Impaired islet turnover in human donor pancreata with aging

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Abstract

Objective: The prevalence of type 2 diabetes mellitus escalates with aging although β-cell mass, a primary parameter of β-cell function, is subject to compensatory regulation. So far it is unclear whether the proliferative capacity of pancreatic islets is restricted by senescence.

Materials and methods: Human pancreatic tissue from n = 20 non-diabetic organ donors with a mean age of 50.2 ± 3.5 years (range 7–66 years) and mean body mass index of 25.7 ± 0.9 kg/m² (17.2–33.1 kg/m²) was morphometrically analyzed to determine β-cell volume, β-cell replication, β-cell apoptosis, islet neogenesis, and pancreatic duodenal homeobox-1 (PDX-1) expression.

Results: Relative β-cell volume in human pancreata (mean 2.3 ± 0.2%) remains constant with aging (r = 0.26, P = ns). β-cell replication (r = 0.71, P = 0.0004) decreases age-dependently, while β-cell apoptosis does not change significantly (r = 0.42, P = 0.08). Concomitantly, PDX-1 expression is downregulated with age in human pancreatic tissue (r = 0.65, P = 0.002). The rate of islet neogenesis is not affected by aging (r = 0.13, P = ns).

Conclusions: In non-diabetic humans, aging is linked with impaired islet turnover possibly due to reduced PDX-1 expression. As β-cell replication is considered to be the main mechanism responsible for β-cell regeneration, these changes restrict the flexibility of the aging human pancreas to adapt to changing demands for insulin secretion and increase the risk for the development of diabetes mellitus in older subjects.

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Introduction

The islet of Langerhans is a self-renewing tissue that undergoes permanent turnover in order to adapt to changing insulin demands, e.g. insulin resistance induced by obesity (1–3). Cell renewal occurs from replication of already existing β-cells, differentiation of stem or progenitor cells and hypertrophy (2, 4). Cell loss on the other hand primarily occurs by apoptosis (1, 5). Dysregulated islet turnover with increased β-cell apoptosis occurs in the context of type 2 diabetes and leads to β-cell deficiency and islet dysfunction (1, 6, 7). In humans, type 2 diabetes occurs more frequently in older subjects (8, 9). This has been attributed to a positive association between aging and insulin resistance, which is primarily due to an increasing body fat content in aging subjects (10). Another aspect that increases the susceptibility for the development of diabetes mellitus in aging humans is a decline of the insulin secretory capacity (11, 12), which, in isolated human islets, is accompanied by a decrease of pancreatic duodenal homeobox-1 (PDX-1) expression (13). PDX-1 is a direct activator of several β-cell specific genes that control glucose metabolism including insulin, glucokinase and SLC2A2 (14, 15). Moreover, PDX-1 promotes β-cell replication and is cytoprotective, e.g. PDX-1 is upregulated in proliferating islets following partial pancreatectomy in rats (16–18). Taken together, it is established that aging is positively associated with the prevalence of type 2 diabetes. However, little is known about the age-related changes occurring on the level of the islet. Therefore, we were analyzing the parameters of islet turnover and PDX-1 expression in human pancreata from non-diabetic, heart-beating organ donors in order to examine the association between islet turnover and aging, as well as the role of PDX-1.

Materials and methods

Subjects

Human pancreatic tissue was obtained from 20 non-diabetic organ donors. The local ethics committee gave
approval and relatives provided written informed consent to use donor organs for research purposes. The subject characteristics are summarized in Table 1. Data about the immediate medical treatment and glucose metabolism prior to organ donation were not available due to constraints imposed by the national transplant agency that is the authority for gathering patient data, and the IRB approval that only allows to use data from the official pancreas donor report sheet provided by the national transplant agency.

**Pancreatic tissue processing**

Each pancreas was sectioned and analyzed in the head, body and tail region. Tissue fixation was performed with formalin (4%) overnight. Tissue sections were dehydrated (Leica ASP 300, Wetzlar, Germany) and embedded in paraffin for subsequent analysis. Sections were cut from these paraffin blocks (Mikromot Leica RM 2165) and immunohistochemically stained for insulin (anti-human insulin monoclonal antibody, Biogenex, San Ramon, CA, USA), glucagon (anti-human glucagon polyclonal antibody, Biogenex), and PDX-1 (anti-human polyclonal antibody, Chemicon International, Temecula, CA, USA) according to the streptavidin-peroxidase/phosphatase method. Replication was determined by immunohistochemistry for Ki-67 (anti-human Ki-67 monoclonal antibody, Dako, Carpinteria, CA, USA), apoptosis using the TUNEL method (in situ cell death detection kit POD, Roche Diagnostics). All tissue sections stained for Ki-67 and TUNEL were double stained for insulin.

**Morphometric analysis**

Since pancreas weight was not available, it was not possible to determine α- and β-cell mass. Therefore, glucagon and insulin content were determined as the percentage of α- or β-cell area of total pancreatic tissue area (= relative α/β-cell volume). Islet size was determined by analysis of the cross-sectional area of individual islets (n = 120 per donor). Islet density was calculated as the number of islets per pancreatic tissue area. PDX-1 staining was confined to islet tissue and was detected in the cytoplasm as well as in the nucleus. In pancreatic β-cells, PDX-1 is rapidly shuttled (30–60 min) from the cytoplasm to the nucleus if glucose levels increase. However, this process is rapidly reversible if glucose levels decrease (19). The expression of PDX-1 is controlled by nutrients and hormones and it has been shown that PDX-1 expression in β-cells is impaired under conditions of glucotoxicity (20) and increased concentrations of fatty acids (21). Owing to this variable regulation of PDX-1, we determined the overall expression level of PDX-1 in donor pancreata by morphometric analysis. Results are expressed as the percentage of PDX-1 positive tissue of total pancreatic area. Slides were analyzed using a Nikon Eclipse TE 2000-E inverted system microscope connected to a Hewlett Packard computer with NIS-Elements AR 2.30 software (Nikon, Düsseldorf, Germany). For the measurement of insulin, glucagon and PDX-1 slides were scanned using a 4× objective. Apoptosis and replication were determined as TUNEL/ Ki-67 positive cells per β-cell area respectively. All islets present in the section were included in this analysis. TUNEL staining occasionally occurred in debris that was no longer a distinct cell; these fragments were not counted. Only discernible cells with TUNEL positive nuclei were included. Three sections per donor (head, body and tail region) were analyzed with 172–900 islets per donor.

Neogenesis was defined as exocrine duct cells positive for insulin. Therefore, the number of exocrine duct cells and exocrine duct cells positive for insulin were determined for each section (head, body, and tail region) to quantify the percentage of insulin positive exocrine duct cells in each donor pancreas. A range of 120–527 ducts per donor with 6–384 cells per duct were analyzed.

**Statistical analysis**

Insulin and glucagon volumes in the pancreatic head, body and tail were compared using student’s t-test. The relationship between age and replication (Ki-67 positive cells), apoptosis (TUNEL positive cells), and PDX-1 was analyzed by linear regression analysis. A p-value of <0.05 was considered to denote a significant difference.

### Results

The mean insulin and glucagon content of human donor pancreata was 2.3 ± 0.2 and 0.5 ± 0.1% (Table 1). Owing to larger individual islets (islet size in head, body and tail region: 6007 ± 1103, 7169 ± 743, 9614 ± 1238 μm²) and an increased islet density in the pancreatic tail the relative percentage of insulin positive tissue was significantly higher in the tail region (2.9 ± 0.3%) compared to pancreatic head (1.8 ± 0.2%) or

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Islet turnover was examined by quantification of β-cell replication, β-cell apoptosis and islet neogenesis expressed as Ki-67 or TUNEL positive cells per β-cell area (Fig. 2) and the percentage of insulin positive duct cells respectively. In general, replication (one positive cell in five islets) and particularly apoptosis (one positive cell in one hundred islets) are rare events (Table 1). Linear regression analysis revealed an age-dependent decrease in β-cell replication while there was no significant change of β-cell apoptosis ($P=0.08$; Fig. 3A and B). β-cell replication was particularly high and β-cell apoptosis was particularly low in the two youngest organ donors. There was no correlation between aging and islet neogenesis (Fig. 3C). In order to identify whether reduced β-cell replication in older individuals is linked to changes of PDX-1 expression, we performed immunohistochemistry for PDX-1. Morphometric analysis shows that in humans PDX-1 is downregulated with aging (Fig. 3D). Consistently, analysis of PDX-1 and β-cell replication shows that replication in human islet tissue is tightly coupled to PDX-1 expression (Fig. 4A). By contrast, there was no significant relationship between PDX-1 and β-cell apoptosis (Fig. 4B). Also, there were no differences of PDX-1 expression in pancreatic head, body or tail (data not shown, $P=\text{ns}$).

**Figure 1** (A) Distribution of β-cell volume, (B) α-cell volume, (C) islet size and (D) islet density in the head, body and tail region of $n=20$ donor pancreata. Data are means±s.e.m. A: *$P<0.05$ head versus tail, # $P<0.05$ body versus tail. C: *$P<0.05$ head versus tail. D: # $P<0.05$ body versus tail. $P$ value derived by ANOVA.

Figure 2 Sections of human pancreata stained for Ki-67 and insulin (A + B), TUNEL and insulin (C + D), PDX-1 (E + F) and insulin (G + H depict insulin positive duct cells). (A) 7-year-old donor, (B) 63-year-old donor, (C) 52-year-old donor, (D) 41-year-old donor, (E) 7-year-old donor, (F) 66-year-old donor, (G) 58-year-old donor, (H) 60-year-old donor. Magnification 200×.
Across the wide age range of organ donors (7–66 years) there was no relationship with relative glucagon or insulin content ($P=\text{ns}$). Similarly, there was no association between BMI and $\alpha$-cell or $\beta$-cell volume ($P=\text{ns}$, data not shown).

**Discussion**

We report that in humans, aging is associated with impaired islet turnover characterized by reduced $\beta$-cell replication while there is no change of islet neogenesis. We provide evidence to suggest that reduced PDX-1 expression is one cause of impaired islet turnover in older subjects. These changes of islet plasticity imply that adaptation of $\beta$-cell mass to changing demands of insulin (e.g. insulin resistance) is attenuated with aging, particularly because $\beta$-cell replication that has recently been hypothesized to be the primary mechanism of islet regeneration (4, 22), is impaired. In consequence, failed islet adaptation probably is one explanation for the increasing prevalence of diabetes mellitus in older individuals.

This notion is supported by data from rat and human islets and human autopsy pancreata. In isolated islets, $\beta$-cell proliferation was negatively associated with donor age (13). We previously reported that in autopsy pancreata the frequency of replicating $\beta$-cells is age-dependently decreasing, although the age range of individuals in that study was shifted to the right (40–94 years, $n=47$) (1). Recent studies suggest that expansion of $\beta$-cell mass and overall pancreatic growth is most prominent during childhood (23, 24), although the former study exclusively analyzed subjects up to 20 years of age. In that study, $\beta$-cell replication determined by Ki-67 staining was highly variable, even in subjects of similar age. One reason for that as shown by the data in the publication is the high number of cases found to have no detectable $\beta$-cell replication at all. In contrast, in the present study we detect Ki-67 positive $\beta$-cells in every case analyzed. This difference may be due to the different aspects associated with autopsy tissue that was obtained up to 24 h after death and may therefore be subjected to postmortem alterations compared to pancreatic tissue obtained from heart-beating organ donors. In the context of the present study, it has to be hypothesized that the most notable decline in $\beta$-cell replication coincides with completion of somatic growth (Fig. 3A). If we systematically compare age-corrected (linear regression function) parameters of islet turnover in human autopsy (tissue obtained within 12 h of death (1)) versus donor pancreata from the present report, we do not find any significant differences. The age-corrected activities of $\beta$-cell replication ($0.05 \pm 0.01$ vs $0.05 \pm 0.01$ cells/islet, donor versus autopsy, $P=\text{ns}$), $\beta$-cell apoptosis ($0.02 \pm 0.003$ vs $0.14 \pm 0.04$ cells/islet, donor versus autopsy, $P=\text{ns}$), and islet neogenesis (percentage of insulin positive ductal epithelial cells: $0.54 \pm 0.16\%$ vs $0.58 \pm 0.10\%$, donor versus autopsy, $P=\text{ns}$) are not different. There also is no difference in the relative $\beta$-cell volume in donor versus autopsy tissue ($2.3 \pm 0.2$ vs $2.3 \pm 0.3\%$, $P=\text{ns}$), suggesting that pancreatic tissue from donor organs obtained within

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**Figure 3** (A) Correlations of age versus $\beta$-cell replication, (B) $\beta$-cell apoptosis, (C) islet neogenesis and (D) PDX-1 expression. Neogenesis is expressed as percentage of insulin positive duct cells. Solid lines are best fit for linear regression analysis with broken lines showing 95% CIs.
12 h after death at autopsy is equally suitable for analysis of β-cell volume and β-cell turnover.

Previously, it has been reported that in animals and humans β-cell mass expands in response to insulin resistance (e.g. obesity) (1, 25, 26). In the present group of subjects, there is no significant association between relative β-cell volume and BMI. This is probably due to the fact that the present subjects are not obese but slightly overweight (Table 1), suggesting that they are not an appropriate group to analyze the relationship between BMI and β-cell mass. Moreover, the present study also includes young subjects, who are still in the process of somatic growth and hence BMI is not an accurate parameter to estimate the degree of insulin resistance. In fact, the two youngest donors 7 and 18 years of age are the ones with the lowest BMI (17.2 kg/m² or 75th–90th age-specific BMI percentile and 19.5 kg/m² or 25th–50th age-specific BMI percentile), but with the highest capacity for islet expansion (high β-cell replication and low β-cell apoptosis). Therefore, it may be concluded that the mechanisms of β-cell expansion are different in young versus obese subjects. This should be analyzed in future studies, designed to specifically include subjects evenly distributed across the full BMI and age range observed in humans.

Aging is associated with a reduced regenerative potential in a number of different tissues like skeletal muscle, bone, liver and macrophages (immune system) (27–29). This poses a threat to the adaptive capacity of these tissues and the whole organism. In the present study, we extend this notion and report that there is an age-related impairment of islet turnover. In consequence, older individuals would be less capable to adapt their endocrine pancreas to changing insulin requirements. Since the islets of Langerhans are a self-renewing tissue, impairment of islet-turnover with reduced β-cell replication would also confound long-term tissue homeostasis resulting in increased susceptibility to diabetes mellitus caused by β-cell deficiency (30). Ideally, these important questions should be analyzed in longitudinal studies examining the time-course of islet morphology and islet turnover in individual humans. Currently, these studies are not feasible due to the difficulties associated with obtaining human pancreatic tissue.

Prior reports suggested that in human autopsy pancreata, isolated human islets and rats there might be a negative association between aging and PDX-1 expression (13, 31). The present study confirms these observations and extends them by using tissue from heart-beating human donors and analyzing islet turnover and PDX-1 expression in identical individuals. We report that reduced β-cell replication in older individuals is linked to decreasing PDX-1 expression. Chronic hyperglycemia (33 mmol/l) has been shown to down-regulate PDX-1 in β-cells and human islets (32). However, the present analysis has been performed with non-diabetic human donor pancreata and PDX-1 expression is low in older individuals despite normoglycemia. Therefore, it is unlikely that chronic changes of glucose metabolism caused downregulation of PDX-1 in the present study. PDX-1 expression in the pancreatic tail region was not different from expression levels in pancreatic head or body (P = n.s.). Therefore, in humans, increased β-cell content of the pancreatic tail region is apparently not due to permanent differences of PDX-1 expression. PDX-1 is functionally localized in the nucleus to affect glucose stimulated gene transcription. As in prior reports, it is difficult to obtain good quality nuclear PDX-1 staining in paraffin embedded tissue sections and perform functional PDX-1 evaluations. New methods for this staining need to be developed in future to obtain data on the functionality of PDX-1.

The present studies confirm earlier reports about the preferential presence of β-cells in the pancreatic tail compared to head and body (33, 34) However, due to different antibody types, precise morphometric analysis techniques and different tissue sources (donor versus autopsy) the relative percentage of β-cells is higher in the present report (∼2.3 vs ∼1.3%).

One limitation of the present study is the incomplete clinical data for the organ donors, due to constraints imposed by the regulations associated with organ donation.
donation and the Institutional Review Board (IRB) approval. Therefore, we cannot exclude that the present collection of non-diabetic individuals includes cases with a positive family history of diabetes mellitus and hence are at risk for defects of glucose metabolism.

In summary, islet turnover is progressively impaired in aging humans concomitantly with decreasing PDX-1 expression. The present data suggest that impaired islet turnover may be one important mechanism for the increasing prevalence of diabetes mellitus with aging. Therefore, strategies aimed at restoration of islet turnover, e.g. induction of PDX-1 expression (incretins), may not only be useful for treatment but also for prevention of type 2 diabetes mellitus occurring in older subjects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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