Measurements of Insulin Secretory Capacity and Glucose Tolerance to Predict Pancreatic β-Cell Mass In Vivo in the Nicotinamide/Streptozotocin Göttingen Minipig, a Model of Moderate Insulin Deficiency and Diabetes

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Knowledge about β-cell mass and/or function could be of importance for the early diagnosis and treatment of diabetes. However, measurement of β-cell function as an estimate of β-cell mass is currently the only method possible in humans. The present study was performed to investigate different functional tests as predictors of β-cell mass in the Göttingen minipig. β-cell mass was reduced in the Göttingen minipig with a combination of nicotinamide (100 [n = 6], 67 [n = 25], 20 [n = 2], or 0 mg/kg [n = 4]) and streptozotocin (125 mg/kg). Six normal pigs were included. An oral glucose tolerance test (OGTT) (n = 30) were performed and pancreata obtained for stereological determination of β-cell mass. During OGTT, fasting glucose (r² = 0.1744, P < 0.01), area under the curve for glucose (r² = 0.2706, P < 0.001), maximum insulin secretion (r² = 0.2160, P < 0.01), and maximum C-peptide secretion (r² = 0.1992, P < 0.01) correlated with β-cell mass. During the insulin secretion test, acute insulin response to 0.3 g/kg (r² = 0.6155, P < 0.0001) and 0.6 g/kg glucose (r² = 0.7321, P < 0.0001) and arginine (67 mg/kg) (r² = 0.7732, P < 0.0001) and maximum insulin secretion (r² = 0.8192, P < 0.0001) correlated with β-cell mass. This study supports the use of functional tests to evaluate β-cell mass in vivo and has established a validated basis for developing a mathematical method for estimation of β-cell mass in vivo in the Göttingen minipig. Diabetes 52:118–123, 2003

Reduction of β-cell mass is a key feature of type 1 diabetes (1–4) and late autoimmune diabetes of the adult (5). However, in type 2 diabetic patients, most (1–3,6–8), but not all (4,9,10), studies have demonstrated only a modest reduction (20–50%) of β-cell mass, whereas a more recent study suggests that the major problem in type 2 diabetes is an abnormal insulin secretion in relation to glycemia rather than a problem of reduced β-cell mass (11). A 20–30% reduction of β-cell mass has been suggested to be sufficient to give rise to very slight increases in glycemic levels (7). Pancreactectomy in humans has been shown to result in increased fasting and postprandial glucose levels and a reduction in fasting and postprandial insulin concentrations (12), whereas a selective 50% reduction of β-cell mass has been reported to induce severe hyperglycemia in baboons (13). In pigs, impairment of glucose tolerance has been shown to be related to the extent of pancreatectomy, with 40% pancreatectomy resulting only in mild changes, whereas a 80% pancreatectomy resulted in significant hyperglycemia (14). However, a greater than 80–90% reduction of β-cell mass seems to be required before overt insulin-dependent diabetes develops in humans (2,3,6) and rats (15). In both humans (16) and pigs (17), there is a strong relation between islet mass used in transplantation studies and metabolic control, as evaluated by glucose levels and insulin secretion. Therefore, in patients with type 2 diabetes, the slight reduction of β-cell mass reported is unlikely to be the only factor responsible for development of hyperglycemia. Most likely, reduced insulin action is also of key importance, but since not all insulin-resistant (i.e., obese) subjects suffer from type 2 diabetes (18–20), it seems that a β-cell dysfunction and/or a reduction of β-cell mass is prerequisite for development of type 2 diabetes.

Knowledge about β-cell mass and/or function, and especially changes in these parameters, could be of great importance for the early diagnosis, as well as for treatment, of diabetes in humans. Several tests are now available for evaluation of β-cell function in humans, including the hyperglycemic clamp (21), the minimal model technique (22), arginine stimulation (23,24), glucagon-like peptide 1 (GLP-1) stimulation (25), and insulin pulse induction (26). However, measurement of β-cell mass in human patients is only possible after autopsy. Recently, estimation of β-cell mass in vivo has been reported by a method using nuclear imaging (27), but whether this technique can be applied in humans still needs to be established. Therefore, measurement of β-cell function as an estimate of...
β-cell mass is, at present, the only method possible in humans, and a validation in animal models is of great importance. Previously, correlations between in vivo functional tests and actual β-cell mass have been reported in primates (13). However, because of the high costs and special ethical considerations when using primates, the use of another animal model should be considered.

For this purpose, the pig is a relevant species because of the many similarities to humans in regard to nutritional requirements and physiology of digestion and metabolism (28–31), which make it useful for studies of fasting and postprandial glucose metabolism and possibly also for studying relations between in vivo functional tests and β-cell mass. The Göttingen minipig offers many advantages in this context because of its well-described biology with respect to glucose and lipid metabolism both in normal animals (32–35) and after induction of diabetes (35,36) or challenge with high-fat diets (32,34,37,38). Furthermore, these animals can be trained to allow experiments to be performed in conscious unanesthetized animals.

To evaluate this concept further, the present study was performed in Göttingen minipigs in vivo to investigate the value of different functional tests for predicting actual β-cell mass, as evaluated by stereology post mortem. To encompass a broad spectrum of β-cell mass, animals dosed with different combinations of nicotinamide (NIA) and streptozotocin (STZ) were used.

**RESEARCH DESIGN AND METHODS**

**Animals.** Adult male Göttingen minipigs, aged 11–16 months, were obtained from the barrier unit at Ellegaard Göttingen minipigs APS (Dalmoose, Denmark). Animals were housed in single pens under controlled conditions (temperature was kept between 18 and 22°C and relative air humidity was 30–70% with four air changes per hour) with a 12 h light:12 h dark cycle and fed 140 g SDS minipig diet (SDS, Essex, U.K.) twice daily and 240 g of a commercial swine fodder (Svnefoder 22, Østsjallands Andel, Karise, Denmark) and allowed free access to water. The pigs were studied at least 2 weeks postsurgery and trained carefully in all experimental procedures before the start of the experiments. A total of 43 animals were used in the studies, weighing 26 ± 4 kg (range 18–33). The type of study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

**Experimental groups.** All 43 animals included in the study underwent a mixed meal oral glucose tolerance test (OGTT) with measurement of glucose and insulin levels, and the pancreata from all animals were evaluated histologically. Furthermore, 20 of the included animals underwent an insulin secretion test (6 normal animals, 20 animals dosed with 67 mg/kg NIA and 125 mg/kg STZ, and 4 animals dosed with 20 mg/kg NIA and 0 mg/kg NIA (n = 3) and 125 mg/kg STZ).

**Surgical implantation of central venous catheters.** Two central venous catheters (Certo 415; B. Braun Melsungen AG, Melsungen, Germany) were inserted surgically under general anesthesia as described previously (35). Postsurgical analgesia was maintained by injection of 0.03 mg/kg buprenorphine (0.3 mg/ml Anorfin; GEA, Frederiksborg, Denmark) and 4 mg/kg carprofen (50 mg/ml Rimadyl vet.; Pfizer, Ballerup, Denmark) intramuscularly before the surgical procedure. The minimal detectable concentration was 3.2 pmol/l, the upper limit was 1,200 pmol/l (no sample dilution), and the inter- and intra-assay variations (at three concentration levels) were 15.3 and 3.2% (at 342 pmol/l), 9.9 and 7.0% (at 235 pmol/l), and 14.0 and 4.4% (at 87 pmol/l). Recovery at high, medium, and low concentration levels was 97.1, 97.0, and 101.0%, respectively. Cross-reactivity against a number of peptides has been tested: IGFI (human) = 0.03%, growth hormone (porcine) = 0.001%, glucagon (porcine) = 0.4%, insulin (rat) = 0.03%, somatostatin = 0.2%, pancreatic polypeptide (porcine) = 0.2%, C-peptide (porcine) = 0.01%, intact proinsulin (human) = 0.3%, 32-33 split proinsulin (human) = 0.3%, des 31-32 split proinsulin (human) = 0.5%, 65-66 split proinsulin (human) = 30%, and des 64-65 split proinsulin (human) = 63% (40).

A commercial kit was used to measure C-peptide concentrations (Porcine C-peptide RIA kit, cat. no. PCC22K; Linco, St. Charles, MO).

**Histological examination of pancreas.** After euthanasia with pentobarbital (20 mg per animal as an intravenous bolus) (20 mg/ml; Pharmacy of the Royal Veterinary and Agricultural University, Copenhagen, Denmark) at the end of the study period, the pancreata were isolated in toto for histological examination, as previously described (35). In animals dosed with NIA plus STZ or STZ alone, pancreata were obtained 35–27 days (range 11–84) after the OGTT and 8–7 days (range 6–27) after the insulin secretion test. In the normal group and in animals dosed with NIA or STZ alone, 8–12 days (range 17–26) after the OGTT and 13 ± 7 days (range 6–23) after the insulin secretion test. In short, pancreata were fixed in parafomaldehyde (Bie & Berntsen, Copenhagen, Denmark), embedded in 3% agar solution (cat. no. 303280; Meco-Benzon, Copenhagen, Denmark), and sectioned as practiced in the smooth fractionator method (41,42). The deparaffinized sections were stained (staining protocols described to physicians, some stained with hematoxyline and Bartholitz); pancreatic polypeptide to visualize β and non-β endocrine cells. Furthermore, sections were counterstained with Mayer’s hematoxyline. β- and non-β
endocrine cell mass was evaluated stereologically in two to three sections with the origin of the sections blinded to the observer. Mass of endocrine cells is expressed in milligrams per kilogram body weight.

Evaluation of results. The area under the curve (AUC) for glucose and insulin during OGTT was calculated using the trapezoidal method (baseline = 0 min). Acute insulin response (AIR) to glucose and arginine was calculated as the average increase in insulin levels during the first 10 min after dosing (AIR = (mean insulin 0–10 min) – (baseline before dosing)). The effect of NIA plus STZ on parameters of glucose tolerance obtained during the OGTT (fasting plasma glucose [FPG], AUC glucose, and maximum insulin and C-peptide levels during the OGTT) was evaluated by comparison to similar parameters obtained in 51 normal animals.

All calculations and statistical evaluation of results were performed using linear and nonlinear regression and one-way ANOVA with Tukey’s multiple comparison test as posttest using Excel (2000) and GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). P values ≤ 0.05 were considered significant. Data are presented as means ± SD.

RESULTS

β-Cell mass. β-Cells accounted for 74 ± 7% of the total endocrine cell mass in the pancreas of the normal minipig, and the pancreatic content of endocrine tissue was 1.5 ± 0.6%. Pancreas weight relative to body weight was 1.6 ± 0.2 g/kg, and the β-cell mass relative to body weight in normal animals was 19.6 ± 6.0 mg/kg. β-Cell mass was reduced significantly by all combinations of NIA and STZ used (P < 0.0001) (Table 1).

Parameters from the OGTT. FPG was increased significantly with falling doses of NIA (P < 0.0001), with the increase in FPG being significant for 67, 20, and 0 mg/kg NIA (Table 1). Similarly, AUC for glucose during the OGTT increased significantly from the normal value when the NIA dose was reduced (P < 0.0001), with the increase being significant in the 67, 20, and 0 mg/kg NIA groups. Furthermore, maximum insulin and C-peptide levels during the OGTT were significantly reduced by a reduction in the NIA dose (P < 0.0001 for both parameters) (Table 1).

Parameters from the insulin secretion test. The AIR to intravenous glucose (0.3 g/kg) was reduced by administration of 67 mg/kg NIA in combination with STZ or 20 or 0 mg/kg NIA in combination with STZ (P < 0.001 for both) versus the normal value. Similarly, AIR to 0.6 g/kg glucose (P < 0.001 for both groups) and arginine (P < 0.01 for 67 mg/kg NIA plus STZ and P < 0.05 for 20 or 0 mg/kg NIA plus STZ), as well as maximum insulin levels during the insulin secretion test (P < 0.001 for 67 mg/kg NIA plus STZ and P < 0.01 for 20 or 0 mg/kg NIA plus STZ), were reduced compared with normal values (Table 2).

Relation between in vivo functional tests and β-cell mass. From the OGTT data, it appears that FPG (r² = 0.1744, P < 0.01), AUC glucose (r² = 0.2706, P < 0.001), and maximum insulin (r² = 0.2160, P < 0.01) and C-peptide (r² = 0.1992, P < 0.01) all correlate with β-cell mass determined postmortem (Fig. 1). Data on FPG and AUC glucose indicated a large compensatory capacity, so that no dramatic change in either of these parameters was seen before β-cell mass was reduced to ~ 5 mg/kg. At this point, there was a sharp increase in both FPG and AUC glucose, with further reduction of β-cell mass. For practical purposes, this relation between β-cell mass and FPG or AUC glucose is illustrated by fitting the data to an exponential function in Fig. 1 (FPG, r² = 0.7848 and AUC glucose, r² = 0.7537).

From the data from the insulin secretion test, it was found that AIR to 0.3 (r² = 0.6155, P < 0.0001) and 0.6 g/kg glucose (r² = 0.7321, P < 0.0001), as well as AIR to arginine (r² = 0.7732, P < 0.0001) and maximum insulin secretion during the test (r² = 0.8192, P < 0.0001), all correlated significantly to β-cell mass. Furthermore, for all parameters derived from the insulin secretion test, except for AIR to 0.3 g/kg glucose, the regression line intercepted the axes close to zero (Fig. 2).

DISCUSSION

The β-cell content of the endocrine tissue in the normal minipig is in the same range as the 60–80% reported in humans (3,4,8), whereas β-cell mass relative to body weight is almost twice as high in the minipig as compared with that in humans (12.7 ± 5.1 mg/kg, P < 0.01) (1). The high β-cell mass relative to body mass in the minipig could indicate a greater insulin secretory reserve compared with the human.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (67 mg/kg)</th>
<th>NIA (20 or 0 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>AIR (0.3 g/kg) (pmol/l)</td>
<td>263 ± 87</td>
<td>117 ± 63*</td>
</tr>
<tr>
<td>AIR (0.6 g/kg) (pmol/l)</td>
<td>354 ± 131</td>
<td>119 ± 68*</td>
</tr>
<tr>
<td>AIR (arginine) (pmol/l)</td>
<td>332 ± 193</td>
<td>104 ± 105†</td>
</tr>
<tr>
<td>Maximum insulin (pmol/l)</td>
<td>1,078 ± 512</td>
<td>448 ± 276*</td>
</tr>
</tbody>
</table>

Data are means ± SD. Animals were either normal or dosed with 67, 20, or 0 mg/kg NIA in combination with streptozotocin (125 mg/kg).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>100 mg/kg</th>
<th>67 mg/kg</th>
<th>20 mg/kg</th>
<th>0 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>25</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>β cell mass (mg/kg)</td>
<td>19.6 ± 6.0</td>
<td>4.6 ± 1.1*</td>
<td>6.2 ± 3.4*</td>
<td>1.5 ± 0.7*</td>
<td>2.5 ± 1.8*</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>3.6 ± 0.7</td>
<td>4.3 ± 0.7</td>
<td>5.1 ± 3.1†</td>
<td>15.4 ± 1.8*</td>
<td>13.4 ± 5.3*</td>
</tr>
<tr>
<td>AUC for glucose (mmol/l × min)</td>
<td>941 ± 193</td>
<td>1,416 ± 525</td>
<td>1,596 ± 817*</td>
<td>4291 ± 139*</td>
<td>3,801 ± 1147*</td>
</tr>
<tr>
<td>Maximum insulin (pmol/l)</td>
<td>558 ± 391</td>
<td>244 ± 85</td>
<td>254 ± 187*</td>
<td>128 ± 65</td>
<td>62 ± 75†</td>
</tr>
<tr>
<td>Maximum C-peptide (pmol/l)</td>
<td>1,031 ± 594</td>
<td>443 ± 81</td>
<td>584 ± 274†</td>
<td>106 ± 62</td>
<td>137 ± 74‡</td>
</tr>
</tbody>
</table>

Data are means ± SD. Animals were either normal or dosed with 100, 67, 20, or 0 mg/kg NIA in combination with streptozotocin (125 mg/kg). *P < 0.001, †P < 0.05, ‡P < 0.01 vs. normal animals (one-way ANOVA with Tukey’s multiple comparison test as posttest).
As previously reported (35), the administration of a combination of NIA and STZ reduces glucose tolerance and \( \beta \)-cell function and mass in the Göttingen minipig. Both fasting and postprandial plasma glucose seem to be relatively resistant to quite considerable reductions in \( \beta \)-cell mass, and are only affected when \( \beta \)-cell mass is reduced to \( \sim 5 \) mg/kg versus the normal value of \( \sim 20 \) mg/kg. The same relationship has been described for \( \beta \)-cell function and FPG in humans with normal insulin sensitivity, where a reduction of \( \beta \)-cell function of \( \sim 75\% \) is seen before overt fasting hyperglycemia occurs (43). The reduction of \( \beta \)-cell mass (\( \sim 75\% \)), resulting in fasting hyperglycemia in the present study, is slightly different from results from a previous study, showing that a \( \beta \)-cell mass reduction of \( \sim 60\% \) results in increased fasting hyperglycemia in minipigs (36). Similar studies in baboons have shown that a reduction of \( \beta \)-cell mass of \( 50–60\% \) causes a dramatic decrease in \( \beta \)-cell function (13), whereas studies in both humans (2,3,6) and rats (15) have indicated that a greater than \( 80–90\% \) reduction of \( \beta \)-cell mass is required before overt insulin-dependent diabetes develops. It cannot be concluded from the present study whether the ability to compensate for a reduction of \( \beta \)-cell mass differs between species. However, most likely, the different results obtained are, to some extent, explained by the relatively small number of observations in each study and the possibility that variability in \( \beta \)-cell function at very low values of \( \beta \)-cell mass seems to occur.

**FIG. 1.** A and B: Maximum insulin (A) and C-peptide (B) response during OGTT. Data are fitted to a linear function. C and D: Relation between \( \beta \)-cell mass and FPG (C) and AUC glucose during an OGTT (D). Data in C and D are fitted to an exponential function, where \( K \) is a rate constant. •, animals dosed with 100 mg/kg NIA and 125 mg/kg STZ \( (n = 6) \); ■, animals dosed with 67 mg/kg NIA and 125 mg/kg STZ \( (n = 25) \); ▲, animals dosed with 20 mg/kg NIA \( (n = 2) \); □, animals dosed with no NIA and 125 mg/kg STZ \( (n = 4) \); ○, normal animals \( (n = 6) \).

**FIG. 2.** AIR to 0.3 (A) and 0.6 g/kg glucose (B) and 67 mg/kg arginine (C). D: Maximum plasma insulin concentration during the insulin secretion test. ■, animals dosed with 67 mg/kg NIA and 125 mg/kg STZ \( (n = 20) \); ▲, animals dosed with 20 mg/kg NIA \( (n = 1) \); □, animals dosed with no NIA and 125 mg/kg STZ \( (n = 3) \); ○, normal animals \( (n = 6) \).
Transplantation studies in both humans (16) and pigs (17) have shown a strong relation between islet mass and metabolic control. Since the β-cell mass (milligrams per kilogram body weight) in pigs is higher than in humans, this could affect the way in which functional tests can be used as a predictor of β-cell mass in each species. However, since a reduction of β-cell mass of ~80–90% is required in both species for development of overt diabetes (2,3,6,14), it seems that effects of a relative reduction of β-cell mass could be compared in the two species. Further studies would have to clarify this in more detail.

Minimal modeling of data obtained during OGTT could be a useful tool for evaluation of β-cell function in nondiabetic humans (44), whereas other methods using OGTT data were not efficient predictors of β-cell function in healthy human subjects (45). In the present study, parameters derived from the OGTT did not show the same strong correlation with actual measures of β-cell mass as those derived from the insulin secretion test. This could be due to the longer time span between OGTT and histology compared with the time between the insulin secretion test and histology. It could also be due to the more acute stimulation of β-cells by the intravenously administered glucose and arginine than by the orally ingested glucose, where several factors influence the rate of appearance of the glucose in the systemic circulation.

A number of the tests available for evaluation of β-cell function in humans, such as the hyperglycemic clamp (21), the minimal model technique (22), and induction of pulsatile insulin secretion (26), rely on intravenous administration of glucose, whereas other tests include administration of arginine (23,24) or GLP-1 (25), and it seems that the insulin secretion induced by intravenous glucose and/or arginine could be a possible tool for predicting β-cell mass in humans (16).

A better correlation between AIR glucose and β-cell mass compared with AIR arginine versus β-cell mass has been reported in β-cell-reduced baboons (13). However, in the present study, AIR to arginine correlated better to β-cell mass than AIR to the same dose of glucose (0.3 g/kg) as used in the baboons (13). This difference could possibly be explained by the higher levels of glucose during arginine stimulation in the present study compared with those in the baboons. In the present study, it seems that maximum insulin concentration during the insulin stimulation test has a slightly higher correlation to β-cell mass than any of the other investigated parameters. Therefore, this would indicate that a maximal stimulation of insulin secretion with a combination of glucose and arginine is a better predictor of β-cell mass in vivo compared with oral or intravenous glucose alone. However, even a simple method, such as the insulin secretory response to a glucose bolus of 0.3 g/kg, could be a significant predictor of β-cell mass.

It should be noted that even though correlations obtained in the present study are highly significant, it is likely that there is considerable biological variation inherent in the technique. Therefore, to further improve the utility of the method, it could be advantageous to study changes over time in individual animals, and this is exactly the strength of the study presented here—it allows for ongoing studies of β-cell function, shown to be a predictor of β-cell mass in live animals, thereby allowing for detection of dynamics of β-cell mass in vivo.

In conclusion, the present study supports the use of functional in vivo tests as a method of evaluating β-cell mass in vivo and has established a validated basis for developing a mathematical method for estimation of β-cell mass in vivo in the Göttingen minipig. This method could be useful for studies of dynamics of β-cell mass in diabetes, with special reference to the pathology and treatment of the disease.

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