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Beta-cell deficit in Obese Type 2 Diabetes, a Minor Role of Beta-cell Dedifferentiation and Degranulation

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Context: Type 2 diabetes is characterized by a beta-cell deficit and a progressive defect in beta-cell function. It has been proposed that the deficit in beta-cells may be due to beta-cell degranulation and transdifferentiation to other endocrine cell types.

Objective: To establish the potential impact of beta-cell dedifferentiation and transdifferentiation on beta-cell deficit in type 2 diabetes, and to consider the alternative that cells with an incomplete identity may be newly forming rather than dedifferentiated.

Design, Setting, And Participants: Pancreata obtained at autopsy were evaluated from 14 non diabetic and 13 type 2 diabetic individuals, from 4 fetal cases and from 10 neonatal cases.

Results: While there was a slight increase in islet endocrine cells expressing no hormone in type 2 diabetes (0.11 ± 0.03 vs 0.03 ± 0.01 cells/islet, $p < 0.01$), the impact on the beta-cell deficit would be minimal. Further, we established that the deficit in beta-cells per islet cannot be accounted for by an increase in other endocrine cell types. The distribution of hormone negative endocrine cells in type 2 diabetes (most abundant in cells scattered in the exocrine pancreas) mirrors that in developing (embryo and neonatal) pancreas, implying that these may represent newly forming cells.

Conclusions: Therefore, while we concur that in type 2 diabetes there are endocrine cells with altered cell identity, this process does not account for the deficit in beta-cells in type 2 diabetes, but may reflect, in part, attempted beta-cell regeneration.

Type 2 diabetes is characterized by a progressive decline in β -cell function (1, 2). In studies of human pancreas obtained at autopsy or from brain dead organ donors there is a deficit in β -cells (3–6). This has been attributed to an imbalance between sufficient β -cell formation, pre- or postnatally, and increased β -cell loss through apoptosis or necrosis. Support for this model of the progressive decline in β -cell function in type 2 diabetes is the striking similarity between the loss of cell mass and function in neurodegenerative diseases such as Alzheimer's disease that share much in common with type 2 diabetes (7). In both the hippocampus in Alzheimer's disease and the islet

in type 2 diabetes, the cells of interest express closely related amyloidogenic proteins (Alzheimer's beta protein and islet amyloid polypeptide) that misfold and form toxic membrane permeant oligomers and accumulate over time as extracellular amyloid. Moreover, the cell signaling changes in β -cells and hippocampal cells in type 2 diabetes and Alzheimer's disease are also shared, with mitochondrial dysfunction, endoplasmic reticulum stress, calpain hyperactivation, accumulation of polyubiquitinated proteins and defective autophagy/lysosomal pathways (7). Further, both the pathological and functional changes in Alzheimer's disease and type 2 diabetes are recapitulated

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Abbreviations:

in models expressing human Alzheimer's beta protein and IAPP respectively (8, 9) accompanied by an increase in cell death (10).

Recently, based initially on genetically manipulated mouse models (11), it has been suggested that the underlying basis of the β -cell deficit in type 2 diabetes is β -cell degranulation and β -cell dedifferentiation and then trans-differentiation, rather than β -cell loss through apoptosis (11). Proponents of this hypothesis have suggested that the therapeutic approach to β -cell dysfunction in type 2 diabetes is best directed at the degranulation/dedifferentiation defects rather than preservation or expansion of β -cell mass (11). The purpose of the present studies was to test the hypothesis that the deficit in β -cells in type 2 diabetes can be accounted for by degranulation of β -cells and/or conversion of β -cells to other endocrine cell types. As a secondary question, we sought to compare human endocrine pancreas during late development and early childhood with that in type 2 diabetes, with consideration that some of the recently reported observations of changes in endocrine identity in diabetes might be a consequence of attempted β -cell regeneration.

RESEARCH DESIGN AND METHODS

Design and Case Selection. For the neonatal and adult subjects, sections of pancreas were obtained from the Mayo Clinic autopsy archives with IRB permission (IRB# 15–004 992). For the adult subjects, two groups were identified: obese nondiabetic (14 subjects) and obese subjects with a documented history of type 2 diabetes (13 subjects). Obesity was defined as a BMI > 27 kg/m². Potential cases were identified by retrospective analysis of the Mayo Clinic autopsy database. To be included, case requirements were a full autopsy within 24 hours of death, a general medical examination including at least one fasting blood glucose documented in the year prior to death and pancreatic tissue stored of adequate size and quality. Exclusion criteria included any potential secondary cause of diabetes, exposure to chronic glucocorticoid treatment, and pancreatic tissue that had undergone autolysis or showed features of pancreatitis. Neonatal autopsy cases (n = 10) were selected to be as recently postdelivery as possible, while using the same inclusion and exclusion criteria for pancreatic tissue quality.

Sections of fetal pancreas (n = 4) were obtained from the Institute of Pathology and the Division of Clinical and Functional Anatomy, Medical University of Innsbruck. They were obtained from miscarriage and legal abortions including parental consent and in compliance with the local governmental and institutional guidelines.

Case Characteristics (Supplemental Tables 1, 2, and 3).

The obese nondiabetic and obese type 2 diabetic groups were matched for age (61.4 ± 2.6 vs 59.1 ± 3.5 years, OT2D vs OND, $p = \text{ns}$). BMI was higher in the OT2D group (43.3 ± 2.0 vs 36.1 ± 1.2 kg/m², OT2D vs OND, $P < .005$). When compared to the obese nondiabetic subjects, the obese diabetic subjects had higher fasting plasma glucose values despite being on treatment (150.9 ± 22.1 vs 100.5 ± 3.8 mg/dl, OT2D vs OND, $P < .05$). Most individuals with type 2 diabetes had been treated with insulin (10 subjects), while one subject had received a sulfonylurea and two had been treated by diet alone.

In order to assure that differences in BMI between the nondiabetic and type 2 diabetic groups were not a factor in the results obtained, a subset of cases (11 obese nondiabetic and 10 obese type 2 diabetic), matched for both age (62.8 ± 2.6 vs 60.1 ± 4.4 years, OT2D vs OND, $p = \text{ns}$) and BMI (40.4 ± 1.4 vs 37.5 ± 1.2 kg/m², OT2D vs OND, $p = \text{ns}$), was examined (Supplemental Tables 4 and 5).

Sections of pancreas from ten neonatal subjects were obtained, death having occurred between 8 hours and 12 weeks postdelivery. Cause of death is noted in Supplemental Table 3. Sections of fetal pancreatic tissue were acquired from four fetuses, at 26, 30, 32 and 34 weeks of gestation.

Pancreatic Tissue Processing and Staining. Paraffin tissue sections from each subject were stained for Chromogranin A, insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin. Standard immuno-histochemistry protocol was used for fluorescent immuno-detection of various proteins in pancreatic sections. One pancreas section obtained from the tail of pancreas was analyzed for each subject. Briefly, slides were incubated at 4°C overnight with a cocktail of primary antibodies prepared in blocking solution (3% BSA in TBST) at the following dilutions: rabbit anti-Chromogranin A (1:200, Novus Biologicals NB120–15 160, Littleton, CO); mouse antiglucagon (1:1000, Sigma-Aldrich G2654-.2ML; St. Louis, MO); guinea-pig anti-insulin (1:200, Abcam7842; Cambridge, MA), rat antisomatostatin (1:300, EMD Millipore MAB354; Billerica, MA), goat antipancreatic polypeptide (1:3000, Everest Biotech; Ramona, CA), rat antighrelin (1:50, R&D Systems MAB8200; Minneapolis, MN). The primary antibodies were detected by a cocktail of appropriate secondary antibodies (Jackson ImmunoResearch, Westgrove, PA) conjugated to Cy3 (1:200, for ChrgA), FITC (1:200 each, to detect glucagon, somatostatin, pancreatic polypeptide, and ghrelin) or Cy5 (1:100, to detect insulin). Slides were counterstained to mark the nuclei using a mounting medium containing DAPI (Vectashield, Vector Labs, Burlingame, CA), and viewed using a Leica

DM6000 microscope (Leica Microsystems, Deerfield, IL) and images were acquired using the 20x objective (200x magnification) using a Hamamatsu Orca-ER camera (C4742–80-12AG, Indigo Scientific, Bridgewater, NJ) and Openlab software (Improvision, Lexington, MA).

Morphometric analysis. Fifty islets per subject were imaged at 20x magnification. An islet was defined as a grouping of four or more chromogranin positive cells. A cluster was defined as a grouping of three or fewer chromogranin positive cells. Islets were selected by starting at the top left corner of the pancreatic tissue section and working across the tissue from left to right and back again in a serpentine fashion, imaging all islets in this systematic excursion across the tissue section. Analysis was performed in a blinded fashion (AEB), and all chromogranin positive hormone negative (CPHN) cells were confirmed by a second observer (SD). The endocrine cells contained within each islet were manually counted and the following data recorded: 1. The number of cells staining only for chromogranin; 2. The number of cells costaining for the endocrine hormone cocktail and chromogranin; 3. The number of cells costaining for insulin and chromogranin.

The mean number of endocrine cells counted within islets for the nondiabetic group was 2240 ± 294 cells per subject and for the type 2 diabetic group was 1696 ± 189 cells per subject. The mean number of cells counted in clusters for the nondiabetic group was 72 ± 9 cells per subject, and for the type 2 diabetic group was 88 ± 8 cells per subject. The mean number of chromogranin positive hormone negative [CPHN] cells per individual identified in islets from the nondiabetic subjects was 1.5 ± 0.5 cells per individual and from the type 2 diabetic group was 5.5 ± 1.5 cells per individual. The mean number of CPHN cells per individual counted in clusters for the nondiabetic group was 2.1 ± 0.3 per individual and for the type 2 diabetic group was 7.8 ± 1.0 cells per individual.

At 200x magnification, using the Leica DM6000 with Hamamatsu Orca-ER camera and a 0.7x C-mount, each field of view was calculated to be 0.292 mm^2 . Within the fields imaged to obtain the fifty islets per subject, all clusters of endocrine cells (one, two, or three adjacent endocrine cells) were counted and recorded as outlined above.

Statistical analysis. Statistical analysis was performed using the Student's *t* test with GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA). Data in graphs and tables are presented as means \pm SEM. Findings were assumed statistically significant at $P < .05$.

Results

Nonhormone expressing endocrine cells are abundant during endocrine pancreas formation, particularly in

small scattered endocrine cell clusters. During late gestation small clusters of pancreatic endocrine cells assemble into small islets and this process continues in early infancy (Figure 1A and B). By 6 months of age most endocrine cells in the pancreas are assembled into islets (12), although infrequent small scattered foci of 1–3 endocrine cells are detectable throughout adult life (Figure 1C, 2A, and 2B).

Within islets, the proportion of endocrine cells expressing no known islet hormone decreases from $\sim 30\%$ to $\sim 5\%$ during the last 10 weeks of gestation, and then further declines from $\sim 4\%$ in early infancy to $\sim 0.1\%$ throughout adult life in nondiabetic individuals (Figure 2C).

The proportion of scattered pancreatic endocrine cells that express no known islet hormone follows a similar pattern, but is consistently higher than in defined islets over the corresponding period, falling from $\sim 60\%$ to $\sim 20\%$ during the last 10 weeks of gestation and then from $\sim 20\%$ to less than $\sim 3\%$ in adults (Figure 2D).

Therefore during development of the human endocrine pancreas, nonhormone expressing endocrine cells, particularly those scattered within the exocrine pancreas, appear to be the predecessors of differentiated endocrine cells that ultimately assemble into well-defined islets.

Nonhormone endocrine cells are more frequent in type 2 diabetes (Figure 3). It has been suggested that dedifferentiation of pancreatic β -cells transforming into hormone negative β -cells might be the predominant cause for the β -cell deficit in type 2 diabetes rather than attrition of the cells through apoptosis (11, 13). We also identify occasional chromogranin positive hormone negative cells in islets in both type 2 diabetes and controls. While these nonhormone expressing endocrine cells are more frequent in islets in type 2 diabetes than controls (0.11 ± 0.03 vs 0.03 ± 0.01 cells/islet, OT2D vs OND, $P < .01$) (Figure 4A and B), they are notably much less frequent than reported in genetically manipulated mouse models with diabetes (11). Since similar cells are characteristic of the developing pancreas in humans, and in particular in the small clusters of endocrine cells that precede mature islet development, we questioned if these cells might represent attempted β -cell regeneration as opposed to dedifferentiation.

Scattered endocrine cells, with a disproportionate increase in nonhormone expressing cells, are more frequent in pancreas of type 2 diabetes. To approach the possibility that hormone negative cells in type 2 diabetes might indicate attempted regeneration, we first quantified the abundance of scattered foci of endocrine (chromogranin positive) cells in pancreas of individuals with type 2 dia-

betes and controls. We find a ~1.7-fold increase in scat-

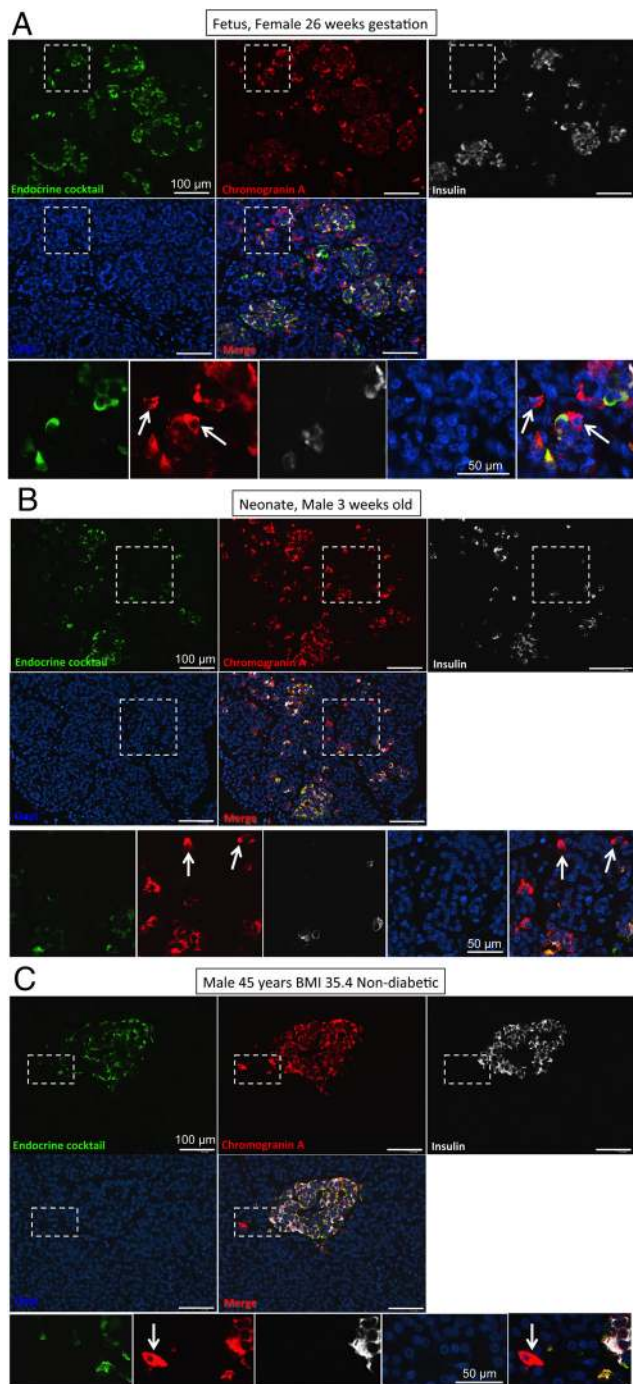


Figure 1. Representative images taken at low power (10x objective) to show pancreas from a fetus at 26 weeks gestation (A), a neonate 3 weeks postdelivery (B) and a 45 year old nondiabetic adult with a BMI of 35.4 (C). Pancreas sections in all cases were stained for Chromogranin A [red], Endocrine cocktail (Glucagon, Somatostatin, Pancreatic Polypeptide and Ghrelin) [green], Insulin [white] and DAPI [blue], and the merged image is shown. Insets in all panels show cells staining for Chromogranin A but none of the other endocrine hormones. These Chromogranin Positive Hormone Negative [CPHN] cells are frequently found in fetal pancreas, become less frequent in neonatal life and are infrequently found in nondiabetic adult human pancreas. Scale bar 100 microns in low power images, 50 microns in high power inset images.

tered foci of pancreatic endocrine cells in type 2 diabetes (13.5 ± 1.3 vs 9.4 ± 1.7 cells/mm² of pancreas, OT2D vs OND, $P < .05$) (Figure 4E and F). Subset analysis likewise revealed an increase in scattered endocrine cells in type 2 diabetes (13.9 ± 1.6 vs 8.8 ± 1.8 cells/mm² of pancreas, OT2D vs OND, $P < .05$).

Moreover, scattered endocrine cells that are nonhormone expressing (chromogranin positive islet hormone negative) are ~6-fold increased in type 2 diabetes (1.54 ± 0.21 vs 0.30 ± 0.05 cells/mm² of pancreas, OT2D vs OND, $P < .0001$) (Figure 4C and D). Subset analysis also revealed an increase in scattered endocrine cells that are nonhormone expressing (chromogranin positive islet hormone negative) in type 2 diabetes (1.43 ± 0.21 vs 0.30 ± 0.05 cells/mm² of pancreas, OT2D vs OND, $P < .0001$). The increase in scattered endocrine cells in type 2 diabetes, including a disproportionate number of nonhormone expressing cells (~12% vs ~3%), is reminiscent of the developing human endocrine pancreas. This raises the possibility that the increase in hormone negative cells in type 2 diabetes might represent attempted β -cell regeneration rather than necessarily being a consequence of β -cell dedifferentiation.

Age, body mass index (BMI) and disease duration do not predict frequency of nonhormone expressing endocrine cells. In order to investigate potential factors that might influence the frequency of nonhormone expressing endocrine cells occurring in clusters of scattered endocrine cells, we evaluated the abundance of these cells in relation to the age, BMI and documented duration of diabetes (Figure 5). None of these variables were related to the frequency of nonhormone expressing endocrine cells. Notably, since BMI was not related to the frequency of hormone negative expressing endocrine cells, the greater abundance of these cells in T2D compared to nondiabetic controls is not attributable to the greater obesity in the former. While 13 type 2 diabetic subjects are included in the study, in only 10 of those subjects was there a documented duration of disease.

The impact of nonhormone expressing endocrine cells on the deficit in β -cell mass in type 2 diabetes is minimal. It has been proposed that the reported deficit in β -cell mass in type 2 diabetes may have been over estimated since the increase in hormone expressing β -cells may be dedifferentiated or degranulated β -cells (11, 13). We addressed this by considering the impact that the hormone negative endocrine cells could have on the deficit in β -cells in type 2 diabetes. The relative abundance of nonhormone expressing endocrine cells per islet vs insulin expressing endocrine cells per islet in type 2 diabetes and controls is

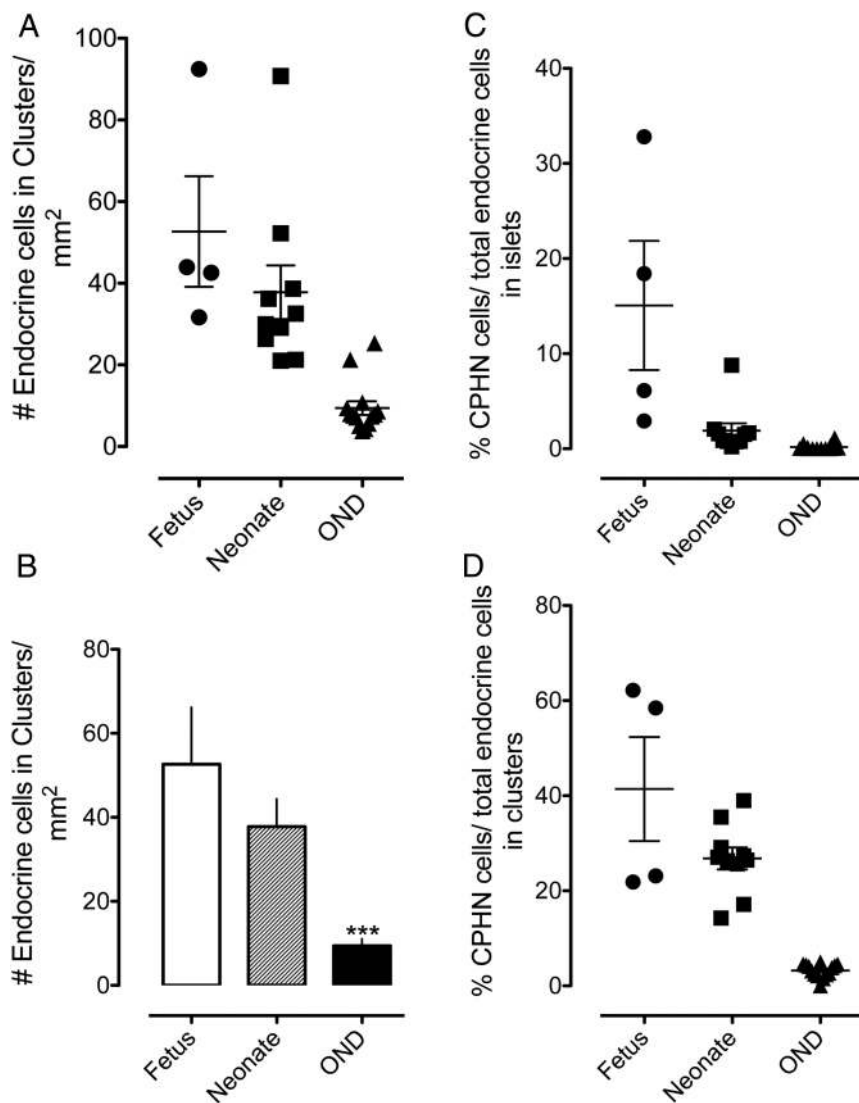


Figure 2. The number of endocrine cells present in scattered clusters of from 1 to 3 cells in the exocrine pancreas parenchyma (Panels A and B). Scattered clusters of endocrine cells are most numerous in sections of fetal pancreas, and the frequency decreases after birth. By adulthood, endocrine clusters are relatively infrequent, though are always present in pancreatic sections. (52.66 ± 13.55 vs 9.41 ± 1.67 endocrine cells/mm², Fetus vs OND, $***P < .0001$; 37.80 ± 6.56 vs 9.41 ± 1.67 endocrine cells/mm², Neonate vs OND, $***P < .0001$) The percentage of endocrine cells that are Chromogranin Positive Hormone Negative [CPHN] in human fetus and neonate compared with nondiabetic adult subjects. The results are grouped according to their location, either within islets (Panel C) or in small clusters of up to 3 endocrine cells (Panel D). CPHN cells are found frequently in fetal and neonatal tissue, with the frequency rapidly declining after birth. Most CPHN cells are found in small clusters of between one and three endocrine cells scattered throughout the exocrine pancreas. Islets: 15.06 ± 6.79 vs 1.89 ± 0.79 vs 0.17 ± 0.09 %CPHN cells in islets, fetus vs. neonate vs OND; $P < .01$, Fetus vs Neonate; $P < .001$, Fetus vs OND; $P < .01$, Neonate vs OND. Clusters: 41.40 ± 10.94 vs 26.79 ± 2.31 vs 3.22 ± 0.37 %CPHN cells in clusters, fetus vs neonate vs OND; $P < .05$, Fetus vs Neonate; Fetus and Neonate vs OND, $P < .0001$. Panels A, C, and D: Black circles = fetus; Black squares = neonate; Black triangles = Obese Nondiabetic [OND] Panel B: White bar = fetus; Striped bar = neonate; Black bar = Obese Nondiabetic [OND]

shown in Figure 6A. If all the nonhormone expressing endocrine cells per islet were added to β -cells per islet, the deficit in β -cells per islet in type 2 diabetes would decrease from 30.5% to 30.1%.

To further address the suggestion that nonhormone ex-

pressing endocrine cells in type 2 diabetes might be degranulated beta cells, we compared these cells to the nascent non hormone expressing endocrine cells in neonates to discern if there was a difference in vesicle density (granulation). As noted in Supplemental Figure 1, the granule density was comparable in neonates and in nondiabetic and individuals with type 2 diabetes. This further supports the possibility that the nonhormone expressing cells in adults with type 2 diabetes are newly formed rather than degranulated beta cells. Taken together, therefore, degranulation of β -cells does not explain the deficit in β -cells in type 2 diabetes in humans.

Islet endocrine cells expressing islet hormones other than insulin are not increased in type 2 diabetes. Advocates of the dedifferentiation hypothesis have also proposed that the deficit in β -cells in type 2 diabetes may be due to transdifferentiation of β -cells to cells expressing other islet hormones such as glucagon (11, 13). To evaluate this, we quantified the endocrine cells expressing islet hormones other than insulin (glucagon, somatostatin, pancreatic polypeptide and ghrelin) in comparison to cells expressing insulin in the same islets in type 2 diabetes vs controls. If the deficit in β -cells is due to transdifferentiation of β -cells to other endocrine cell types, then there should be a corresponding increase in endocrine cells expressing other islet hormones to match the deficit in β -cells. However, there is no increase in islet endocrine cells that do not express insulin in type 2 diabetes (Figure 6B). Collectively, while compelling evidence has been presented that there is a loss of cellular identity (as defined

by hormone and transcriptional factor expression) in a subset of endocrine cells in type 2 diabetes (13–16), the deficit in β -cells in type 2 diabetes is not explained by either degranulated β -cells or conversion of β -cells to

other endocrine cell types. Moreover, the deficit in β -cells in type 2 diabetes is accounted for by both a decrease in β -cells per islet and a decrease in islet density (4, 13). Studies that focus on the former only, underestimate the β -cell deficit.

Discussion

Type 2 diabetes is characterized by a progressive decline in β -cell function in vulnerable individuals in the face of insulin resistance (1, 2). In cross-sectional studies of human

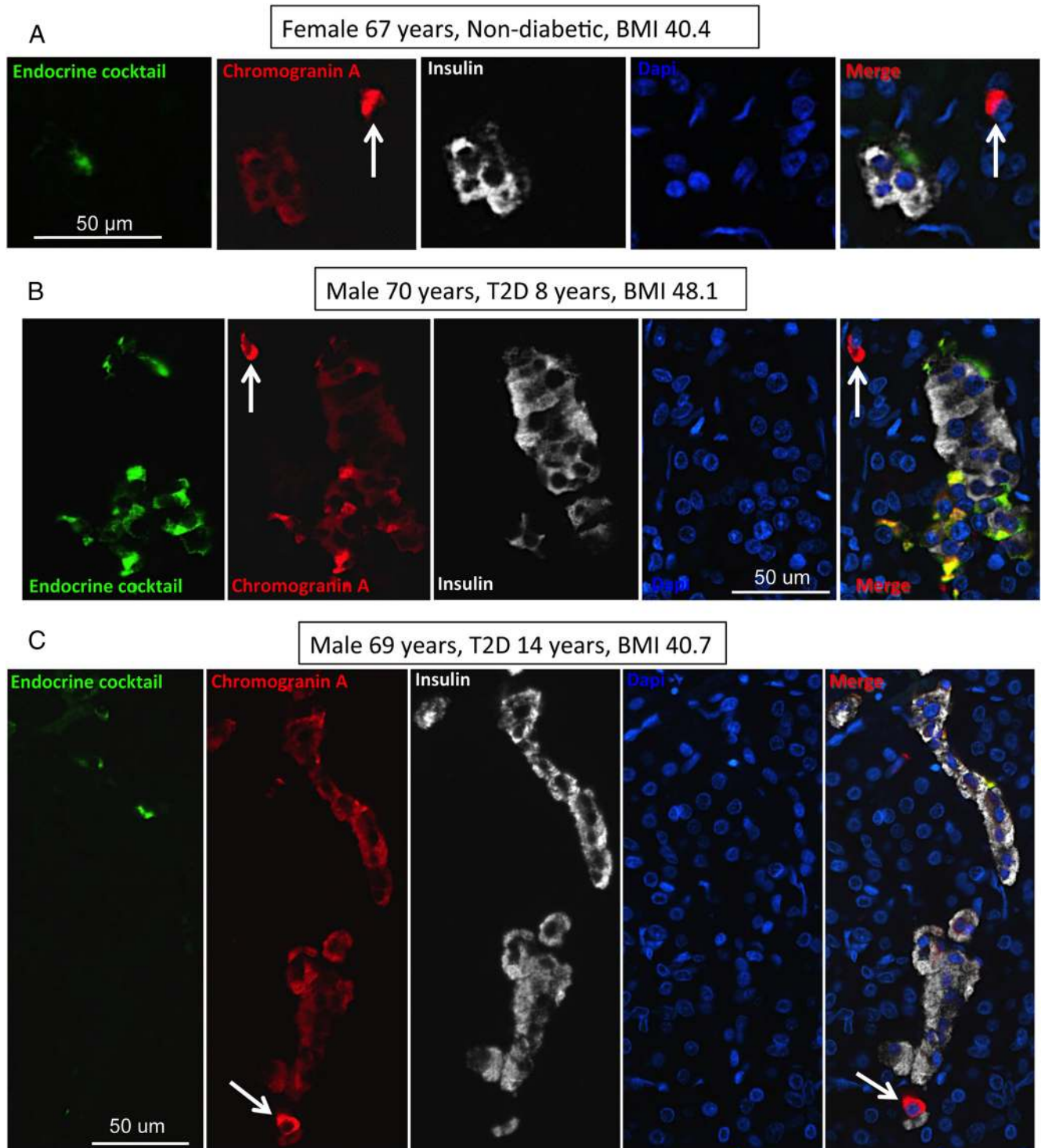


Figure 3. Examples of Chromogranin Positive Hormone Negative [CPHN] cells in pancreas from an Obese Non-Diabetic [OND] subject (A) and two Obese type 2 Diabetic [OT2D] subjects (B and C). Individual layers stained for Endocrine cocktail (Glucagon, Somatostatin, Pancreatic Polypeptide and Ghrelin) [green], Chromogranin A [red], Insulin [white] and DAPI [blue] are shown along with the merged image. Arrows indicate Chromogranin Positive Hormone Negative [CPHN] cells. Scale bar is 50 microns.

pancreas obtained at autopsy or from brain dead organ donors, there is a β -cell deficit in type 2 diabetes (3–6). In humans, pigs, and nonhuman primates there is a wide variance in β -cell mass in nondiabetic individuals. However, once the β -cell mass approaches $\sim 50\%$ of the mean, an apparent threshold is reached beyond which there is a steep rise in fasting blood glucose (and therefore decline in β -cell function) for any further decrement in β -cells (17–20). Collectively, these findings highlight the importance of a sufficiency of β -cells and that an insufficient β -cell mass, even if induced by a partial pancreatectomy (18, 21), leads to β -cell dysfunction.

Recently, the basis of the loss of β -cell mass in type 2 diabetes has been an area of increased interest. We and others have reported that β -cells in type 2 diabetes are

characterized by endoplasmic reticulum stress and an increase in β -cell apoptosis (22–24). In species vulnerable to type 2 diabetes, islet pathology is notable for the presence of islet amyloid deposits derived from islet amyloid polypeptide, a protein that forms toxic oligomers in β -cells in type 2 diabetes (25). While it is not possible to perform prospective studies comparing islet pathology with evolving diabetes in humans, in a unique prospective study of a nonhuman primate (*Macaca nigra*) there was a progressive increase in islet amyloid and loss of islet cells in relation to the transition from nondiabetic through borderline diabetes to diabetes (26). A recently published cross sectional study of both humans and nonhuman primates with and without type 2 diabetes, reported an increased proportion of β -cells that display altered identity in type 2

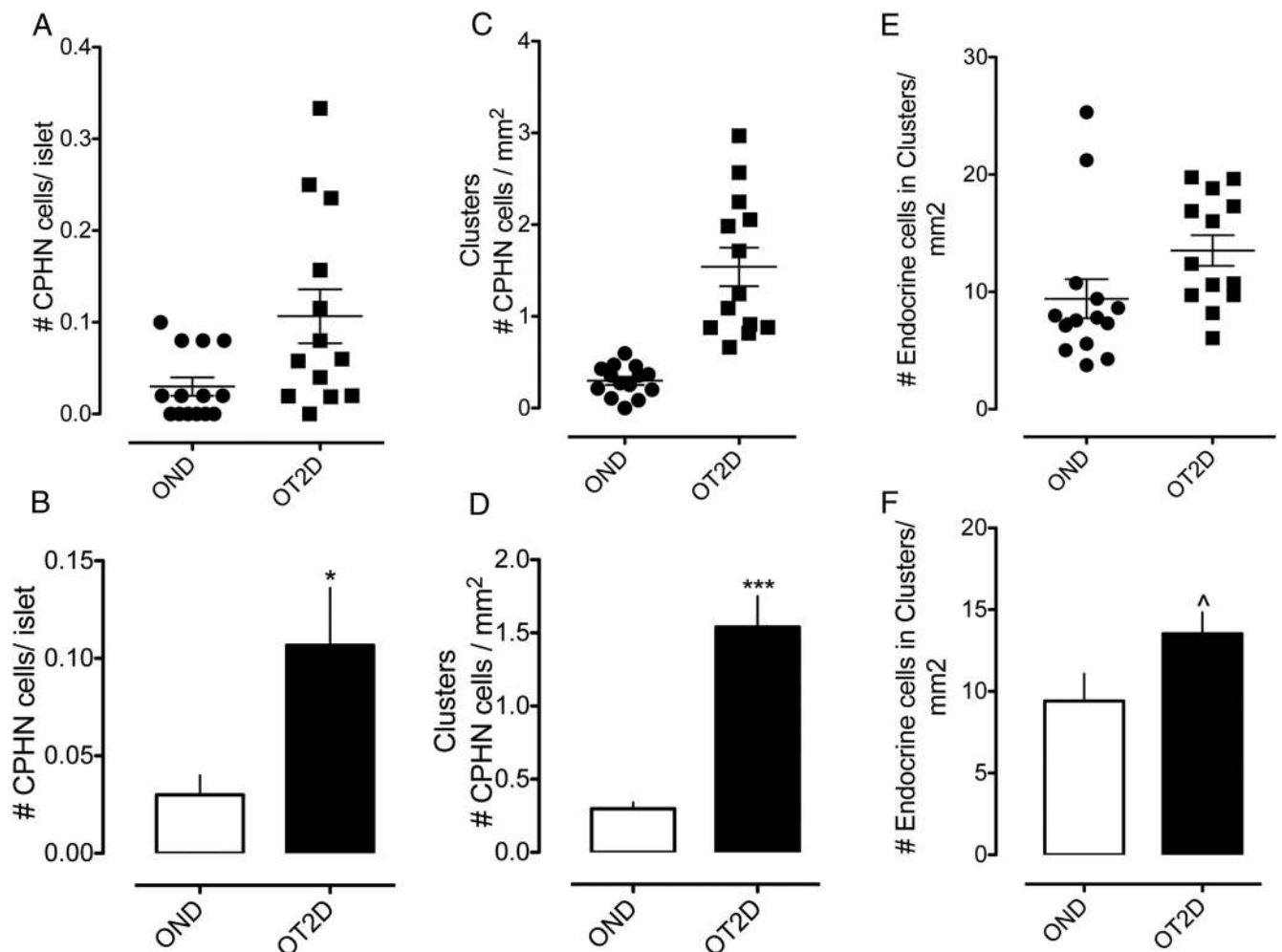


Figure 4. The number of nonhormone expressing endocrine cells (Chromogranin Positive Hormone Negative [CPHN] cells) in obese nondiabetic subjects [OND] and obese subjects with type 2 diabetes [OT2D]. (A and B) The number of nonhormone expressing endocrine cells (Chromogranin Positive Hormone Negative [CPHN] cells) per islet cross section is increased in the obese type 2 subjects (0.11 ± 0.03 vs 0.03 ± 0.01 cells/islet, OT2D vs OND, $*P < .01$). (C and D) The number of nonhormone expressing endocrine cells (Chromogranin Positive Hormone Negative [CPHN] cells) present in scattered clusters of endocrine cells is increased in OT2D (1.54 ± 0.21 vs 0.30 ± 0.05 cells/mm², OT2D vs OND, $***P < .0001$). (E and F) The number of endocrine cells (both hormone expressing and nonhormone expressing) present in scattered clusters of endocrine cells is increased in OT2D (13.53 ± 1.31 vs 9.41 ± 1.67 cells/mm² of pancreas, OT2D vs OND, $P < .05$). Panels A, C and E: Black circles = Obese Nondiabetic [OND] subjects; Black squares = Obese type 2 Diabetic [OT2D] subjects. Panels B, D and F: White bars = Obese Nondiabetic [OND] subjects; Black bars = Obese type 2 Diabetic [OT2D] subjects.

diabetes, and that these cells were more abundant in proximity to islet amyloid deposits (16). Loss of cell identity in that study was characterized by cells that expressing both insulin and glucagon, or by cells expressing the β -cell transcription factor Nkx6.1 and glucagon, both of which were increased in type 2 diabetes. We previously reported a comparable increased proportion of β -cells ($\sim 5\%$) coexpressing glucagon in type 2 diabetes (6). Perhaps more significant as regards the impact on functional β -cell mass, the β -cell transcriptional factor musculoaponeurotic fibrosarcoma oncogene family A (MafA) was detectable in less than 2% of β -cells in humans with type 2 diabetes compared to $\sim 46\%$ in nondiabetic controls, a change that has most often been attributed to glucotoxicity (15). The challenge with ascribing these changes in cell identity to cause or consequence in type 2 diabetes in humans is that all studies of human pancreas are necessarily cross-sectional. Moreover, while partially differentiated endocrine cells may represent dedifferentiation, an alternative hypothesis is that they are derived from newly forming cells, or regeneration (16, 27).

To further consider that possibility, we examined human pancreas obtained through the third trimester of human development and early infancy as a period of known new islet cell formation from endocrine precursors to probe for parallels with the findings in pancreas of adults with type 2 diabetes. As previously reported, newly forming islets in late gestation and early infancy appear to form via the coalescence of scattered foci of endocrine cells (28). Consistent with the assumption that scattered cells in the exocrine pancreas are the precursors of the developing islets, in the fetal pancreas $\sim 40\%$ of these scattered endocrine cells marked as chromogranin A positive did not yet express a known islet hormone. These chromogranin A positive hormone negative cells were also observed

within the islets, amounting to $\sim 15\%$ of islet endocrine cells. By infancy, the corresponding values were $\sim 20\%$ of scattered endocrine cells and 4% of islet endocrine cells that expressed no known islet hormone. Collectively these findings support the concept that newly forming human islet cells arise from dispersed foci in pancreas, initially expressing chromogranin A but no known islet hormone, and then coalescing to form islets, while differentiating to an endocrine cell lineage. Given that the distribution of hormone negative chromogranin A positive cells in type 2 diabetes mirrors that present during islet formation during development, it is plausible that the pattern in type 2 diabetes signals ongoing attempted beta cell regeneration. Of note, while scattered hormone negative endocrine cells are more abundant in type 2 diabetes than nondiabetics, the magnitude of these are still much less than in the developing pancreas, consistent with a relatively low, and apparently insufficient, rate of regeneration. Of interest, the increase in β -cell mass in pregnant humans is also characterized by an increase in small scattered foci of β -cells (29). Taken together, these data suggest that there may be a relatively low attempted regeneration of beta cells in adults with type 2 diabetes. On the other hand, an alternative explanation for the predominantly scattered localization of these hormone negative endocrine cells within pancreas could be that these cells are the product of β -cell dedifferentiation that have lost cell-cell contact and migrated (30). In the absence of lineage tracing in humans, however, it is unknown if the hormone negative endocrine cells, and those with a mixed identity, in type 2 diabetes arise from degranulation of previously mature endocrine cells, or are incompletely mature newly formed endocrine cells.

While our data question the concept that the measured deficit in β -cells in type 2 diabetes in humans can be pri-

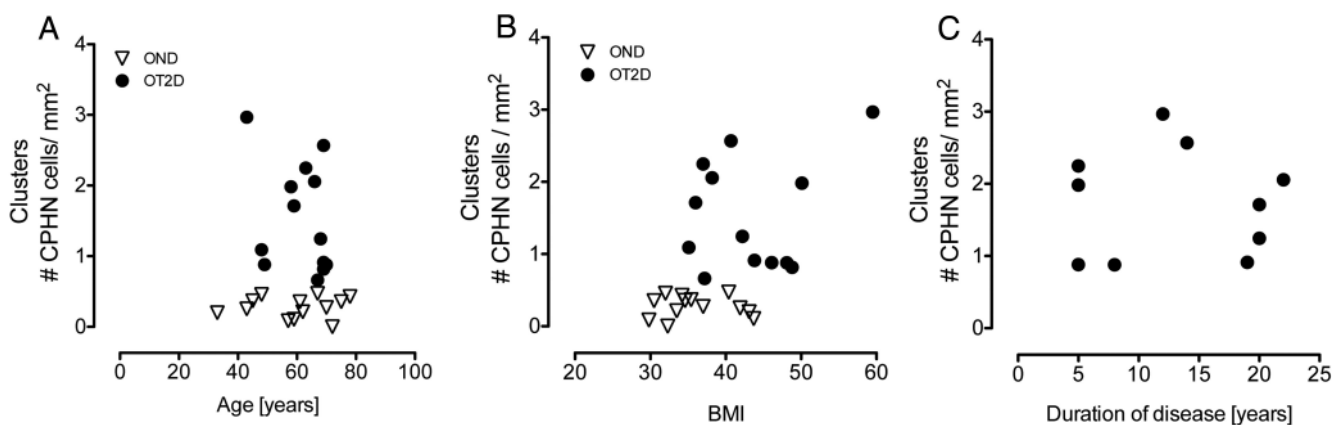


Figure 5. The relationship between the scattered clusters of nonhormone expressing endocrine cells (Chromogranin Positive Hormone Negative [CPHN] cells) in obese nondiabetic subjects and obese subjects with type 2 diabetes with age, BMI and duration of disease. There was no relationship of the CPHN cells with age (A), BMI (B) or duration of disease (C). Panels A and B show the 13 OT2D subjects included in the study; panel C shows only 10 OT2D subjects as duration of disease was not known in 3 OT2D subjects. In all panels: Open triangles = Obese Nondiabetic [OND] subjects; Black circles = Obese type 2 Diabetic [OT2D] subjects.

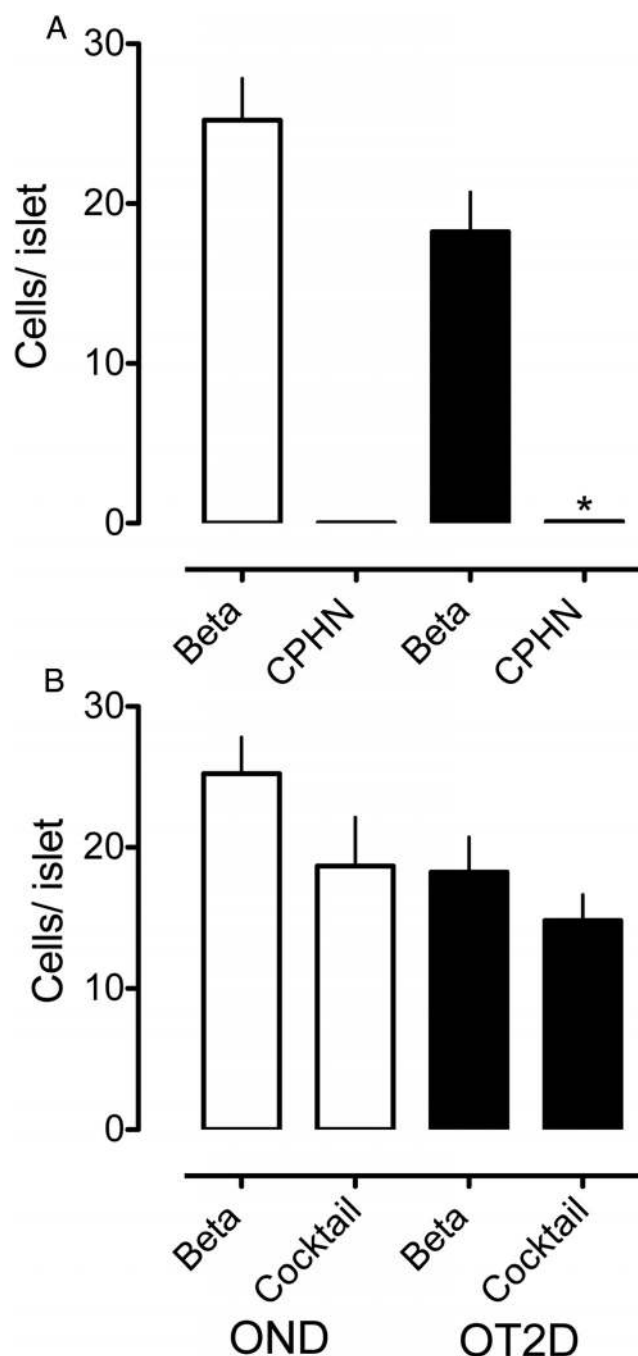


Figure 6. The impact of nonhormone expressing endocrine cells on the deficit in β -cell mass in type 2 diabetes is minimal. The number of cells/islet of the CPHN cell fraction is very small when compared with the number of β -cells/islet in the OND, and the deficit in β -cells in the OT2D subjects cannot be accounted for by the increase in CPHN cells in type 2 diabetes. A. There is an approximate 30% deficit in β -cells in the islets from the subjects with type 2 diabetes (18.23 ± 2.49 vs 25.23 ± 2.59 β -cells/islet, OT2D vs OND, $P < .05$). The number of CPHN cells/islet is increased in type 2 diabetes (0.11 ± 0.03 vs 0.03 ± 0.01 CPHN cells/islet, OT2D vs OND, $*P < .01$) but this increase does not account for the β -cell deficit seen in type 2 diabetes. B. Islet endocrine cells expressing islet hormones other than insulin are not increased in type 2 diabetes (14.83 ± 1.83 vs 18.67 ± 3.47 endocrine cocktail cells/islet, OT2D vs OND, $p=ns$). Therefore, the β -cell deficit in type 2 diabetes (18.23 ± 2.49 vs 25.23 ± 2.59 β -cells/islet, OT2D vs OND, $P < .05$) cannot be explained by conversion of β -cells to other endocrine cells. White bars = Obese Non-Diabetic [OND] subjects;

marily explained by degranulation or dedifferentiation, we agree that the partial loss of molecular identity of β -cells in diabetes likely plays a major role in β -cell dysfunction, most likely in both type 1 and 2 diabetes as well as secondary diabetes (for example in pancreatitis) (11, 16). Moreover, we agree with the group of Accili that reversal of β -cell deficit through the induction of endogenous β -cell formation without correcting the alterations in cell identity may be therapeutically unsuccessful (16, 31). In a small study of brain dead organ donors, we previously reported that while prior GLP-1 based therapy in type 2 diabetes was associated with a complete reversal of the deficit in β -cell mass, the individuals still had diabetes (6). Of interest, GLP-1 based therapy in humans was also associated with a marked increase in the percentage of cells coexpressing insulin and glucagon, further suggesting that these cells with a mixed identity may be newly formed rather than formed by β -cell dedifferentiation (6).

In conclusion, based on the present studies, the deficit in β -cells in type 2 diabetes is not attributable alone to degranulation or dedifferentiation of β -cells. As previously discussed, given the cross-sectional nature of human studies, it is not possible to know if the deficit in β -cells precedes disease onset, and contributes to diabetes risk, or is only a consequence of loss of β -cells (32). Given the closely shared molecular and pathological characteristics of the islet in humans with type 2 diabetes and the relevant neurological tissue in neurodegenerative diseases, the concept that β -cell dysfunction and attrition occurs through mechanisms related to protein misfolding is not inconsistent with studies reporting changes in β -cell transcriptional networks and cellular identity. Moreover, type 2 diabetes is a heterogeneous disease, and it is plausible that there are other groups of patients (perhaps lean patients, as opposed to the obese patients reported here) in which β -cell dedifferentiation and/or transdifferentiation play a more prominent role towards β -cell deficit. Moreover, given that the remaining beta cells in type 2 diabetes, as in the remaining neurons in neurodegenerative diseases, are dysfunctional and have an altered transcriptional identity (14, 15, 16), we agree with the proposition that efforts to reverse those functional changes, perhaps by addressing the altered molecular identity (11), is a worthy therapeutic target.

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Legend to Figure 5 Continued. . .

Black bars = Obese type 2 Diabetic [OT2D] subjects.

and performed the morphological analysis. AEB, SD, HF, JJM, RAR, PCB researched data, wrote the manuscript, reviewed the manuscript, edited the manuscript, and contributed to the discussion. We thank Bonnie Lui for editorial assistance. Funding for these studies was from NIH/NIDDK Grant #DK077967 and the Larry Hillblom Foundation Grant #2014-D-001-NET.

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