6</td>
<td>71.5 ± 74.3</td>
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<td>Serum urea [mg/dl]</td>
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</tr>
<tr>
<td>Serum creatinine [mg/dl]</td>
<td>0.97 ± 0.11</td>
<td>0.99 ± 0.08</td>
<td>1.0 ± 0.06</td>
<td>0.98</td>
</tr>
<tr>
<td>Triglycerides [mg/dl]</td>
<td>100.3 ± 34.9</td>
<td>121.9 ± 49.8</td>
<td>155.2 ± 72.1</td>
<td>0.13</td>
</tr>
<tr>
<td>Cholesterol [mg/dl]</td>
<td>209.9 ± 37.0</td>
<td>207.2 ± 45.5</td>
<td>204.5 ± 56.0</td>
<td>0.97</td>
</tr>
<tr>
<td>HDL-cholesterol [mg/dl]</td>
<td>64.0 ± 22.6</td>
<td>52.4 ± 24.0</td>
<td>44.7 ± 14.5</td>
<td>0.20</td>
</tr>
<tr>
<td>LDL-cholesterol [mg/dl]</td>
<td>136.2 ± 36.8</td>
<td>143.5 ± 28.0</td>
<td>137.6 ± 39.3</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Means ± SD; Statistics: ANOVA or X-Square test
*: significantly different vs. controls (Duncan’s post hoc test)
Figure 1: Concentrations of glucose (A), insulin (B), and C-peptide (C) in eight individuals with normal glucose tolerance (NGT), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT), and 11 patients with diabetes following the oral ingestion of 75 g glucose. Data are presented as means ± SEM. Statistics were carried-out using repeated measures ANOVA and denote A: differences between the experiments, B: differences over time and AB: differences due to the interaction of experiment and time. Asterisks (*) indicate significant (p < 0.05) differences versus control subjects at individual time points (one-way ANOVA and Duncan’s post hoc test).
Figure 2: Fractional beta-cell area (A), fasting insulin/glucose ratio (B), insulin/glucose ratio 30 min after oral glucose ingestion (C), HOMA beta-cell function (excluding two outlier patients) (D), fasting C-peptide/glucose ratio (E), and C-peptide/glucose ratio 30 min after oral glucose ingestion (F) in eight individuals with normal glucose tolerance (NGT), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT), and 11 patients with diabetes. Data are presented as means ± SEM. Statistics were carried-out by ANOVA.
Figure 3: Correlation analyses between fractional beta-cell area (A, B, C) or the plasma C-peptide levels (D, E, F) and the basal blood glucose levels (A, D), the blood glucose levels 120 min after oral glucose ingestion and (B, E), and the HbA1c levels (C, F) in eight individuals with normal glucose tolerance (NGT; circles), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT; diamonds), and 11 patients with diabetes (triangles). r = correlation coefficient. p-values were calculated by linear regression analysis. r and p-values in panels A and D were calculated by linear regression analyses. r-values in panels B, C, E and F were calculated by non-linear 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.
Figure 4: Linear regression analyses between fractional beta-cell area and established indices of insulin secretion. Panels A-F show the correlations with the fasting insulin/glucose ratio (A), the plasma insulin levels 30 min after oral glucose administration (B), the insulin/glucose ratio 30 min after oral glucose administration (C), the fasting C-peptide/glucose ratio (D), the plasma C-peptide levels 30 min after oral glucose administration (E), and the C-peptide/glucose ratio 30 min after oral glucose administration (F) in eight individuals with normal glucose tolerance (NGT; circles), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT; diamonds), and 11 patients with diabetes (triangles). Dashed lines denote the respective upper and lower 95% confidence intervals; r = correlation coefficient.
Figure 5: Linear regression analyses between fractional beta-cell area and the HOMA index of beta-cell function in eight individuals with normal glucose tolerance (NGT; circles), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT; diamonds), and 11 patients with diabetes (triangles). Panel A shows the correlation including all patients examined, panel B shows the correlation after excluding two outlier patients. Dashed lines denote the respective upper and lower 95% confidence intervals; $r =$ correlation coefficient.
Figure 6: Correlation coefficients (squared) for the relationship between fractional beta-cell area and the respective insulin, C-peptide and glucose levels obtained before and after oral glucose ingestion in eight individuals with normal glucose tolerance (NGT), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (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).

Figure 7: Linear regression analyses between fractional beta-cell area and the glucose concentrations 60 min after oral glucose administration (A), and the C-peptide/glucose ratio 15 min after oral glucose administration (B) in eight individuals with normal glucose tolerance (NGT; circles), 14 individuals with impaired fasting glucose (IGT) and/or impaired glucose tolerance (IGT; diamonds), and 11 patients with diabetes (triangles). These parameters were identified as strongest predictors of beta-cell area based on the respective r-values. Dashed lines denote the respective upper and lower 95% confidence intervals; r = correlation coefficient.