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# Original Article

## Aging Correlates With Decreased β-Cell Proliferative Capacity and Enhanced Sensitivity to Apoptosis

## A Potential Role for Fas and Pancreatic Duodenal Homeobox-1

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Type 2 diabetes is characterized by a deficit in  $\beta$ -cell mass, and its incidence increases with age. Here, we analyzed  $\beta$ -cell turnover in islets from 2- to 3- compared with 7- to 8-month-old rats and in human islets from 53 organ donors with ages ranging from 17 to 74 years. In cultured islets from 2- to 3-month-old rats, the age at which rats are usually investigated, increasing glucose from 5.5 to 11.1 mmol/l decreased  $\beta$ -cell apoptosis, which was augmented when glucose was further increased to 33.3 mmol/l. In parallel,  $\beta$ -cell proliferation was increased by both 11.1 and 33.3 mmol/l glucose compared with 5.5 mmol/l. In contrast, in islets from 7- to 8-month-old rats and from adult humans. increasing glucose concentrations from 5.5 to 33.3 mmol/l induced a linear increase in  $\beta$ -cell death and a decrease in proliferation. Additionally, in cultivated human islets, age correlated positively with the sensitivity to glucose-induced  $\beta$ -cell apoptosis and negatively to baseline proliferation. In rat islets, constitutive expression of Fas ligand and glucose-induced Fas receptor expression were observed only in 7- to 8-month-old but not in 2- to 3-month-old islets, whereas no age-dependent changes in the Fas/Fas ligand system could be detected in human islets. However, pancreatic duodenal homeobox (PDX)-1 expression decreased with age in pancreatic tissue sections of rats and humans. Furthermore, older rat islets were more sensitive to the high-glucose-mediated decrease in PDX-1 expression than younger islets. Therefore, differences in glucose sensitivity between human and 2- to 3-month-old rat islets may be due to both differences in age and in the genetic background. These data provide a possible explanation for the increased incidence of type 2 diabetes at an older age and support the use of islets from older rats as a more appropriate model to study glucose-induced  $\beta$ -cell apoptosis. Diabetes 55:2455-2462, 2006

he prevalence of type 2 diabetes increases with age due to alteration or insufficient compensation of  $\beta$ -cell functional mass in the face of increasing insulin resistance (1–4). While the existence of an inadequate adaptation of  $\beta$ -cell mass in patients with type 2 diabetes is beyond controversy (5–9), the effect of age on the sensitivity to proapoptotic stimuli of the human  $\beta$ -cell remains to be investigated.

Changes in glucose concentrations are key regulators of β-cell proliferation and apoptosis. Indeed, graded increases in glucose concentrations induce  $\beta$ -cell apoptosis in cultured islets from the diabetes-prone Psammomys obesus and from human islets (10-13). In contrast, in islets from 2- to 3-month-old rats, the age at which rats are usually investigated, an increase in glucose concentrations to 11.1 mmol/l promotes  $\beta$ -cell survival (10,14). When glucose concentrations are further increased, glucose proves to be pro- or antiapoptotic depending on culture conditions (10,14,15). Investigations of  $\beta$ -cell proliferation revealed induction of proliferation by glucose in rat, P. obesus, and human islets (10,11,16). Nevertheless, unlike the long-lasting effect in rat islets, only a transient and reduced proliferative response was observed in P. obesus and human islets. Previous studies have shown that  $\beta$ -cell replication declines as rodents age and stabilizes at a rate of 1-3% per day (17-19) or even lower according to recent data (20). However,  $\beta$ -cell turnover in aging humans remains unclear.

In human islets, the mechanism underlying glucoseinduced  $\beta$ -cell apoptosis and impaired proliferation involves the upregulation of the Fas receptor, which interacts with the constitutively expressed Fas ligand on neighboring  $\beta$ -cells (11,21). In an interesting study, Hanke (22) detected expression of Fas ligand in  $\beta$ -cells of rats aged  $\geq 6$  months, while Fas ligand was not expressed in younger animals. It is not known whether glucose-induced Fas is age dependent.

Pancreatic duodenal homeobox (PDX)-1 is a  $\beta$ -cell–specific transcription factor regulating  $\beta$ -cell differentiation and secretory function (23). Furthermore, PDX-1 promotes  $\beta$ -cell replication and is cytoprotective (24–26). Interestingly, in human pancreatic islets, PDX-1 may mediate deleterious effects of high glucose concentrations (27).

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KRB, Krebs-Ringer bicarbonate buffer; PDX, pancreatic duodenal homeobox; TUNEL, transferase-mediated dUTP nick-end labeling.

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FIG. 1. Response to glucose-induced changes in  $\beta$ -cell apoptosis and proliferation are age dependent. Human (A and B; mean age 48.1 years [range 17–67]) and 2- to 3-month-old rat (C, D, and G) and 7- to 8-month-old rat (E, F, and G) pancreatic islets were cultured on extracellular matrix-coated dishes, exposed for 4 days to media containing 5.5, 11.1, or 33.3 mmol/l glucose, and double immunostained for insulin and DNA fragmentation by TUNEL assay (A, C, E, and G) or for proliferation by the Ki-67 antibody (B, D, F, and G). Results are means  $\pm$  SE of the relative percentage of TUNEL-positive or Ki-67-positive  $\beta$ -cells versus control incubations at 5.5 mmol/l glucose alone

Therefore, we investigated the changes in  $\beta$ -cell turnover in relation to age. We show that age correlates with decreased proliferative activity and enhanced sensitivity to glucose-induced apoptosis. In parallel, constitutive expression of Fas ligand and inducible Fas expression appeared along with decreased expression of PDX-1 under normal and glucotoxic conditions.

#### **RESEARCH DESIGN AND METHODS**

Islet isolation and culture. Human islets were isolated from pancreata of 53 organ donors. Islet purity was greater than 95%, as judged by dithizone staining (if this degree of purity was not achieved by routine isolation, islets were handpicked). The donors, aged 17-74 years, were heart-beating cadaver organ donors, and none had a previous history of diabetes or metabolic disorders. Male Sprague Dawley rats, aged 2-3 months (200-220 g) and 7-8 months (500-600 g), were anesthetized and islets isolated from the pancreata as previously described (28). The islets were cultured on extracellular matrix-coated plates derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel), allowing the cells to attach to the dishes and spread. This mono- or bilayer of cells have direct access to nutrients and oxygen, which allows long-term preservation of their functional integrity (31). Human islets were cultured in CMRL 1066 medium containing 5.5 mmol/l glucose, and rat islets were cultured in RPMI 1640 medium containing 11.1 mmol/l glucose, both supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (Invitrogen, Carlsbad, CA), hereafter referred to as culture medium. Two days after plating, when most islets were attached and had begun to flatten, the medium was changed to culture medium containing 5.5, 11.1, or 33.3 mmol/l glucose for 4 days.

Detection of Fas-, Fas ligand-, and PDX-1-expressing β-cells. Pancreata from routine necropsies were immersion fixed in formalin followed by paraffin embedding. Sections were deparaffinized and rehydrated and endogenous peroxidase blocked by submersion in 0.3% H<sub>2</sub>O<sub>2</sub> for 15 min; after that, sections were incubated in methanol for 5 min and double stained with rabbit anti-PDX-1 antibody (provided by Christopher Wright, Vanderbilt University Medical Center, Nashville, TN) and insulin. Islet cultures were fixed in 4% paraformaldehyde (30 min at room temperature) followed by permeabilization with 0.5% triton X-100 (4 min at room temperature) and were double labeled for Fas receptor, Fas ligand, or PDX-1 by 1-h exposure to 10% BSA, followed by incubation with rabbit anti-Fas (FL-335; Santa Cruz Biotech, Santa Cruz, CA), mouse anti-Fas ligand (Transduction Laboratories, Lexington, KY), and detection using the streptavidin-biotin-peroxidase complex (Zymed, San Francisco, CA) or donkey anti-mouse Cy3 conjugated antibody (Jackson Immunoresearch Laboratories, West Grove, PA). Subsequently, the specimens were stained for insulin as previously described (10,11). The specificity of the Fas and Fas ligand antibodies was assessed previously (11). Saturation of staining was analyzed using analySIS 3.1 software (Soft Imaging System, Muenster, Germany).

**β-Cell replication and apoptosis.** For β-cell replication and apoptosis studies, islet cells were double stained with either a monoclonal antibody against the human or rat Ki-67 antigen (Zymed) or by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) according to the manufacturer's instructions (In Situ Cell Death Detection Kit; Boehringer, Mannheim, Germany) and for insulin as described above.

Western blot analysis. Islets were cultured in suspension in RPMI 1640 (rat) or CMRL 1066 (human) medium as described above. Equivalent amounts of protein from each treatment group were run on 15% SDS polyacrylamide gels as described previously (29). Proteins were electrically transferred to nitrocellulose filters and incubated with rabbit anti-Fas antibody (FL-335; Santa Cruz Biotech), mouse anti–Fas ligand (Transduction Laboratories), or mouse anti–PDX-1 (provided by Stefan Zahn, Novo Nordisk, Bagsværd, Denmark) or rabbit anti-actin (Cell Signaling Technology, Danvers, MA) antibodies followed by incubation with horseradish peroxidase–linked anti-rabbit or antimouse IgG (Santa Cruz Biotechnology). Immune complexes were detected by chemiluminescence using LumiGLO (Cell Signaling Technology).

**RNA extraction and quantitative RT-PCR.** Total RNA was extracted from the cultured islets by using the RNeasy mini kit (Qiagen, Basel, Switzerland),

(100%). In absolute values: 0.43% TUNEL-positive  $\beta$ -cells (A); 0.53% Ki67-positive  $\beta$ -cells (B); 0.82% TUNEL-positive  $\beta$ -cells (C); 2.06% Ki67-positive  $\beta$ -cells (D); 1.1% TUNEL-positive  $\beta$ -cells (C); 2.06% Ki67-positive  $\beta$ -cells (D); 1.1% TUNEL-positive  $\beta$ -cells (C); and 0.83% Ki67-positive  $\beta$ -cells (F) at 5.5 mmol/ glucose alone. The mean number of islets scored was A: 81, B: 74, C: 65, D: 55, E: 71, and F: 76 for each treatment condition from each donor. Islets were isolated from seven human organ donors and from 32 rats aged 2–3 and 7–8 months. \*P < 0.05 vs. islets at 5.5 mmol/l glucose; \*\*P < 0.05 vs. islets at 11.1 mmol/l glucose.



FIG. 2. Aging correlates with enhanced sensitivity to glucose-induced β-cell apoptosis and decreased baseline proliferation. Human pancreatic islets were isolated from 53 organ donors of ages ranging from 17 to 74 years and cultured on extracellular matrix-coated dishes, exposed 4 days to media containing 5.5 or 33.3 mmol/l glucose, and double immunostained for insulin and TUNEL assay (A, B, and E) or the Ki-67 antibody (C, D, and F). Correlation between age and means of the percentage of TUNEL-positive or Ki-67–positive  $\beta$ -cells at baseline 5.5 mmol/l glucose (A and C) or at 33.3 mmol/l glucose versus control incubations at 5.5 mmol/l glucose alone. Each point represents an independent experiment of one organ donor. Solid lines are best fit, with broken lines showing 95% CIs. Analysis of the data according to groups of similar ages for basal (1; 5.5 mmol/l glucose), glucosestimulated ( $\equiv$ ; 33.3 mmol/l), and the ratio of basal to stimulated ( $\Box$ )  $\beta$ -cell apoptosis (E) and proliferation (F). \*P < 0.05 vs. islets from the youngest quartile (aged 17-25 years).

and RT-PCR was performed using the SuperScript Double-Stranded cDNA synthesis kit according to the manufacturer's instructions (Life Technologies, Gaithersburg, MD). For quantitative analysis, we used the LightCycler quantitative PCR system (Roche, Basel, Switzerland) with a commercial kit (Light Cycler-DNA Master SYBR Green I; Roche). The primers were 5'CCACCTTG

GACCTGTTTAG3' and 5'TGATGCCAGAGGAAGAGGAG3' (human PDX-1), 5'GAGGACCCGTACAGCCTACA3' and 5'CGTTGTCCCGCTACTACGTT3' (rat PDX-1), 5'TTCTTCTACACACCCA3' and 5'CTAGTTGCAGTAGTTCT3' (insulin), 5'AGAGTCGCGCTGTAAGAAGC3' and 5'TGGTCTTGTCACTTGGCATC3' ( $\alpha$ -Tubulin), and 5'AACAGCGACACCCACTCCTC3' and 5'GGAGGGGAGATTC AGTGTGGT3' (glyceraldehyde-3-phosphate dehydrogenase).

**Glucose-stimulated insulin secretion.** For acute insulin release in response to glucose, islets were washed and preincubated (30 min) in Krebs-Ringer bicarbonate buffer (KRB) containing 2.8 mmol/l glucose and 0.5% BSA. KRB was then replaced by KRB 2.8 mmol/l glucose for 1 h (basal), followed by an additional 1 h in KRB 16.7 mmol/l glucose. Islets were extracted with 0.18 NHCl in 70% ethanol for determination of insulin content. Insulin was determined using a human insulin radioimmunoassay kit (CIS Bio International, Gif-Sur-Yvette, France), which has similar affinity for both rat and human insulin.

**Evaluation and statistical analysis.** Samples were evaluated in a randomized manner by a single investigator (K.M.) blind to the treatment conditions. Data were analyzed by Student's t test, by ANOVA with a Bonferroni correction for multiple-group comparisons, or by regression analysis using GraphPad Prism version 3.0 (San Diego, CA).

#### RESULTS

Distinct effects of glucose-induced changes in  $\beta$ -cell apoptosis and proliferation are not species but age dependent. Analysis of human islets cultured for 4 days at 11.1 and 33.3 mmol/l glucose revealed a 2.0- and 2.8-fold increase, respectively, in  $\beta$ -cell nuclei with DNA fragmentation (TUNEL positive, Fig. 1A) versus islets at 5.5 mmol/l glucose. In parallel,  $\beta$ -cell proliferation was reduced 1.5and 2.7-fold by 11.1 and 33.3 mmol/l glucose, respectively (Fig. 1B). In contrast, exposure of 2- to 3-month-old rat islets to increasing glucose concentrations resulted in an inverse bell-shaped response. The lowest incidence of apoptotic β-cell nuclei occurred at a glucose concentration of 11.1 mmol/l and increased 3.6- and 3.0-fold at 5.5 and 33.3 mmol/l glucose, respectively (Fig. 1C and G). Proliferation of 2- to 3-month-old rat  $\beta$ -cells increased 1.9and 1.8-fold at 11.1 and 33.3 mmol/l glucose, respectively, compared with islets at 5.5 mmol/l glucose (Fig. 1D and G). However, islets from 7- to 8-month-old rats behaved similarly to human islets, with a progressive induction of  $\beta$ -cell apoptosis of 1.3- and 2.9-fold by 11.1 and 33.3 mmol/l glucose, respectively, and a 1.4-fold decrease of  $\beta$ -cell proliferation at 33.3 mmol/l glucose (Fig. 1E-G). Note that the images in Fig. 1G are from whole islets cultured on extracellular matrix-coated dishes and not from sections, explaining the irregular appearance of the staining.

Aging correlates with enhanced sensitivity to glucose-induced β-cell apoptosis and decreased baseline **proliferation.** Since the incidence of diabetes increases with age, we were interested to know whether this correlates with changes in the proliferative capacity and in the sensitivity to glucose-induced apoptosis of  $\beta$ -cells. Therefore, we analyzed baseline and glucose-stimulated apoptosis and proliferation rates of  $\beta$ -cells from cultivated islets from 53 organ donors aged 17-74 years. Mean baseline  $\beta$ -cell apoptosis at 5.5 mmol/l glucose after 4 days of culture was 0.43% TUNEL-positive  $\beta$ -cells and did not significantly change with age (Fig. 2A). However, there was a significant correlation between the age of the donor and the sensitivity to glucose-induced apoptosis (Fig. 2B). In contrast, the baseline proliferation rate of cultured human  $\beta$ -cells was negatively correlated with increasing age of the donor, whereas the deleterious effect of glucose on the replicating rate of  $\beta$ -cells was age independent (Fig. 2C and D). These changes in cell turnover were also apparent when the data were grouped according to age: the ratio of the percentage of TUNEL-positive  $\beta$ -cells at



FIG. 3. Age-dependent appearance of the Fas/Fas ligand system. Pancreatic islets isolated from 2- and 8-month-old rats (A and B) and from 21and 71-year-old humans (C and D) were cultured on extracellular matrix-coated dishes (A and C) or in suspension (B and D) and exposed for 30–96 h to media containing 5.5, 11.1, or 33.3 mmol/l glucose. A and C: Double immunostaining for insulin (in green) and Fas ligand or the Fas receptor (in red). B and D: Western blot analysis of Fas ligand, Fas, and actin. The antibodies were blotted on the same membrane after stripping. One representative of three experiments from isolated islets from 2- to 3- and 7- to 8-month-old rats and from 18- to 21- and 60- to 71-year-old human organ donors is shown, respectively. E: In parallel,  $\beta$ -cell secretion assays from the isolated rat and human islets were performed. Basal and glucose, normalized to insulin content and expressed as percent change from basal secretion. Islets were isolated from four 2- to 3-month-old and four 7- to 8-month-old rats and from four 18- to 24-year-old and four 60- to 71-year-old human organ donors and plated in four dishes per experiment. \*P < 0.05 vs. stimulated insulin secretion from 2- to 3-month-old rats and 18- to 24-year-old humans, respectively. F: Double immunostaining for Fas ligand or Fas (in red) and insulin (in green) on pancreatic sections from 5-day and 5-year-old humans.



FIG. 4.  $\beta$ -cell expression of PDX-1 decreases with age. A: Pancreatic islets isolated from 2- and 8-month-old rats were cultured in suspension and exposed for 30 or 96 h to media containing 5.5, 11.1, or 33.3 mmol/l glucose. Western blot analysis of PDX-1 and actin. The antibodies were blotted on the same membrane after stripping. One of three experiments is shown. B: RT-PCR quantification of PDX-1 and insulin mRNA expression in total RNA isolated from 2- and 8-month-old rat islets following overnight incubation at 11.1 mmol/l glucose. The levels of PDX-1 and insulin expression were normalized against tubulin. Double immunostaining for PDX-1 in red and insulin in green on pancreatic sections from rats (C and D) and humans (E and F). Intensity of PDX-1 immunostaining versus background in  $\beta$ -cells identified by double staining for insulin in rat (D) and human (F) sections. D: Results are shown as mean from five rats ±SE. F: Results are a correlation between PDX-1 and age from 18 human pancreas sections. Ten islets were scored in each section. +P < 0.05 vs. islets at 2 months of age. G and H: Correlation between age and RT-PCR quantification of PDX-1 (G) and insulin (H) mRNA expression in total RNA isolated from 23 human islet isolations. The level of PDX-1 and insulin expression was normalized against tubulin. Each point represents an independent experiment of one organ donor. Solid lines are best fit, and broken lines show 95% CIs.

33.3 mmol/l glucose versus control incubations at 5.5 mmol/l glucose increased 1.6-, 1.9-, and 2.2-fold in the age-groups 26–49, 50–60, and 61–74 years, respectively, compared with the 17- to 25-year age-group (Fig. 2*E*). In parallel,  $\beta$ -cell proliferation at 5.5 mmol/l glucose decreased by 1.5-, 2.1-, and 5.4-fold and at 33.3 mmol/l glucose by 1.9-, 2.2-, and 5.7-fold in the age-groups 26–49, 50–60, and 61–74, respectively, versus the 17- to 25-year age-group (Fig. 2*F*).

Age-dependent appearance of the Fas/Fas ligand system in rat but not in human islets. In human islets, the mechanism underlying glucose-induced β-cell apoptosis and impaired proliferation involves the upregulation of Fas, which interacts with constitutively expressed Fas ligand on neighboring  $\beta$ -cells (11). Therefore, we hypothesized that age-dependent changes in Fas and Fas ligand expression could explain differences in  $\beta$ -cell apoptosis and proliferation seen in rats of different ages. Double immunostaining of cultivated islets from 2- to 3-month-old rats revealed neither expression of the Fas receptor nor of Fas ligand at different glucose concentrations (Fig. 3A and *B*). In contrast,  $\beta$ -cells from 7- to 8-month-old rats constitutively expressed Fas ligand at low and high glucose concentrations (Fig. 3A and B). Moreover, similar to human  $\beta$ -cells (11), glucose-induced expression of Fas at 11.1 mmol/l (data not shown) and 33.3 mmol/l glucose concentrations (Fig. 3A) compared with 5.5 mmol/l (Fig. 3A). Age-dependent Fas receptor and Fas ligand expression was confirmed by Western blot analysis of lysates from 2- to 3- and 7- to 8-month-old rat islets cultured in suspension at 5.5, 11.1, and 33.3 mmol/l glucose (Fig. 3*B*). After 30 and 96 h of culture, Fas ligand was present in the 7- to 8-month-old rats at all glucose concentrations but not in the 2- to 3-month-old rats. Fas receptor was upregulated in the 7- to 8-month-old rats by 11.1 and 33.3 compared with 5.5 mmol/l glucose after 30 h and at higher levels after 96 h of culture. Finally, Fas receptor was almost undetectable in the islets of 2- to 3-month-old rats cultured at low or high glucose concentrations for 30 or 96 h. In contrast to the rats, in human islets, Fas ligand was already present at 5 days of age (for representative images see Fig. 3F), and no change in expression levels could be detected with age (Fig. 3C). We also performed analysis for the Fas receptor in humans at different ages from 5 days to 71 years. No Fas staining was detectable in human islets cultured at 5.5 mmol/l glucose (Fig. 3C) and in human pancreatic sections from nondiabetic individuals (Fig. 3F). Increasing glucose concentrations induced Fas receptor upregulation, but no age-dependent differences could be quantified (Fig. 3D). In parallel,  $\beta$ -cell secretory function was analyzed. Glucose-stimulated insulin secretion decreased with age in rats and in humans (Fig. 3E).

**β-Cell expression of PDX-1 decreases with age.** We next studied the influence of aging on PDX-1 expression under normal and glucotoxic conditions. β-Cell expression of PDX-1 was strongly decreased in islets of 7- to 8-month-old rats compared with younger rats. This was observed by Western blotting of cultured islet lysates (Fig. 4*A*), quantitative RT-PCR (Fig. 4*B*), and by immunostaining of tissue sections (Fig. 4*C* and *D*). In contrast, insulin mRNA was not changed (Fig. 4*B*). Exposure of the islets to increasing glucose concentrations for 30 and 96 h decreased PDX-1 expression in 7- to 8-month-old islets whereas in 2- to 3-month-old islets, a decrease occurred only after prolonged culture for 96 h but not after 30 h (Fig. 4*A*). Subsequently, we analyzed tissue sections from nec-

ropsies of 18 human pancreata from nondiabetic individuals aged from 5 days to 76 years. PDX-1 was clearly expressed in  $\beta$ -cells of young individuals, whereas it was barely detectable in pancreata of old individuals (for representative images, see Fig. 4*E*). Quantitative analysis of the intensity of the PDX-1 staining revealed a significant negative correlation between the age of the patients and PDX-1 expression (Fig. 4*F*). Finally, PDX-1 and insulin mRNA expression levels from 23 human islet isolations were studied and also revealed a negative correlation of PDX-1 expression with age, both compared with tubulin (Fig. 4*G*) or glyceraldehyde-3-phosphate dehydrogenase (data not shown), whereas insulin mRNA expression did not correlate with age (Fig. 4*H*).

#### DISCUSSION

Aging is a major risk factor for the development of type 2 diabetes. In cultured human islets, aging was negatively correlated with baseline  $\beta$ -cell proliferative activity, which was further decreased by high concentrations of glucose independent of the age of the donor. Moreover, aging positively correlated with enhanced sensitivity to glucose-induced apoptosis. These in vitro observations are supported by Butler et al. (6) who show a trend for decreased  $\beta$ -cell replication with age. Thus, the limited adaptive capacity of aging  $\beta$ -cells may contribute to the risk of developing type 2 diabetes, as observed in elderly patients.

Many differences exist between rat and human islets that are usually attributed to differences in the genetic background. Indeed, islets from 2- to 3-month-old rats and human islets responded differently to glucose-induced changes in cell turnover. However, while 2- to 3-month-old rats are often considered to be adult, this is certainly not the case in many aspects, including linear growth. No striking cell cycle differences were apparent when comparing human islets and islets from rats aged >6 months. This can be explained partly by the appearance of the Fas/Fas ligand system. Indeed, Fas ligand is constitutively expressed by  $\beta$ -cells from rats aged  $\geq 6$  months but not in younger rats, as previously shown (22) and confirmed in the present study. Similarly, glucose-induced Fas expression occurred only in  $\beta$ -cells from 7- to 8-month-old rats and not at 2-3 months. Therefore, differences between human islets, usually emanating from adult organ donors, and rodent islets may be due to differences in age and not in genetic background. However, in human  $\beta$ -cells, Fas ligand and upregulation of the Fas receptor by chronic exposure to increased glucose concentrations were present at each age analyzed. Therefore, some intrinsic genetic differences in the proliferative capacity between the species may exist in addition to the role of age.

In human islets, baseline  $\beta$ -cell proliferation decreased with age. Furthermore, prolonged exposure to 33.3 mmol/l glucose decreased  $\beta$ -cell proliferation of cultured islets from 7- to 8-month-old rats and from adult humans. In parallel, both aging and prolonged exposure to glucose were associated with decreased expression of PDX-1. Interestingly, islets from 2- to 3-month-old rats displayed a reduced expression of PDX-1 only following a 96-h exposure to high glucose but not after 30 h. Therefore, changes in PDX-1 activity may contribute to these changes in proliferation rates. Supporting this notion, PDX-1 has been shown to be important for  $\beta$ -cell replicative activity and survival (24–26). Furthermore, chronic exposure of cultured human pancreatic islets to high glucose lowers the activity of PDX-1 (27). Yet, our data are correlative, and a causative proof remains to be shown.

The observed changes in  $\beta$ -cell turnover most probably did not result in significant changes in  $\beta$ -cell area in vitro. Indeed, a 4-day exposure of islets to elevated glucose concentrations induced changes in apoptosis and proliferation in only 1–2% of the  $\beta$ -cells. However, these findings may be relevant for the in vivo situation. Indeed, a net change of ±1% in cell turnover will lead to a doubling or 50% decrease in  $\beta$ -cell mass within 3–4 months. Such impressive changes in  $\beta$ -cell mass may occur in vivo over a similar time period (e.g., during obesity) (6). Nevertheless, in parallel to changes in  $\beta$ -cell turnover, aging is associated with a progressive decrease in  $\beta$ -cell function, as previously described (2,30) and confirmed in the present study.

In conclusion, we demonstrate that there is a progressive impairment in  $\beta$ -cell turnover with age, characterized by decreased baseline proliferation and enhanced sensitivity to glucose-induced  $\beta$ -cell apoptosis. This impairment is associated with decreased PDX-1 activity. Furthermore, in rat islets, appearance with age of the Fas/Fas ligand pathway parallels changes in sensitivity to glucose-induced apoptosis and decreased proliferation. Therefore, changes in  $\beta$ -cell plasticity may be a predisposing factor to the development of diabetes in elderly subjects. Finally, islets of older rats may be a more appropriate model than younger islets to study glucose-induced  $\beta$ -cell apoptosis.

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