Partial Pancreatectomy in Adult Humans Does Not Provoke β-Cell Regeneration

Bjoern A. Menge,[1] Andrea Tannapfel,[2] Orlin Belyaev,[3] Robert Drescher,[4] Christophe Müller,[3] Waldemar Uhl,[3] Wolfgang E. Schmidt,[1] and Juris J. Meier[1]

OBJECTIVE—β-Cell regeneration has been proposed as a possible treatment for diabetes, but the capacity for new β-cell formation in humans is yet unclear. In young rats, partial pancreatectomy prompts new β-cell formation to restore β-cell mass. We addressed the following questions: In adult humans: *1*) Does partial pancreatectomy provoke new β-cell formation and increased β-cell mass? *2*) Is β-cell turnover increased after partial pancreatectomy?

RESEARCH DESIGN AND METHODS—Protocol 1: human pancreatic tissue was collected from 13 patients who underwent two consecutive partial pancreas resections, and markers of cell turnover were determined in both tissue samples, respectively. Protocol 2: pancreas volumes were determined from abdominal computer tomography scans, performed in 17 patients on two separate occasions after partial pancreatectomy.

RESULTS—Protocol 1: fasting glucose concentrations increased significantly after the 50% pancreatectomy ($P = 0.01$), but the fractional β-cell area of the pancreas remained unchanged ($P = 0.11$). β-Cell proliferation, the overall replication index (Ki67 staining), and the percentage of duct cells expressing insulin were similar before and after the partial pancreatectomy. The overall frequency of apoptosis (terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling) was slightly increased following the partial pancreatectomy ($P = 0.02$). Protocol 2: pancreatic volume was ~50% reduced to 35.6 ± 2.6 ccm^3 by the partial pancreatectomy. The total pancreatic volume was unchanged after an interval of 247 ± 160 days (35.4 ± 2.7 ccm^3; $P = 0.51$).

CONCLUSIONS—Unlike in rodents, a 50% pancreatectomy does not prompt β-cell regeneration in adult humans. This explains the high incidence of diabetes after pancreatic resections. Such differences in β-cell turnover between rodents and humans should be born in mind when evaluating new treatment options aiming to restore β-cell mass in patients with diabetes.
***Diabetes* 57:142–149, 2008**

Diabetes develops if β-cell mass is diminished below a critical threshold required to sustain adequate insulin secretion at a given level of insulin resistance (1–3). Based on cross-sectional studies (3,4) in human autopsy pancreata, glucose levels start to rise when β-cell mass is reduced by more than ~50% of normal. Likewise, studies (5,6) in patients with new-onset type 1 diabetes have reported a 50–70% β-cell deficit around the onset of hyperglycemia. The loss of insulin-secreting β-cells in both types of diabetes provides a rationale for novel therapeutic approaches aiming to restore β-cell mass in diabetic patients.

Given the obvious inaccessibility of human pancreas tissue for repeated biopsy sampling in clinical studies, most studies about the dynamics and the mechanisms of β-cell regeneration available so far have been carried out in rodent models (7). Among those, the partial pancreatectomy model has been commonly utilized to induce β-cell regeneration in mice and rats (8,9). Indeed, following a 90% pancreatectomy in rats, an up to 200% increase in β-cell number has been observed in the pancreas remnants (10), and, also, less extensive pancreas resections (40–60%) has been associated with increased β-cell proliferation (11–13). In addition to the gain in β-cell mass, a substantial proliferation of small ductules, as well as the appearance of small islets and β-cell clusters adjacent to exocrine ducts, has been described following partial removal of the pancreas (8). This has led to the hypothesis that new β-cell formation is partly driven by the neogenesis of islets from putative precursor cells arising from the ductal epithelium (the presence of which is yet unconfirmed) (8). Altogether, these studies have lent hope to the idea of fostering β-cell regeneration as a potential treatment of diabetes (7). However, despite the undisputed importance of such rodent studies for the elucidation of the potential mechanisms involved in the regulation of β-cell mass and turnover, the transferability of these data to the human situation is questionable. In fact, the overall capacity for regeneration in mice appears to far exceed that in humans, where β-cell replication is found rather infrequently (4). Furthermore, unlike humans, mice and rats do not develop diabetes spontaneously unless challenged by specific dietary regimens or genetic, chemical (e.g., streptozotocin), or surgical (e.g., duct ligation, pancreatectomy) alterations. Finally, functional studies in humans have shown that a hemi-pancreatectomy leads to significant impairments in insulin secretion and increases the risk of developing diabetes (14). However, the morphological changes in β-cell mass and turnover following a partial pancreatectomy have not yet been evaluated in humans.

Therefore, we examined whether a partial pancreatec-

From the [1]Department of Medicine I, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany; the [2]Institute for Pathology, Ruhr-University Bochum, Bochum, Germany; the [3]Department of Surgery, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany; and the [4]Department of Radiology, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany.

Address correspondence and reprint requests to Dr. Juris J. Meier, Department of Medicine I, St. Josef-Hospital, Ruhr-University Bochum, Gudrunstr. 56, 44791 Bochum, Germany. E-mail: juris.meier@rub.de.

Received for publication 11 September 2007 and accepted in revised form 15 October 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 24 October 2007. DOI: 10.2337/db07-1294.

CT, computed tomography; IPMT, intraductal papillary mucinous tumor; TUNEL, terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling.

tomy in adult humans would also prompt β-cell proliferation in the pancreas remnant. For these purposes we utilized a combination of computed tomography (CT) scan–derived pancreatic volume measurements performed longitudinally in patients after partial pancreatectomy and a histological examination of pancreatic tissue collected from patients who had undergone repeated pancreatic surgery on two separate occasions. Using this resource, we addressed the following questions: In adult humans, *1*) Does partial pancreatectomy provoke new β-cell formation and increased β-cell mass? *2*) Is β-cell turnover increased after partial pancreatectomy?

RESEARCH DESIGN AND METHODS

To assess changes in pancreatic mass and cell turnover following partial pancreatectomy, two separate studies were performed. In protocol 1, 13 patients who had undergone repeated pancreatic surgery on two separate occasions were included. Pancreatic tissue for histological evaluation was collected on both occasions. Fractional β-cell area, as well as measures of replication, new islet formation, and apoptosis, was quantified and compared between both tissue specimens, respectively. In protocol 2, pancreatic volumes were determined longitudinally in 17 patients, in whom abdominal CT scans had been performed on two separate occasions following partial pancreatectomy. The study protocols were approved by the ethics committee of the Ruhr-University Bochum (registration nos. 2392 and 2528).

Protocol 1

Patients. A total of 13 patients (11 male and 2 female) who had undergone repeated pancreatic resections between the years 2001 and 2006 were included. Potential case subjects were identified by retrospective analysis of the patient database of the Department of Surgery, St. Josef-Hospital, Ruhr-University Bochum. To be included, case subjects were required to have pancreatic tissue stored that was of adequate size and quality on both operations. The diagnoses leading to pancreas surgery were chronic pancreatitis in eight case subjects, pancreatic carcinoma in three case subjects, pancreatic metastases of a renal cell carcinoma in one case subject, and intraductal papillary mucinous tumor (IPMT) in one case subject. Patients with acute pancreatitis were excluded from the analyses. Diabetes was present in five patients, and one patient had impaired glucose tolerance. The mean age of the patients at the time of first surgery was 51.9 ± 11.3 years (range 39–72) and BMI was 23.5 ± 3.6 kg/m^2 (19.3–30.0). The interval between both operations was 1.8 ± 1.2 years (8 days to 3.5 years).

At the time of first surgery, the pancreas head was removed in 10 case subjects, whereas the tail region was resected in the other three case subjects. The amount of pancreas tissue removed during the first surgery was 50% in all case subjects. The reasons leading to relaparatomy included continued abdominal pain and pancreatic duct obstruction in patients with chronic pancreatitis and the suspicion of a tumor relapse in the other case subjects.

Pancreatic tissue processing. Pancreata were fixed in formaldehyde and embedded in paraffin for subsequent analysis as previously described (15). Sequential 5-μm sections were stained as follows: *1*) hematoxylin and eosin for light microscopy, *2*) insulin (peroxidase staining) and Ki67 (alkaline phosphatase/red) for light microscopy, and *3*) insulin, terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL), and DAPI for immunofluorescence. The following primary antibodies were used: guinea pig anti-insulin, 1:400 (no. A 0564, lot no. 00001500; Dako); and mouse Ki67, 1:400 (MIB-1, no. M 7240, lot no. 00014101; Dako). Secondary antibodies labeled with peroxidase or alkaline phosphatase/red were obtained from Dako (DAKO Real Envision Detection System, nos. K 5007 and K 5005, lot nos. 00025382 and 00025812, respectively). Secondary antibodies labeled with Cy3 were obtained from The Jackson Laboratories (no. 106-165-003, lot no. 67623) and used at 1:800 dilution, and secondary antibodies labeled with fluorescein isothiocyanate were obtained from Santa Cruz Biotechnology (no. sc-2090, lot no. L271) and used at 1:100 dilution.

For TUNEL staining, the in situ cell death detection kit (cat. no. 11 684 809 910, lot no. 13184900; Roche Diagnostics, Mannheim, Germany) was used according to the manufacturer's recommendations. Tissue samples were excluded from the determination of apoptosis if the pancreas sections exhibited areas of tissue damage or necrosis caused by autolysis.

Morphometric analysis. For the determination of the fractional β-cell area, the entire pancreatic section was imaged using a Zeiss Axioplan microscope equipped with a motorized-stage ×50 magnification (5 × objective). A tile image of the tissue section was generated using the Mosaix tool of the software Axiovision (version 4.5). The fractional area of the pancreas stained positive for insulin was digitally quantified using a color-based threshold using Zeiss Axiovision software, as previously described (15).

For the estimation of the overall proliferative activity of the pancreas, 10 random locations in each section stained for insulin and Ki67 were imaged at ×200 magnification (20 × objective), and the total number of Ki67-positive cells per field was quantified. To determine the frequencies of replication in β-cells and duct cells, the number of β-cells costaining with Ki67, as well as the number of duct cells positive for Ki67, was quantified in 10 fields each and expressed as a percentage of the total number of β-cell and duct cells, respectively. To estimate the contribution of possible new β-cell formation from duct cells, the number of duct cells expressing insulin was quantified in 10 fields each and expressed as a percentage of the total number of duct cells per field.

For the determination of the overall rate of apoptosis, 15 random locations in each section stained for insulin, TUNEL, and DAPI were imaged at ×200 magnification (20 × objective), and the total number of TUNEL-positive cells per field was quantified. To determine the frequency of β-cell apoptosis, the number of β-cells positive for TUNEL was quantified in each field and expressed as percentage of the total number of β-cells, respectively. To determine the mean β-cell nuclear diameter, a total of 100 β-cell nuclei per section were encircled manually, and the nuclear diameter was calculated as described (16). In a similar fashion, the mean overall cell diameter was determined in 100 β-cells per section.

All analyses were performed in a blinded fashion. To ensure reproducibility of morphometric analyses, intraobserver variability of the proliferation index was calculated by comparing the results obtained from counting 10 different fields in five representative cases. These analyses showed an intraobserver variability of 6.5%.

Protocol 2

Patients. To determine the changes in pancreatic volume following partial pancreatectomy, abdominal CT scans from 17 patients (9 male and 8 female) were analyzed. Case subjects were identified from the database of the Department of Radiology of the University Hospital, St. Josef-Hospital Bochum. To be included, case subjects were required to have had abdominal CT scans <100 days after partial pancreatectomy, as well as a second abdominal CT scan within the following 2 years. In five case subjects, CT scans determined before the partial pancreatectomy were also available. Patients were aged (means ± SD) 57.5 ± 14.6 years, weighted 65.1 ± 9.1 kg, and were 1.73 ± 0.07 m tall. The diagnoses leading to pancreatic surgery were chronic pancreatitis in six case subjects, pancreatic carcinoma in seven case subjects, benign pancreatic adenoma in two case subjects, and IPMT in two case subjects. Diabetes was present at the time of the first CT scan in 7 case subjects, whereas 10 case subjects were nondiabetic. Among the 17 patients, 13 had undergone pancreas head resections, and in 4 patients the pancreatic tail was removed. The mean (±SD) interval between pancreatic surgery and the first CT scan was 33 ± 26 days (range 6–83), and the interval between the first and the second postoperative CT scan was 247 ± 160 days (88–633). Case subjects were excluded if they had any signs of acute pancreatitis, pancreatic edema, or peritonitis or if precise delineation of pancreas from adjacent structures was not possible. In all cases, the abdominal CT was evaluated by an independent radiologist to confirm the absence of acute pancreatitis.

Determination of pancreatic volume. CT images were acquired with a standard clinical abdominal CT protocol utilizing a multidetector Somatom Sensation 16 CT scanner (Siemens Medical Solutions, Munich, Germany). Patients were administered oral contrast (barium sulfate). After infusion of intravenous contrast (2 ml/kg body wt Omnipaque), contiguous 2.5- or 5-mm axial images of the abdomen and pelvis were obtained. The images were transferred to a personal computer workstation and analyzed using SynGo Somaris 5 software (Siemens Medical Solutions). Pancreas was identified based on the typical landmarks (splenic vein, superior mesenteric artery) and carefully hand outlined along with parenchymal pancreas on each image. The total pancreas area (cm^2) on each image was computed as the actual area or the respective pixel area that is the product of the number of pixels in the outline, respectively. Pancreas volume per section was calculated as the product of each pancreas area and the CT section thickness. The total pancreatic volume was then computed by summing the volume from each section that included a piece of pancreas tissue.

Statistical analysis. Subject characteristics are reported as means ± SD, and results are presented as means ± SE. Statistical calculations were carried out by paired *t* test using GraphPad Prism 4 (San Diego, CA). A P value <0.05 was taken to indicate significant differences. Regression analyses were carried out using GraphPad Prism 4.

RESULTS

Protocol 1: histological changes in cell mass and turnover. Diabetes or impaired glucose tolerance was present in 6 of 13 patients (46%) before the initial 50% partial pancreatectomy. This percentage increased to 93%

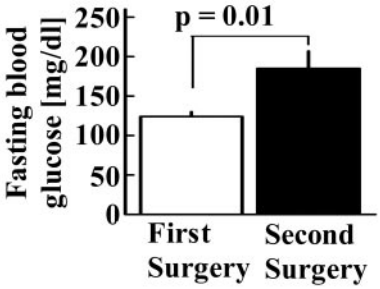

FIG. 1. Fasting glucose concentrations in 13 patients who underwent two consecutive pancreas resections, determined before the first and second operation, respectively. Data are reported as means ± SE. The *P* value was calculated using the paired *t* test.

(12 of 13 patients) at the time of the redo pancreas resection. Thus, fasting glucose concentrations increased from 124 ± 6 to 185 ± 22 mg/dl in between the two surgical interventions ($P = 0.01$) (Fig. 1).

The overall pancreas morphology was rather normal in the examined tumor-free tissue sections of the patients presenting with pancreatic tumors or metastases, whereas the pancreas of the patients with chronic pancreatitis exhibited characteristic changes, such as fibrosis and depletion of acinar tissue. There were no obvious differences in the overall pancreas morphology between the tissue samples collected from each patient on occasion of the initial 50% partial pancreatectomy and the subsequent redo operation, respectively.

Fractional β-cell area was 1.1 ± 0.3% in the pancreas specimens collected during the initial 50% partial pancreatectomy (Fig. 2). This percentage was unchanged in the specimens collected on occasion of the redo operation (0.7 ± 0.2%; $P = 0.11$) (Fig. 2). Likewise, the number of islets per pancreatic tissue area was not significantly increased by the partial pancreatectomy (3.3 ± 0.7 vs. 4.4 ± 1.1 islets/mm^2 pancreatic tissue, respectively; $P = 0.36$). There also were no differences in the mean β-cell diameter (12.4 ± 0.05 vs. 12.5 ± 0.06 μm, respectively; $P = 0.80$) or the mean β-cell nuclear diameter (7.5 ± 0.05 vs. 7.5 ± 0.05 μm, respectively; $P = 0.65$).

The overall expression of Ki67 in the pancreas was not different between the pancreas tissue removed during the 50% partial pancreatectomy and the subsequent redo operation (27.2 ± 7.2 vs. 17.3 ± 5.5 cells/field, respectively; $P = 0.30$) (Figs. 3 and 4). There also were no differences in the expression of Ki67 in β-cells (0.66 ± 0.11 vs. 0.48 ± 0.12%, respectively; $P = 0.31$) or duct cells (4.3 ± 0.9 vs. 3.3 ± 1.0%, respectively; $P = 0.16$) (Figs. 3 and 4). The frequency of replication was significantly higher in duct cells than in β-cells ($P < 0.0001$). The overall replication index, as well as the percentage of β-cells expressing Ki67 in the tissue specimens collected on occasion of the redo operation, were unrelated to the time interval between both operations (Fig. 5).

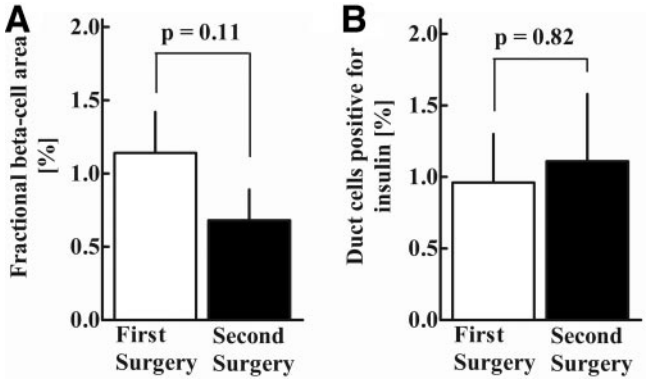

FIG. 2. Fractional area of the pancreas positive for insulin (*A*) and percentage of duct cells expressing insulin (*B*) in 13 patients who underwent two consecutive pancreas resections, determined in the tissue samples collected on occasion of the initial 50% partial pancreatectomy (first operation) and the respective redo pancreatectomy (second operation). Data are reported as means ± SE. *P* values were calculated by paired *t* tests.

Since prior studies had suggested an increased formation of new islets from exocrine duct cells, the expression of insulin in ducts was measured in all tissue specimens. There were no differences in the percentage of duct cells expressing insulin between the pancreas samples collected during the initial 50% partial pancreatectomy and the redo operation ($P = 0.82$) (Fig. 2).

The overall frequency of TUNEL staining was relatively low in all pancreas specimens examined, suggesting a generally low rate of cell death (Fig. 6). The number of TUNEL-positive cells per field was increased following the 50% partial pancreatectomy ($P = 0.02$) (Fig. 7). However, there was no detectable increase in the frequency of β-cell apoptosis following the intervention ($P = 0.09$) (Fig. 7).

Protocol 2: longitudinal assessment of pancreas volume. The mean preoperative pancreatic volume (available in five patients only) was 76.2 ± 2.8 ccm^3 (range 72–79). Following partial pancreatectomy (determined <100 days after the operation), pancreas volume was reduced to 35.6 ± 2.6 ccm^3 (20–52) ($P < 0.01$), consistent with an ~50% pancreatectomy. The pancreas volume was unchanged at the time of the second CT scan, performed after an interval of 247 ± 160 days following the initial assessment (35.4 ± 2.7 ccm^3 [20–54]; $P = 0.51$) (Fig. 8).

DISCUSSION

The present studies were designed to examine whether a 50% partial pancreatectomy would provoke a compensatory regeneration of β-cell mass in humans. Since any alteration of the pancreas for purely experimental purposes in humans is not feasible due to ethical reasons, we identified patients who had undergone two consecutive pancreatic resections in order to examine whether the initial partial pancreatectomy would prompt an increase in β-cell cell mass and turnover in the pancreas remainder. In addition, changes in total pancreatic mass following partial pancreatectomy were assessed longitudinally using CT-based digital pancreas volume measurements. We report that in humans *1*) β-cell mass and new β-cell formation are not increased after a 50% partial pancreatectomy and *2*) β-cell turnover is unchanged by a 50% partial pancreatectomy.

The present data are at variance with a number of previous studies in rodents uniformly reporting a high capacity for β-cell regeneration after partial pancreatectomy. Thus, an approximately three- to fourfold increase in β-cell mass has been reported after a 90% pancreatectomy in rats (10), and, even following less extensive surgical resections (40–50%), a modest augmentation of β-cell mass, accompanied by an induction of β-cell and ductal proliferation, has been noted in the pancreas remnants (11,17,18). These studies have led to the assumption that the endocrine pancreas harbors some capacity for adaptive expansion in response to various secretory de-

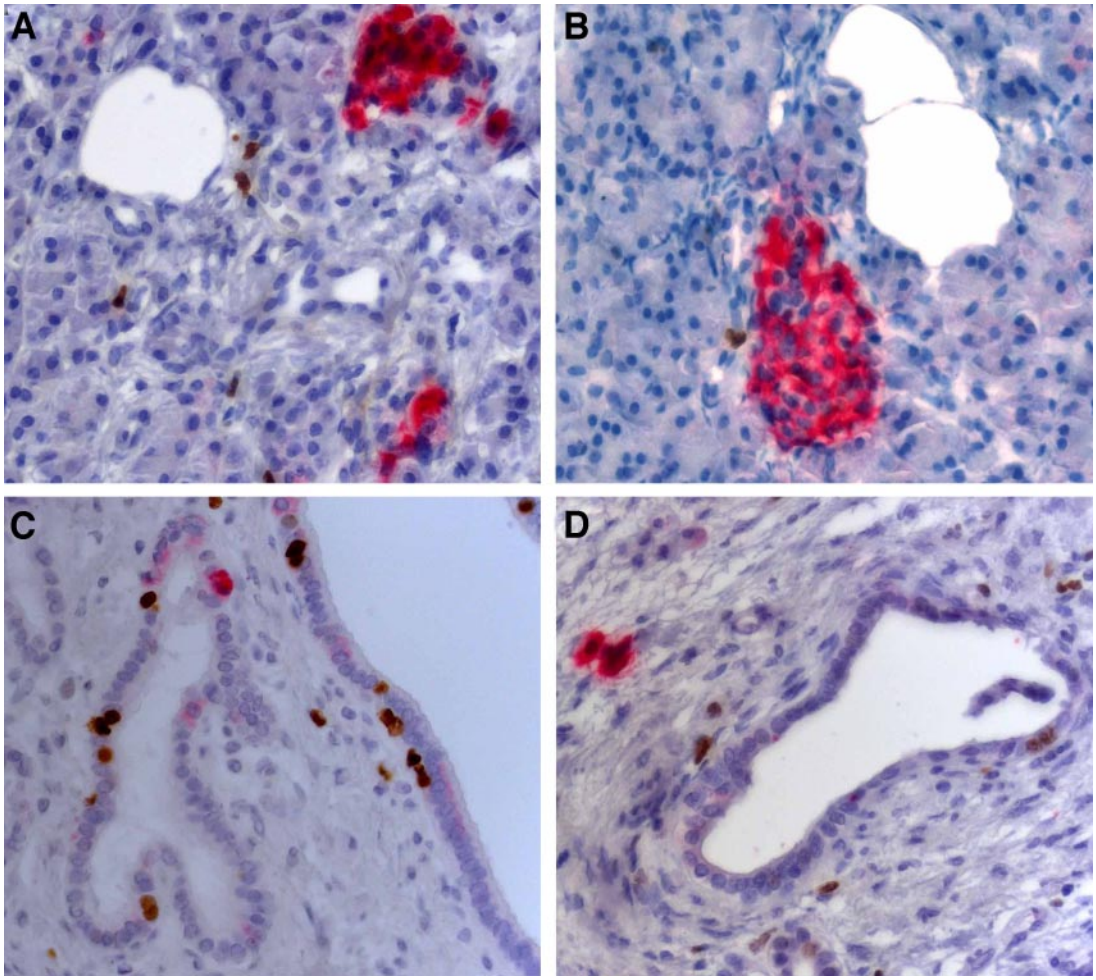

FIG. 3. Pancreatic sections from a patient (female, aged 73 years) who underwent two consecutive partial pancreas resections for the treatment of an IPMT at a 1-week interval (*A* and *B*) and from a patient (male, aged 54 years) who underwent two consecutive pancreas resections for the treatment of an pancreatic adeno-carcinoma at a 6-month interval. *A* and *C*: The respective parts of the pancreas removed on occasion of the initial 50% partial pancreatectomy. *B* and *D*: The pancreatic tissue removed on occasion of the respective redo pancreatectomy. Sections were stained for insulin (red), Ki67 (brown), and hematoxylin (purple) and imaged at ×40 objective magnification. (Please see http://dx.doi.org/10.2337/db07-1254 for a high-quality digital representation of this figure.)

mands (19). Restoration of glucose control and partial regeneration of β-cell mass after partial pancreatectomy have also been reported in some studies in larger animals. Notably, a transient development of glucosuria and polydipsia, followed by a partial recovery of glucose control (as judged by the absence of glucosuria) after subtotal

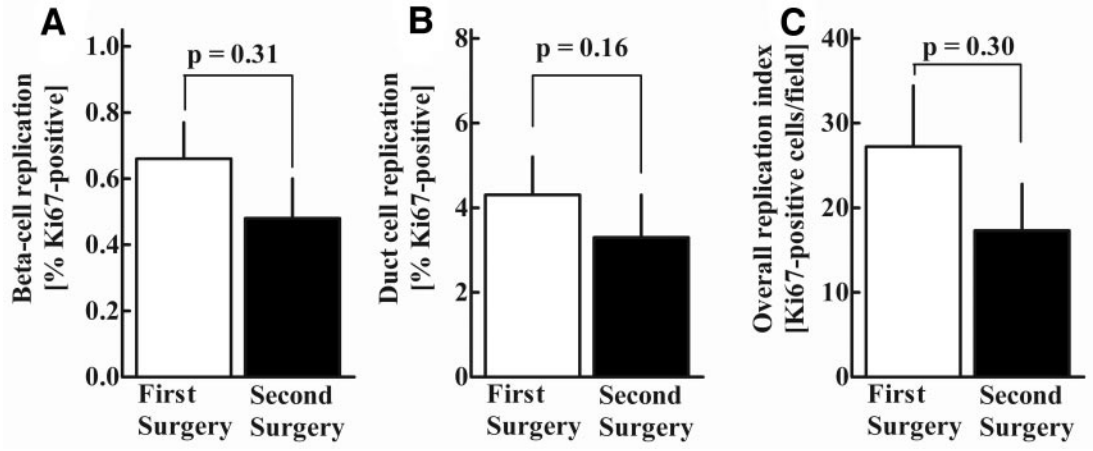

FIG. 4. Percentage of β-cells (*A*) or duct cells (*B*) expressing the replication marker Ki67 as well as overall replication index (number of Ki67-positive cells per field) (*C*) in 13 patients who underwent two consecutive pancreas resections, determined in the tissue samples collected on occasion of the initial 50% partial pancreatectomy (first operation) and the respective redo pancreatectomy (second operation), respectively. Data are reported as means ± SE. *P* values were calculated by paired *t* tests.

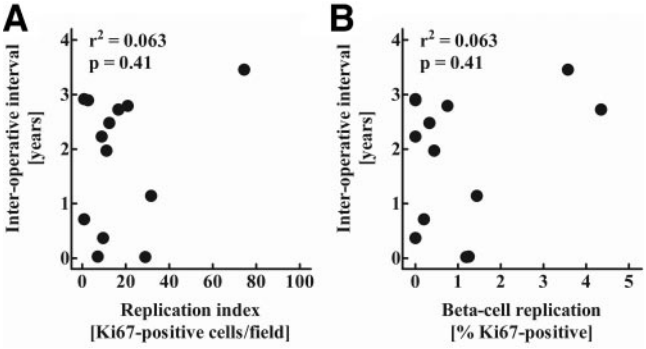

FIG. 5. Relationship between the overall replication index (number of Ki67-positive cells per field) (*A*) or the percentage of replicating β-cells (*B*), determined in tissue pancreatic tissue samples collected on occasion of a redo pancreas resection after a previous 50% partial pancreatectomy, and the respective interval between the 50% partial pancreatectomy and the redo pancreas resection in 13 patients with various pancreatic diseases. *P* values and r^2 values were determined by linear regression analysis.

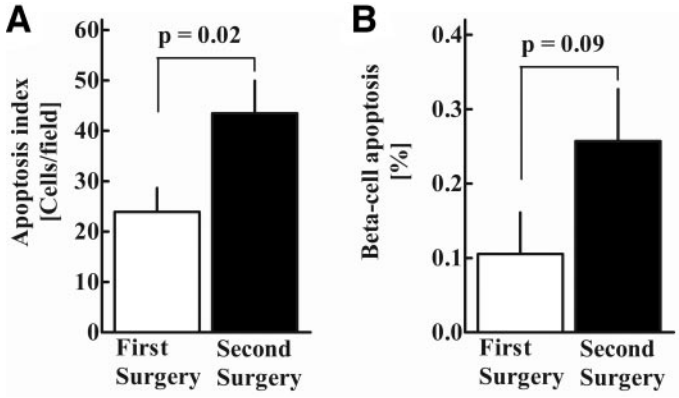

FIG. 7. Overall apoptosis index (number of TUNEL-positive cells per 10 fields) (*A*) and percentage of apoptotic β-cells in 11 patients who underwent two consecutive pancreas resections, determined in the tissue samples collected on occasion of the initial 50% partial pancreatectomy (first operation) and the respective redo pancreatectomy (second operation), respectively. Data are reported as means ± SE. *P* values were calculated by paired *t* tests.

pancreatectomy in dogs, has been described as early as 1688 by Johann Conrad Brunner (20). In contrast, more recent studies (21–24) focused on the functional consequences of reduced β-cell mass have found significant impairments in insulin secretion, hyperglucagonemia, and abnormal glucose control following 50–60% reductions of β-cell mass in pigs, dogs, and baboons. In a study specifically designed to examine the potential for β-cell regeneration, Löhr et al. (25) found a 19% gain in β-cell mass 6 weeks after a 60% pancreatectomy in 7-month-old pigs, while an 80% pancreatectomy elicited a 56% increase in β-cell mass. Interestingly, when adult dogs were subjected to a similar 80% pancreatectomy, no recovery of insulin

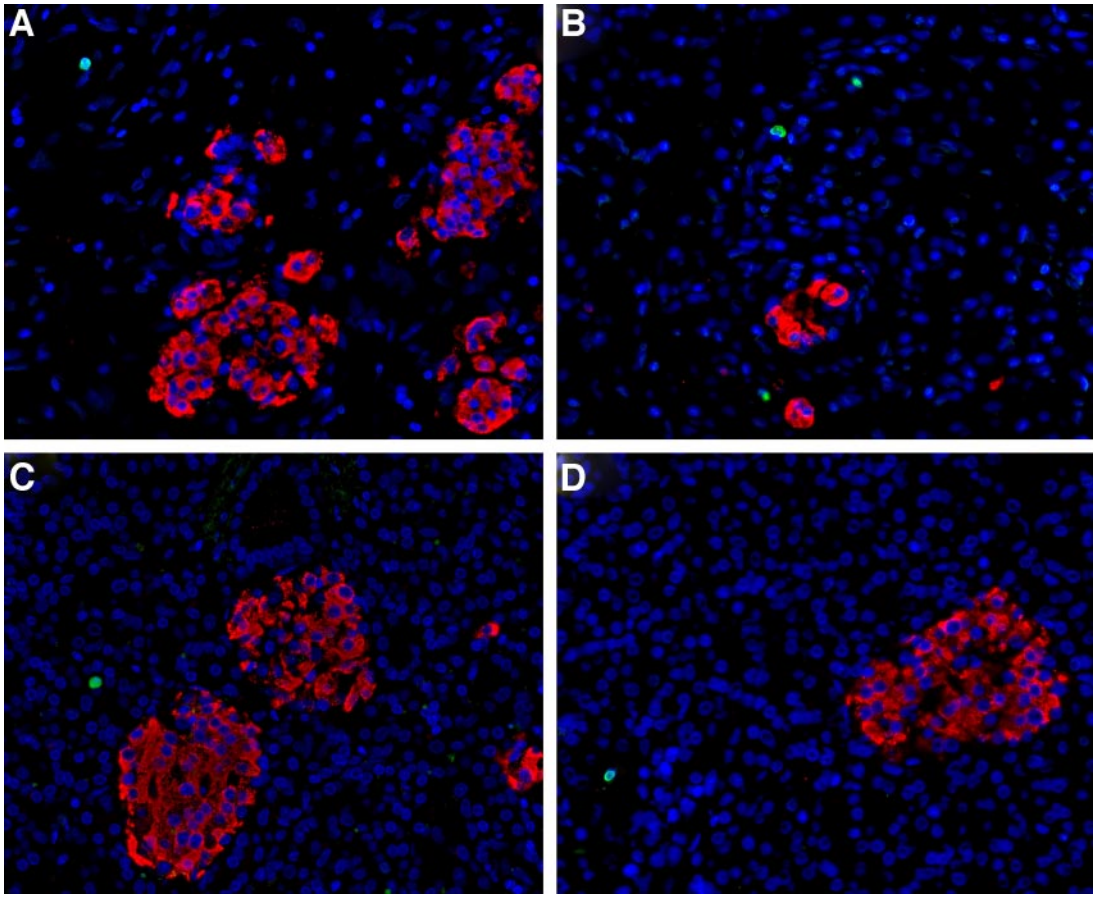

FIG. 6. Pancreatic sections from a patient (male, aged 54 years) who underwent two consecutive partial pancreas resection for the treatment of pancreatic carcinoma at a 6-month interval (*A* and *B*) and from a patient (male, aged 67 years) who underwent two consecutive partial pancreas resection for the treatment of chronic pancreatitis at an 8-day interval. *A* and *C*: The respective parts of the pancreas removed on occasion of the initial 50% partial pancreatectomy. *B* and *D*: The pancreatic tissue removed on occasion of the subsequent redo operation. Sections were stained for insulin (red), TUNEL (green), and DAPI (blue) and imaged at ×40 objective magnification. (Please see http://dx.doi.org/10.2337/db07-1254 for a high-quality digital representation of this figure.)

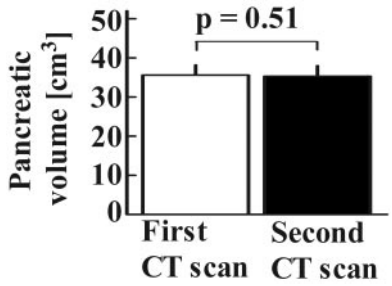

FIG. 8. Pancreatic volume determined from abdominal CT scans in 17 patients at two consecutive occasions after partial pancreatectomy at a mean interval of 247 ± 41 days. Data are reported as means ± SE. *P* values were calculated by paired *t* tests.

secretion or β-cell mass was found in the pancreas remnant (26). The most obvious explanation for the discrepancies between these studies is that the capacity for new β-cell formation declines with aging. Consistent with this, the frequency of β-cell proliferation in young children has been shown to far exceed that in older humans (27–29). Furthermore, in children with congenital hyperinsulinism of infancy, a subtotal pancreatectomy is often tolerated without the immediate development of overt hypergycemia, and the subsequent risk for developing diabetes later in life has been shown to be lowest in the children undergoing surgery early in life (30,31).

However, it is difficult to translate these results to the situation in healthy humans, since the overall frequency of β-cell replication is significantly increased in children with hyperinsulinism of infancy (27). Nevertheless, the available data suggest that the capacity for β-cell replication in humans is highest immediately after birth and significantly declines thereafter. It is therefore possible that a similar 50% pancreatectomy as performed in this group of ~52-year-old patients might have been compensated for in a more pronounced fashion during childhood or adolescence.

Does the lack of β-cell regeneration after an ~50% pancreatectomy in these patients imply that promoting new β-cell formation in adult humans is not possible at all? A number of points argue against such reasoning. First, β-cell mass in humans has been shown to increase with obesity, as well as during pregnancy, even though to a considerably smaller extent than in rodents (4,32,33). Second, in isolated human islets studied under in vitro conditions, β-cell replication can be enhanced by a variety of growth factors, such as gastrin, growth hormone, IGF-1, and others (34,35). Third, despite the ongoing autoimmune islet cell destruction, small numbers of β-cells can still be found even in the pancreas of patients with long-standing type 1 diabetes, thereby suggesting a concomitant provision of new β-cells (15). Fourth, β-cell replication can be detected occasionally in adult human pancreas, and the frequency of β-cell replication was found to be increased in patients with hypergastrinemia, as well as around the onset of type 1 diabetes (6,36). Taken together, these findings provide strong evidence that while the overall plasticity of β-cell mass is much smaller than in rodents, there is still some capacity for β-cell regeneration in adult humans. In the present studies, a mean frequency of β-cell replication of ~0.5% was found. This percentage appears to slightly exceed the numbers reported in some previous studies in adult human pancreas obtained at autopsy (29). One potential explanation for this relatively high frequency of β-cell proliferation might be low-grade inflammation in the pancreas of these patients (37). However, differences in β-cell turnover between pancreas specimens obtained at autopsy or at surgery cannot be excluded.

Another question arising from these studies is whether the development of diabetes following partial pancreatectomy in humans might be prevented by an active induction of β-cell regeneration using, for example, gastrin, growth factors, or glucagon-like peptide 1 analogs. Indeed, all of these compounds have been shown to promote β-cell regeneration in rodent models of diabetes (38,39). Whether a similar therapeutic induction of β-cell regeneration can also be achieved in humans cannot be answered yet.

While most studies agree that β-cells can be regenerated to some extent even in adult individuals, the underlying sources are highly disputed. In particular, replication of existing β-cells and the formation of new islets from putative ductal precursors have been suggested based on rodent studies (8,40–42). In the present study, β-cell replication was found at relatively low rates both before and after partial pancreatectomy. Replication was also abundantly detected in duct cells, and the expression of insulin was noted in ~1% of duct cells. However, whether these insulin-positive duct cells have indeed arisen from the transdifferentiation of epithelial cells into endocrine cells or whether their occurrence inside the ductal tree was purely incidental cannot be answered from these studies.

In light of the lack of increased β-cell proliferation after a 50% partial pancreatectomy in this study, one question arising is, through what other mechanisms can new β-cell formation be enhanced in humans? One obvious candidate would be hyperglycemia leading to an increased secretory demand on the β-cells. However, despite the significant increase in glucose concentrations following the 50% partial pancreatectomy, β-cell replication was completely unchanged in this study, and the frequency of β-cell proliferation was unrelated to the ambient glucose concentrations (details not shown). Furthermore, the fact that β-cell mass is increased in nondiabetic humans with obesity or during pregnancy would not support this hypothesis (4,33). Alternatively, a number of endocrine hormones might be involved in the control of β-cell mass and turnover. Among those, the gut hormones gastrin, glucagons-like protein-1, as well as the lactogenic hormones placental lactogen and prolactin, have been suggested to enhance β-cell replication (43–45). In addition, inflammatory cytokines such as interleukin 1-β, interferon-inducible protein 10, and others might play a role (46,47).

The present findings are in line with prior functional studies on insulin secretion in healthy, nondiabetic humans who donated ~50% of their pancreas for the treatment of relatives with type 1 diabetes. Follow-up examinations of this cohort revealed abnormalities in both insulin secretion and glucose homeostasis in a substantial percentage of patients (14). Interestingly, a similar 50% reduction of β-cell mass has been associated with the presence of impaired glucose tolerance based on autopsy studies (3).

One potential limitation of the present study might be seen in the fact that all patients examined had some underlying pancreas pathology. Arguably, β-cell regeneration might be more prominent in healthy pancreatic tissue. Indeed, the overall frequency of apoptosis in the pancreas specimens was increased on occasion of the redo operation, consistent with the progressive nature of pancreatic

diseases. Against this, the results were similar throughout a broad variety of disease entities, including chronic pancreatitis, pancreatic cancer or metastasis, or benign pancreatic lesions (IPMT). Another open question is whether a more extensive pancreatectomy would have provoked a more extensive regeneration of β-cell mass. However, the significant increase in blood glucose levels after the hemi-pancreatectomy in this study, as well as in previous studies, shows that the actual 50% reduction of β-cell mass was sufficient and functionally relevant, even though in the absence of a control group deteriorations of glucose control due to the natural progression of the disease cannot be excluded with certainty. Finally, minor increases in β-cell mass and turnover might have been overlooked because of the limited number of patients (n = 13) and the heterogeneity of the underlying pancreas pathology. To minimize the variability implied by these points, all statistical comparisons were performed intra-individually in a paired testing procedure.

Since all CT scans in protocol 2 were performed for purely clinical reasons, the mean interval between the partial pancreatectomy and the CT assessment of pancreas volume in this study was already 33 days. Therefore, early postoperative changes in pancreatic mass cannot be excluded with certainty, although even in the cases with the shortest interval between surgery and CT examination (6 and 13 days), there was no indication of pancreatic regeneration during the subsequent follow-up period. Along the same lines, it is possible that in protocol 1 a transient increase in β-cell replication immediately after partial pancreatectomy was missed in the pancreatic specimens obtained after a follow-up period of 1.8 ± 1.2 years. However, in that case, fractional β-cell area would still be expected to be increased in the pancreas specimens obtained on occasion of the redo operation. Furthermore, none of the patients undergoing redo surgery after an interval of 8 days to 3.5 years exhibited any signs of increased β-cell proliferation.

In conclusion, in humans an ~50% partial pancreatectomy does not prompt increased β-cell mass or regeneration. Therefore, the potential induction of long-term deteriorations of glucose control should be born in mind when subjecting patients to partial pancreatectomy. Furthermore, differences in β-cell turnover between rodents and humans should be considered when evaluating new treatment options aiming to restore β-cell mass in patients with diabetes.

ACKNOWLEDGMENTS

These studies were supported by grants from the Ruhr-University Bochum (FoRUM to J.J.M.) and the Deutsche Forschungsgemeinschaft (Me 2096/5-1 to J.J.M.).

The excellent technical assistance of Kirsten Mros, Birgit Baller, Sabine Richter, and Gudrun Müller is gratefully acknowledged.

REFERENCES

1. Stumvoll M, Goldstein BJ, van Haeften TW: Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365:1333–1346, 2005
2. Meier JJ, Butler PC: Insulin secretion. In *Endocrinology.* 5th ed. DeGroot LJ, Jameson JL, Eds. Philadelphia, Elsevier Saunders, 2005, p. 961–973
3. Ritzel RA, Butler AE, Rizza RA, Veldhuis JD, Butler PC: Relationship between β-cell mass and fasting blood glucose concentration in humans. *Diabetes Care* 29:717–718, 2006
4. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC: β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–110, 2003
5. Kloppel G, Drenck CR, Oberholzer M, Heitz PU: Morphometric evidence for a striking B-cell reduction at the clinical onset of type 1 diabetes. *Virchows Arch A Pathol Anat Histopathol* 403:441–452, 1984
6. Meier JJ, Lin JC, Butler AE, Galasso R, Martinez DS, Butler PC: Direct evidence of attempted beta-cell regeneration in an 89-year old patient with recent onset type 1 diabetes. *Diabetologia* 49:1838–1844, 2006
7. Halban PA: Cellular sources of new pancreatic beta cells and therapeutic implications for regenerative medicine. *Nat Cell Biol* 6:1021–1025, 2004
8. Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE: A second pathway for regeneration of adult exocrine and endocrine pancreas: a possible recapitulation of embryonic development. *Diabetes* 42:1715–1720, 1993
9. Bonner-Weir S: β-Cell turnover: its assessment and implications. *Diabetes* 50 (Suppl. 1):S20–S24, 2001
10. Plachot C, Movassat J, Portha B: Impaired beta-cell regeneration after partial pancreatectomy in the adult Goto-Kakizaki rat, a spontaneous model of type II diabetes. *Histochem Cell Biol* 116:131–139, 2001
11. Lee HC, Bonner-Weir S, Weir GC, Leahy JL: Compensatory adaption to partial pancreatectomy in the rat. *Endocrinology* 124:1571–1575, 1989
12. Peshavaria M, Larmie BL, Lausier J, Satish B, Habibovic A, Roskens V, Larock K, Everill B, Leahy JL, Jetton TL: Regulation of pancreatic β-cell regeneration in the normoglycemic 60% partial-pancreatectomy mouse. *Diabetes* 55:3289–3298, 2006
13. Pearson KW, Scott D, Torrance B: Effects of partial surgical pancreatectomy in rats. I. Pancreatic regeneration. *Gastroenterology* 72:469–473, 1977
14. Kendall DM, Sutherland DE, Najarian JS, Goetz FC, Robertson RP: Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. *N Engl J Med* 322:898–903, 1990
15. Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC: Sustained beta-cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 48:2221–2228, 2005
16. Meier JJ, Butler AE, Galasso R, Butler PC: Hyperinsulinemic hypoglycemia after gastric bypass surgery is not accompanied by islet hyperplasia or increased β-cell turnover. *Diabetes Care* 29:1554–1559, 2006
17. Jetton TL, Liu YQ, Trotman WE, Nevin PW, Sun XJ, Leahy JL: Enhanced expression of insulin receptor substrate-2 and activation of protein kinase B/Akt in regenerating pancreatic duct epithelium of 60%-partial pancreatectomy rats. *Diabetologia* 44:2056–2065, 2001
18. Jetton TL, Lausier J, LaRock K, Trotman WE, Larmie B, Habibovic A, Peshavaria M, Leahy JL: Mechanisms of compensatory β-cell growth in insulin-resistant rats: roles of Akt kinase. *Diabetes* 54:2294–2304, 2005
19. Bonner-Weir S, Weir GC: New sources of pancreatic beta-cells. *Nat Biotechnol* 23:857–861, 2005
20. Brunner JC: De experimentis circa pancreas novis confirmatis. *Misc Cur Ephemerid Nat Cur* 1688, 132:243–248
21. Matveyenko AV, Veldhuis JD, Butler PC: Mechanisms of impaired fasting glucose and glucose intolerance induced by an approximate 50% pancreatectomy. *Diabetes* 55:2347–2356, 2006
22. Kjems LL, Kirby BM, Welsh EM, Veldhuis JD, Straume M, McIntyre SS, Yang D, Lefèbvre P, Butler PC: Decrease in β-cell mass leads to impaired pulsatile insulin secretion, reduced postprandial hepatic insulin clearance, and relative hyperglucagonemia in the minipig. *Diabetes* 50:2001–2012, 2001
23. Meier JJ, Kjems LL, Veldhuis JD, Lefèbvre P, Butler PC: Postprandial suppression of glucagon secretion depends on intact pulsatile insulin secretion: further evidence for the intraislet insulin hypothesis. *Diabetes* 55:1051–1056, 2006
24. Larsen MO, Juhl CB, Pørksen N, Gotfredsen CF, Carr RD, Ribel U, Wilken M, Rolin B: Beta-cell function and islet morphology in normal, obese, and obese beta-cell mass-reduced Gottingen minipigs. *Am J Physiol Endocrinol Metab* 288:E412–E421, 2005
25. Lohr M, Lubbersmeyer J, Otremba B, Klapdor R, Grossner D, Kloppel G: Increase in B-cells in the pancreatic remnant after partial pancreatectomy in pigs: an immunocytochemical and functional study. *Virchows Arch B Cell Pathol Incl Mol Pathol* 56:277–286, 1989
26. Marincola F, Cobb LF, Horaguchi A, Maeda M, Merrell R: Accommodation to a reduced islet cell mass in dogs. *Am J Physiol* 247:E456–E461, 1984
27. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B: β-Cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 49:1325–1333, 2000
28. Maedler K, Schumann DM, Schulthess F, Oberholzer J, Bosco D, Berney T, Donath MY: Aging correlates with decreased β-cell proliferative capacity and enhanced sensitivity to apoptosis: a potential role for Fas and pancreatic duodenal homeobox-1. *Diabetes* 55:2455–2462, 2006
29. Meier JJ, Butler AE, Monchamp T, Galasso R, Rizza RA, Butler PC: β-Cell

replication is the primary mechanism for postnatal expansion of β-cell mass in humans (Abstract). *Diabetes* 56 (Suppl. 1):A47, 2007
30. de Lonlay-Debeney P, Poggi-Travert F, Fournet JC, Sempoux C, Vici CD, Brunelle F, Touati G, Rahier J, Junien C, Nihoul-Fékété C, Robert JJ, Saudubray JM: Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med* 340:1169–1175, 1999
31. de Lonlay-Debeney P, Poggi-Travert F, Fournet JC, Sempoux C, Vici CD, Brunelle F, Touati G, Rahier J, Junien C, Nihoul-Fékété C, Robert JJ, Saudubray JM: The outcome in Australian children with hyperinsulinism of infancy: early extensive surgery in severe cases lowers risk of diabetes. *Clin Endocrinol (Oxf)* 58:355–364, 2003
32. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S: Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab* 88:2300–2308, 2003
33. Van Assche FA, Aerts L, De Prins F: A morphological study of the endocrine pancreas in human pregnancy. *Br J Obstet Gynaecol* 85:818–820, 1978
34. Rhodes CJ: IGF-I and GH post-receptor signaling mechanisms for pancreatic beta-cell replication. *J Mol Endocrinol* 24:303–311, 2000
35. Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S. Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun* 341:291–298, 2006
36. Meier JJ, Butler AE, Galasso R, Rizza RA, Butler PC: Increased islet beta cell replication adjacent to intrapancreatic gastrinomas in humans. *Diabetologia* 49:2689–2696, 2006
37. Sherry NA, Kushner JA, Glandt M, Kitamura T, Brillantes AM, Herold KC: Effects of autoimmunity and immune therapy on β-cell turnover in type 1 diabetes. *Diabetes* 55:3238–3245, 2006
38. Suarez-Pinzon WL, Yan Y, Power R, Brand SJ, Rabinovitch A: Combination therapy with epidermal growth factor and gastrin increases β-cell mass and reverses hyperglycemia in diabetic NOD mice. *Diabetes* 54:2596–2601, 2005
39. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B: Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the β-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes* 51:1443–1452, 2002
40. Dor Y, Brown J, Martinez OI, Melton DA: Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429:41–46, 2004
41. Georgia S, Bhushan A: Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass. *J Clin Invest* 114:963–968, 2004
42. Bouwens L, Pipeleers DG: Extra-insular beta cells associated with ductules are frequent in adult human pancreas. *Diabetologia* 41:629–633, 1998
43. Nielsen JH, Svensson C, Galsgaard ED, Moldrup A, Billestrup N: Beta cell proliferation and growth factors. *J Mol Med* 77:62–66, 1999
44. Meier JJ, Nauck MA: Glucagon-like peptide 1(GLP-1) in biology and pathology. *Diabetes Metab Res Rev* 21:91–117, 2005
45. Drucker DJ: Glucagon-like peptide-1 and the islet beta-cell: augmentation of cell proliferation and inhibition of apoptosis. *Endocrinology* 144:5145–5148, 2003
46. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, Kaiser N, Halban PA, Donath MY: Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110:851–860, 2002
47. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY: Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356:1517–1526, 2007