Pancreatic β-Cells Express the Fetal Islet Hormone Gastrin in Rodent and Human Diabetes

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β-Cell failure in type 2 diabetes (T2D) was recently proposed to involve dedifferentiation of β-cells and ectopic expression of other islet hormones, including somatostatin and glucagon. Here we show that gastrin, a stomach hormone typically expressed in the pancreas only during embryogenesis, is expressed in islets of diabetic rodents and humans with T2D. Although gastrin in mice is expressed in insulin+ cells, gastrin expression in humans with T2D occurs in both insulin+ and somatostatin+ cells. Genetic lineage tracing in mice indicates that gastrin expression is turned on in a subset of differentiated β-cells after exposure to severe hyperglycemia. Gastrin expression in adult β-cells does not involve the endocrine progenitor cell regulator neurogenin3 but requires membrane depolarization, calcium influx, and calcineurin signaling. In vivo and in vitro experiments show that gastrin expression is rapidly eliminated upon exposure of β-cells to normal glucose levels. These results reveal the fetal hormone gastrin as a novel marker for reversible human β-cell reprogramming in diabetes.

Failure of pancreatic β-cells to compensate for increased demand is a central event in the pathogenesis of type 2 diabetes (T2D). It is thought that a vicious cycle of glucotoxicity harms β-cells and further increases glucose levels and metabolic load, but the underlying mechanisms remain incompletely understood. β-Cell failure may result from chronic endoplasmic reticulum (ER) stress or oxidative stress, leading to stunned β-cells that fail to secrete bioactive insulin (1,2). Alternatively, β-cell failure was proposed to result from β-cell death or failed β-cell replication, leading to reduced β-cell mass. This view is supported by autopsy studies, which suggested that people with T2D have, on average, a 50% reduction in β-cell mass compared with BMI-matched control subjects without T2D (3). More recently, Talchai et al. (4) proposed that β-cell failure occurs to a large extent via dedifferentiation, causing an apparent decrease of β-cell mass. According to this model, most β-cells remain alive in T2D but lose the ability to express insulin and other hallmarks of differentiation and revert to a fetal-like state characterized by expression of the endocrine progenitor regulator neurogenin3 (NeuroG3), subsequently gaining expression of other islet hormones such as glucagon and somatostatin (4). The idea of β-cell dedifferentiation, followed by expression of noninsulin hormones, was supported by several additional studies, which also showed that normalization of glycemia reverses the phenomenon (5,6). However, controversy remains, in particular regarding the existence and magnitude of the phenomenon in human diabetes (7,8). Notably, all solid demonstrations of dedifferentiation so far have been based on analysis of genetically engineered mouse models, where genetic lineage tracing could prove that preexisting β-cells are losing cell-specific identity and turning on non-β-cell genes. Current evidence for dedifferentiation in spontaneous models of diabetes in rodents and humans is indirect, relying mostly on observations of cells coexpressing insulin and glucagon or somatostatin, a phenomenon

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that could be explained in multiple ways (e.g., preexisting α- or δ-cells gaining expression of insulin) (9).

We previously characterized the developmental determinants of pancreatic G cells expressing the hormone gastrin (10). These cells form abundantly during embryonic development of the pancreas from the same NeuroG3+ endocrine progenitor cells that give rise to all islet cells. Around birth, however, all pancreatic gastrin+ cells disappear and are never seen in the adult pancreas other than in rare pancreatic gastrinomas.

Here we report that gastrin expression is induced in β-cells in multiple settings of diabetes, including human T2D. We demonstrate that gastrin expression depends on glucose metabolism acting via membrane depolarization and calcineurin signaling and is reversible upon normalization of glycemia. We also show that dedifferentiation to a fetal progenitor state is not involved. In addition to these molecular insights, gastrin expression provides a valuable biomarker for β-cell reprogramming, or loosened identity, in human T2D.

**RESEARCH DESIGN AND METHODS**

**Immunostaining**

Primary antibodies used in this study included rabbit anti-gastrin (1:200; Cell Marque), guinea pig anti-insulin (1:400; Dako), mouse anti-glucagon (1:800; Abcam), mouse anti-somatostatin (1:400; BCBC), goat anti-green fluorescent protein (GFP) (1:400; Abcam), mouse anti-α-nx6.1 (1:200; BCBC), rabbit anti-mafA (1:300; Bethyl), goat anti-pdx1 (1:2,500, a gift from Chris Wright), and mouse anti-NeuroG3 (1:500; Hybridoma Bank). Secondary antibodies were from Jackson ImmunoResearch. Fluorescent images were taken on a Nikon C1 confocal microscope at original magnification ×40.

**Proximity Ligation Assay**

After incubation with primary antibodies rabbit anti-gastrin (1:1,500) and mouse anti-insulin (1:10,000; Abcam), proximity ligation assay (PLA) was performed (Duolink In Situ Orange Starter Kit Mouse/Rabbit, DUO92102; Sigma-Aldrich) according to the manufacturer’s instructions. Briefly, slides were washed and incubated in PLA solution for 1 h at 37°C. Slides were washed, and ligation was performed at 37°C for 30 min, followed by incubation in amplification-polymerase solution for 100 min at 37°C. Secondary antibodies were added and incubated at room temperature for 2 h. Slides were washed and mounted with Duolink In Situ Mounting Medium with DAPI and visualized as described above.

**Real-Time PCR**

RNA was isolated and purified from fresh islets with TRI Reagent (Sigma-Aldrich) and an RNaseasy Micro Kit (Qiagen). cDNA was prepared from 50 ng RNA by a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For quantitative real-time PCR, we used SYBR Green mix (Quanta Biosciences) and the following primers:

*Gastrin (5’-GCTGGCTAGCTCCTCC-3’, 5’-TGCTTC TTGGACAGTGCTGCA-3’), NeuroG3 (5’-ACTAGCCTGCTCCTATTTTT-3’, 5’-GGGCCCTCTGCTGCTC-3’), Oct4 (5’-AGAGGAACCTCCTCAGACG-3’, 5’-CCTGG GAAAGGTCTGCTGTA-3’), Nanog (5’-CTGAGGAACATGCAATTCTG-3’, 5’-TGAAGGCCAGTCTCCAGAGG-3′), and β-actin (5’-CACAGCTTTTCTAGCTCCT-3’, 5’-GTC ATCCATGGCGAACTG-3’).

Reactions were performed in triplicate in 96-well plates using the CFX96 Real-Time System (Bio-Rad). Reactions were performed in triplicate with biological replicates. The relative amount of mRNA was calculated using the comparative Ct method after normalization to β-actin. Gastrin cycles in purified islets ranged between 23.2 and 24.2 (db/db 3 months old) and 28.4 and 30.7 (controls), 23.2 (Akita) and 25.6 and 29.4 (controls), 26 and 29 (S961), and 31 and 32.2 (PBS). Actin cycles were between 21 and 25. Gastrin cycles in cultured islets ranged from 29.1 to 30.5 (25 mmol/L glucose) to 32.4 to 34 (5 mmol/L glucose), whereas actin in these conditions was seen after 24.2–25 cycles.

**Animals**

Mouse strains used in this study included lepRmut/mut (db/db) (The Jackson Laboratory), Ins2WT/C96Y (Akita) (The Jackson Laboratory), Insulin-tTA,TET-DTA (BDTA) (11), Insulin-CreER (12), NeuroG3lox/lox (13), Rosa26-LSL-Kir6.2-V59M (14), and Rosa26-LSL-YFP (15). As controls we used wild-type or single transgenic littermates. Psammomys obesus (desert gerbil) were raised on low- or high-calorie diets, as described (16). The joint ethics committee (Institutional Animal Care and Use Committee) of the Hebrew University and Hadassah Medical Center approved the study protocol for animal welfare. The Hebrew University is an Association for Assessment & Accreditation of Lab Animal Care Internationally accredited institute.

**Human Samples**

We used paraffin sections that were obtained from the pancreata of brain-dead patients, after institutional review board permission was granted. Maximal warm ischemia time before fixation was 6 h. Patient details are presented in Supplementary Table 1.

**Mouse Procedures**

Tamoxifen (20 mg/mL in corn oil; Sigma-Aldrich) was injected subcutaneously to normoglycemic adult mice (1 month old). Two daily doses of 8 mg were used to achieve GFP marking and/or deletion of NeuroG3 or activation of βKir6.2-V59M transgene in β-cells.

Insulin receptor antagonist S961 was a gift from Novo Nordisk. Vehicle (PBS) or S961 (12 nmol) was loaded into an ALZET osmotic pump (model 2001) and implanted subcutaneously on the back of 6-week-old ICR mice. Diabetes-prone male P. obesus (Hebrew University Colony; Harlan, Jerusalem, Israel) were fed low-energy
normoglycemia-maintaining diet (9.96 kJ/g; Koffolk, Petach-Tikva, Israel) or a high-energy diet (14.23 kJ/g; Teklad Global Diets) leading to diabetes (defined as glucose levels >200 mg/dL).

Doxycycline (Dexon) was given to 1-month-old male Insulin−rtTA;TET−DTA mice in the drinking water (200 µg/mL doxycycline, 2% w/v sucrose) for 7 days. Severely hyperglycemic mice (blood glucose >500 mg/dL) were implanted subcutaneously with insulin pellets (LinBit implants; LinShin, Scarborough, ON, Canada; two implants for the first 20 g body weight and another pellet for each additional 5 g) or left untreated. Mice were sacrificed 2 months after implantation.

Long-term insulin detemir (Levemir) was injected subcutaneously to diabetic db/db mice, twice daily for 8–16 days, to normalize glycemia. Insulin doses were adjusted depending on measured blood glucose levels.

**Islet Procedures**

Islets were isolated using collagenase P (Roche Applied Science) injected to the pancreatic duct, followed by Histopaque (1119 and 1077; Sigma-Aldrich) gradient. Islets were incubated overnight in standard RPMI-1640 medium (Biological Industries) supplemented with 10% FBS, L-glutamine, and penicillin-streptomycin in a 37°C, 5% CO₂ incubator. Hand-picked islets (30–70 islets) were placed for 42–48 h in 5 mmol/L or 25 mmol/L glucose RPMI medium and treated with 325 µmol/L diazoxide (Sigma-Aldrich), 10 µmol/L nifedipine (Alomone Labs), 37 nmol/L tacrolimus (Astellas Pharma), 0.5 µmol/L gliburide (Sigma-Aldrich), or 3 µmol/L Bay-K8644 (Alomone Labs).

**RESULTS**

**Gastrin Expression in β-Cells of Diabetic db/db Mice**

Immunostaining for gastrin revealed abundant expression in islets of diabetic db/db mice, which lack the leptin receptor (Fig. 1A). The vast majority of gastrin-expressing cells were β-cells, as judged by coexpression with insulin, proinsulin, Pdx1, and Nkx6.1 (Fig. 1B and Supplementary Fig. 1). No gastrin staining was observed in age-matched wild-type controls and in young, normoglycemic db/db mice, suggesting gastrin expression is linked to metabolic status rather than being caused by a deficiency in leptin signaling (Fig. 1C). Gastrin protein was found in ~9% of β-cells in diabetic db/db mice (Fig. 1D). The induction of gastrin expression was validated by real-time PCR of islet cDNA (Fig. 1E). Islets of young, mildly hyperglycemic db/db mice showed a small elevation of gastrin mRNA, although we were not able to observe gastrin protein staining in these mice.

To determine the cellular origins of gastrin-expressing cells, we traced their lineage using the Cre-lox system. We generated db/db mice that express tamoxifen-dependent Cre recombinase in β-cells (Insulin-CreER) (17), as well as a fluorescent Cre reporter (Rosa26-LSL-YFP) (15). We injected tamoxifen at 1 month of age to permanently pulse-label β-cells with YFP and sacrificed the animals 2.5 months later, after they had developed diabetes. Half of the gastrin+ cells expressed YFP, proving that they derived from preexisting β-cells (Fig. 1F and G). The gastrin+ cells that were not labeled with YFP could theoretically derive from non-β-cells or from β-cells that were not labeled at the time of the tamoxifen injection. Costaining for gastrin, insulin, glucagon, and somatostatin revealed that gastrin colocalized only with insulin, further supporting its exclusive association with β-cells (Supplementary Fig. 1). To determine whether insulin and gastrin reside in the same cellular compartment, we used PLA, an immunostaining technique that indicates whether two proteins are located less than 40 nm apart (18). Insulin and gastrin antibodies generated a clear signal in the β-cells of diabetic db/db mice (Fig. 1H), suggesting colocalization, likely within the same granules (average granule diameter, 300 nm [19]). These results reveal that β-cells in diabetic db/db mice turn on expression of gastrin mRNA and protein.

**Gastrin Expression in Multiple Rodent Models of Hyperglycemia**

We conducted experiments to determine whether the induction of gastrin expression in β-cells was specific to the db/db model or a generalized response to hyperglycemia. Conditional ablation of the majority of β-cells in mice using doxycycline-induced expression of diphtheria toxin, led to severe hyperglycemia (11), and induced gastrin expression in ~5% of surviving β-cells as early as 10 weeks after the onset of hyperglycemia (Fig. 2A and B). A subset of β-cells in hyperglycemic Akita mice, a model of diabetes resulting from misfolding of insulin (20), also expressed gastrin protein, and Akita islets had more gastrin mRNA than islets of control mice (Fig. 2C–E).

Further, treatment of wild-type adult mice with the insulin receptor antagonist S961, which causes severe insulin resistance and hyperglycemia (21), induced gastrin expression in a similar subset of β-cells (Fig. 2F and G). Finally, P. obesus, a model of acute diet-induced diabetes (22), showed gastrin expression in β-cells when fed a high-calorie but not a low-calorie diet (Fig. 2H). Thus, expression of gastrin in a subset of β-cells appears to be a general feature of rodent β-cells exposed to hyperglycemia.

**Gastrin Expression in Islets of Humans With T2D**

We asked whether islet gastrin expression occurs also in humans. Although no gastrin expression was found in islets of individuals without T2D, we observed gastrin expression in ~2.5% of islet cells in patients with T2D (Fig. 2I and J). These findings are consistent with a significant elevation of gastrin mRNA in one published transcriptome of islets from humans with T2D (23) (false discovery rate = 0.04274, P = 0.00969), although no significant change was observed in two other data sets (24,25).

Costaining experiments revealed that 72% of gastrin+ cells in human T2D islets coexpressed somatostatin, whereas 17% coexpressed insulin and 11% were negative for both insulin and somatostatin (Fig. 2K and L).
Figure 1—Gastrin is expressed in β-cells of diabetic db/db mice. A: Costaining for gastrin (Gast), insulin (Ins), and Pdx1 in islets of 3-month-old (3M) control and diabetic db/db mice. Arrows point to gastrin+ cells. B: High magnification confocal images show that gastrin is coexpressed with insulin, Nkx6.1, and Pdx1 in db/db mice. C: Gastrin is not expressed in β-cells of 1-month-old (1M) db/db mice, before the onset of hyperglycemia. Right panel shows only the gastrin and Pdx1 channels, emphasizing the lack of gastrin staining. D: Percentage of β-cells stained positive for gastrin in 3- to 4-month-old control and diabetic db/db mice. ***P < 0.001. E: Quantitative real-time PCR analysis of gastrin mRNA expression in islets of 1-month-old db/db mice and in 3-month-old db/+ (heterozygous) mice, C57/BL6 mice, and diabetic db/db mice (n = 4 in each group). Results were normalized to β-actin. *P < 0.05; ***P < 0.001. F: Lineage tracing experiment demonstrates that gastrin is expressed in β-cells that existed before diabetes. Immunostaining for insulin, gastrin, and GFP in the pancreas of db/db;Insulin-CreER;Rosa26-LSL-YFP mice injected with tamoxifen at 1 month and sacrificed when severely diabetic at 3.5 months of age. Arrows point to gastrin+YFP+ cells. YFP was detected using an anti-GFP antibody. G: Efficiency of β-cell Cre-mediated labeling (percentage of insulin+ cells that stain for GFP) and fraction of gastrin+ cells that carry the mark of preexisting β-cells in db/db mice. Four mice were analyzed, and >3,000 insulin+ and >130 gastrin+ cells were counted per mouse. **P < 0.01. H: PLA to assess proximity of gastrin and insulin proteins in diabetic db/db mice. GastPLA and InsPLA are controls where only the indicated primary antibody was added. Scale bars = 20 μm.
Figure 2—Expression of gastrin in other diabetes models and in patients with T2D. A: Gastrin (Gast) expression in β-cells of diabetic Insulin-rtTA;TET-DTA mice, 11.5 weeks after administration of doxycycline for 1 week. Arrows in all panels point to gastrin+ cells. B: Quantification of the percentage of β-cells expressing gastrin in Insulin-rtTA;TET-DTA (n = 4 mice). In each mouse, >1,800 β-cells were counted. Blood glucose levels were >580 mg/dL. *P < 0.05. C: Expression of gastrin in islets of 5.5-month-old diabetic Akita mice. Gastrin is coexpressed with both insulin (Ins) and Pdx1. D: Percentage of β-cells stained for gastrin in 3- to 5.5-month-old Akita mice. *P < 0.05. E: Quantitative real-time PCR analysis of gastrin mRNA in islets isolated from diabetic Akita mice (n = 3; average blood glucose, 591 mg/dL) and control mice (n = 3; average blood glucose, 170 mg/dL). Results were normalized to β-actin. *P < 0.05. F: Gastrin...
Thus, expression of gastrin marks a subset of islet cells in human T2D. We could not determine the origins of human gastrin+ islet cells, but the coexpression patterns suggest that, unlike the situation in mice (likely pure β-cell origins), human gastrin+ islet cells may derive from both δ- and β-cells. Because gastrin is not expressed in islets of healthy adult humans, its presence provides a novel and convenient biomarker for deregulated hormone expression in T2D islets, including β-cells.

**NeuroG3 Is Not Required for Gastrin Expression in β-Cells of Adult db/db Mice**

Previous studies suggested that β-cell dedifferentiation in diabetes involves the expression, and presumably the activity, of the embryonic progenitor cell determinant NeuroG3 (4–6). That β-cell dedifferentiation involved the pluripotency markers Nanog and Oct4 was also proposed (4). To examine this idea, we investigated the expression of these factors in models of spontaneous diabetes in mice. We did not observe expression of Oct4 or Nanog mRNA in islets of db/db mice, Akita mice, and wild-type mice treated with S961, nor in islets cultured in high glucose, although we did detect high-level expression in embryonic stem cells (Supplementary Fig. 2). Similarly, expression of NeuroG3 was undetectable in islets of db/db mice, using both immunostaining and quantitative real-time PCR (Fig. 3A and B), in islets of Akita and S961-treated mice, and in islets cultured in high glucose (Supplementary Fig. 2), whereas NeuroG3 protein and mRNA were readily detectable in the fetal pancreas.

The inability to detect NeuroG3 in db/db islets argues against involvement of this factor in β-cell dedifferentiation or reprogramming but does not rule out this possibility because transient expression of NeuroG3 could have a biological effect and still be missed by expression analysis. We examined this idea more rigorously by deleting NeuroG3 in β-cells of db/db mice before the onset of hyperglycemia (or gastrin expression). We generated db/db mice that contained a floxed allele of NeuroG3 (NeuroG3<sup>lox/lox</sup>), an Insulin-CreER transgene, and a Rosa26-LSL-YFP lineage reporter. Tamoxifen injection at 4 weeks of age should result in β-cell–specific deletion of NeuroG3 and expression of YFP. We examined the pancreas for evidence of recombination and gastrin expression 5–8 weeks after the tamoxifen injection, when the mice were severely diabetic. Widespread expression of YFP indicated efficient Cre-mediated recombination and hence NeuroG3 deletion in β-cells (Fig. 3C). Islets of diabetic NeuroG3-deleted db/db mice had abundant gastrin+ cells (Fig. 3D and E), and quantification revealed that the numbers of gastrin+ cells were similar to diabetic db/db animals, which have an intact NeuroG3 gene (Fig. 3F). To more directly look at β-cells that have lost NeuroG3, we costained for insulin, YFP, and gastrin. YFP+gastrin+ cells were readily detected, including some that were negative for insulin. This result further indicates that NeuroG3-deficient β-cells are capable of turning on gastrin expression (Fig. 3D).

**Gastrin Expression in β-Cells Is Triggered by Glucose and Requires Membrane Depolarization, Calcium Influx, and Calcineurin Signaling**

To understand how diabetes causes gastrin expression in β-cells, we tested the role of elevated glucose. We incubated wild-type mouse islets in normal and high concentrations of glucose and determined gastrin expression by quantitative real-time PCR. As early as 48 h after exposure to high glucose (25 mmol/L), there was a massive upregulation of gastrin mRNA in islets, which increased further after 72 h (Fig. 4A). These results show that high levels of glucose are sufficient to trigger gastrin expression in islet cells.

To further examine how glucose drives gastrin expression, we examined the role of membrane depolarization, a key step in the pathway leading from glucose uptake to insulin secretion. We generated db/db mice with a β-cell–specific, conditional allele that causes hyperactivation of the K<sub>ATP</sub> channel (Insulin-CreER;Rosa26-LSL-Kir6.2-V59M). β-Cells expressing the mutant K<sub>ATP</sub> channel do not depolarize when exposed to glucose, and hence fail to activate voltage-gated calcium channels. Consequently, they fail to secrete insulin (14). Tamoxifen injection in 1-month-old db/db mice containing both Insulin-Cre and Kir6.2 transgenes led to immediate hyperglycemia, similar to that which gradually developed in unmanipulated db/db mice. Strikingly, the presence of mutant Kir6.2 reduced the fraction of gastrin+ β-cells in diabetic db/db mice by 70% (Fig. 4B and C). Mice expressing the Kir6.2 mutant in the background of wild-type leptin receptor still had a small fraction of gastrin+ cells (compared with zero in wild-type mice), potentially resulting from the effect of systemic hyperglycemia on non-recombined β-cells (Fig. 4B). Further supporting these findings, treatment of cultured wild-type islets with diazoxide, a K<sub>ATP</sub> channel opener, prevented glucose-stimulated expression of gastrin (Fig. 4D). Finally, we incubated islets with glyburide, a drug that forces K<sub>ATP</sub> channel closure. This
treatment was not sufficient to induce gastrin expression on the background of 5 mmol/L glucose (Fig. 4E). These findings indicate that closure of K<sub>ATP</sub> channels is a necessary but not sufficient step in the pathway by which high glucose stimulates gastrin expression in β-cells.

Downstream of K<sub>ATP</sub> channels, the glucose-stimulated insulin secretion pathway in β-cells involves calcium influx. The calcium channel blocker nifedipine blocked glucose-induced gastrin expression in islets (Fig. 4F), but the calcium channel opener BayK8644 did not induce gastrin expression in islets cultured in 5 mmol/L glucose (Fig. 4G). Thus, calcium influx is necessary but not sufficient for gastrin expression in adult islets. Finally, we treated islets with tacrolimus, an inhibitor of calcineurin, a major signaling pathway that acts downstream to calcium. Tacrolimus effectively inhibited glucose-stimulated gastrin expression (Fig. 4H).

These findings reveal a pathway for gastrin expression in islets involving glucose metabolism, membrane depolarization, calcium entry, and calcineurin signaling.

**Gastrin Expression Is Reversible Upon Normalization of Glycemia**

Previous reports showed that several aspects of β-cell dedifferentiation—specifically the appearance of insulin ‘glucagon’ cells and NeuroG3<sup>+</sup> cells—are reversible upon normalization of blood glucose levels (5,6). To determine whether gastrin expression in diabetic β-cells in vivo is similarly reversible, we treated 3.5-month-old diabetic db/db mice (which had been severely diabetic for ~4 weeks) with insulin injections to lower their blood glucose levels. We examined their islets 8 or 16 days later (Fig. 5A). Insulin treatment led to a dramatic reduction in the islet area staining for glucagon as well as increased expression of MafA, as previously reported (Fig. 5B). Strikingly, gastrin expression was almost completely eliminated after 8 days of insulin treatment (Fig. 5C). Insulin injections in db/db mice led to a variable degree of glycemic correction. Plotting the percentage of gastrin<sup>+</sup> cells as a function of glucose suggested a cutoff where islets of mice with blood glucose levels <300 mg/dL had much less gastrin expression than islets of mice with blood glucose >400 mg/dL (Fig. 5D).
We further examined the reversibility of gastrin expression in the Insulin-rtTA;TET-DTA model. We treated severely hyperglycemic DTA mice with insulin pellets and examined gastrin expression. Insulin-treated DTA mice had average glucose levels of 244 mg/dL compared with 580 mg/dL in untreated mice. No gastrin+ cells were observed in the treated mice (Fig. 5E), consistent with the findings in db/db mice. We also asked whether glucose-induced gastrin expression in cultured islets was reversible. Islets from wild-type mice cultured for 2 days in 25 mmol/L glucose showed a dramatic elevation in gastrin mRNA, which was reversed upon shifting to 5 mmol/L glucose for an additional 3 days (Fig. 5F). Finally, cultured islets from mice with NeuroG3-deficient β-cells induced gastrin and reversed its expression similarly to wild-type islets (Fig. 5G), indicating that NeuroG3 is not involved in...
gasolin induction or its silencing. In all of these experiments, reversal to normal glucose levels did not seem to involve massive β-cell death. Thus, although we cannot exclude the possibility that some gastrin+ cells die after normalization of glucose, it is more likely that they remain alive and shut off gastrin expression. We conclude that β-cell gastrin expression in diabetes is readily reversible upon normalization of glycemia.

**DISCUSSION**

We demonstrate in this report that in T2D, β-cells may turn on expression of the fetal islet hormone gastrin.
The reversibility of gastrin expression upon normalization improves after normalization of glycemia (31–33). An important open question is whether reprogrammed β-cells in diabetes remain permanently capable of reversing to a normal phenotype or whether dysfunction/reprogramming become permanent at some point under certain metabolic conditions. The expression of gastrin in metabolically stressed β-cells in vivo and in vitro provides a convenient experimental system to address this question, which has clear implications for the understanding of β-cell failure in T2D.

The ability to detect gastrin expression in metabolically stressed β-cells provides a novel and convenient biomarker for the study of β-cell reprogramming in T2D, in particular in humans where lineage tracing approaches are not available. Gastrin may not be just a marker, however. It is a biologically active peptide that may influence islet biology in a paracrine manner. The receptor for gastrin (CCKB-R) is reported to be expressed in α- and δ-cells (34,35), and gastrin was reported to influence glucagon expression and secretion (34,36). Gastrin was also proposed to promote β-cell replication and β-cell neogenesis (37–40), although these effects remain controversial. Future work will determine whether β-cells expressing gastrin also process and secrete it and under which conditions and whether locally produced gastrin affects islet biology.

Finally, our results highlight the competence of islet cells to change aspects of their terminal differentiation, namely, the specific hormone that they produce. Notably, reprogrammed islet cells tend to retain an endocrine identity. The underlying reason for the conservation of endocrine phenotype is likely the extensive similarity of the transcriptome, chromatin structure, and DNA methylation patterns among different islet cell types, which puts a lower bar for changes of terminal identity. Although this phenomenon may partly explain β-cell failure in diabetes, it also presents opportunities for therapeutic approaches using non-β-cells as a source for the generation of new β-cells (9,41).

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References
1. Leibowitz G, Kaiser N, Cerasi E. β-Cell failure in type 2 diabetes. J Diabetes Invest 2011;2:82–91
2. Halban PA, Polonsky KS, Bowden DW, et al. β-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. Diabetes Care 2014;37:1751–1758
3. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102–110
4. Talchai C, Xuan S, Lin HV, Susel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of β-cell failure. Cell 2012:150:1223–1234
5. Brereton MF, Iberti M, Shimonura K, et al. Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. Nat Commun 2014;5:4639
6. Wang Z, York NW, Nichols CG, Remedi MS. Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. Cell Metab 2014;19:872–882
7. Cinti F, Bouchi R, Kim-Muller JY, et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. J Clin Endocrinol Metab 2016;101:1044–1054
8. Butler AE, Dhawan S, Hoang J, et al. β-cell deficit in obese type 2 diabetes, a minor role of β-cell dedifferentiation and degranulation. J Clin Endocrinol Metab 2016;101:523–532
9. Thorel F, Népote V, Avril I, et al. Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. Nature 2010;464:1149–1154
10. Suisa Y, Magenheim J, Stolovich-Rain M, et al. Gastrin: a distinct fate of neurogenin3 positive progenitor cells in the embryonic pancreas. PLoS One 2013;8:e70397
11. Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. J Clin Invest 2007;117:2553–2561
12. Dor Y, Brown J, Martinez Ol, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429:41–46
13. Wang S, Jensen JN, Seymour PA, et al. Sustained Neurula expression in hormone-expressing islet cells is required for endocrine maturation and function. Proc Natl Acad Sci U S A 2009;106:9715–9720
14. Girard CA, Wunderlich FT, Shimomura K, et al. Expression of an activating mutation in the gene encoding the KATP channel subunit Kir6.2 in mouse pancreatic beta cells recapitulates neonatal diabetes. J Clin Invest 2009;119:80–90
15. Šrůnivá S, Watramo T, Lin CS, et al. CRE reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 2001;1:4
16. Kaiser N, Cerasi E, Leibowitz G. Diet-induced diabetes in the sand rat (Psammomys obesus). Methods Mol Biol 2012;933:89–102
17. Wicksteed B, Brissova M, Yan W, et al. Conditional gene targeting in mouse pancreatic β-cells: analysis of ectopic CRE transgene expression in the brain. Diabetes 2010;59:3090–3098
18. Gullberg M, Güdström SM, Shollmeiner E, et al. Cytokine detection by antibody-based proximity ligation. Proc Natl Acad Sci U S A 2004;101:8420–8424
19. Hou JC, Lin L, Pessin JE. Insulin granule biogenesis, trafficking and exocytosis. Vitam Horm 2009;80:473–506
20. Izumi T, Yokota-Hashimoto H, Zhao S, Wang J, Halban PA, Takeuchi T. Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. Diabetes 2003;52:409–416
21. Vikram A, Jena G. S961, an insulin receptor antagonist causes hyperinsulinemia, insulin-resistance and depletion of energy stores in rats. Biochem Biophys Res Commun 2010;398:260–265
22. Gross DJ, Leibowitz G, Cerasi E, Kaiser N. Increased susceptibility of islets from diabetes-prone Psammomys obesus to the deleterious effects of chronic glucose exposure. Endocrinology 1996;137:5610–5615
23. Bugliini M, Liechti R, Cheon H, et al. Microarray analysis of isolated human islet transcriptome in type 2 diabetes and the role of the ubiquitin-proteasome system in pancreatic beta cell dysfunction. Mol Cell Endocrinol 2013;367:1–10
24. Marselli L, Thorne J, Dahya S, et al. Gene expression profiles of beta-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. PLoS One 2010;5:e11499
25. Fadista J, Vikman P, Laakso EO, et al. Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism. Proc Natl Acad Sci U S A 2014;111:13924–13929
26. Dor Y, Glauser B. β-cell dedifferentiation and type 2 diabetes. N Engl J Med 2013;368:572–573
27. Bader E, Migliorini A, Gegg M, et al. Identification of proliferative and mature β-cells in the islets of Langerhans. Nature 2016;535:430–434
28. Dorrell C, Schug J, Canaday PS, et al. Human islets contain four distinct subtypes of β cells. Nat Commun 2016;7:11756
29. Wang YJ, Schug J, Won KJ, et al. Single-cell transcriptomics of the human endocrine pancreas. Diabetes 2016;65:3028–3038
30. Johnston NR, Mitchell RK, Haythome E, et al. Beta cell hubs dictate pancreatic islet responses to glucose. Cell Metab 2016;24:389–401
31. Ilkova H, Glauser B, Tunçkaile A, Bagriać N, Cerasi E. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients by transient intensive insulin treatment. Diabetes Care 1997;20:1353–1356
32. Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. Lancet 2008;371:1753–1760
33. Ryan EA, Imes S, Wallace C. Short-term intensive insulin therapy in newly diagnosed type 2 diabetes. Diabetes Care 2004;27:1028–1032
34. Leung-Thueong Long S, Roulet E, Clerc P, et al. Essential interaction of Egr-1 at an islet-specific response element for basal and gastrin-dependent glucagon gene transactivation in pancreatic alpha-cells. J Biol Chem 2005;280:7984–7984
35. Morisset J, Julien S, Lainé J. Localization of cholecystokinin receptor subtypes in the endocrine pancreas. J Histochem Cytochem 2003;51:1513–1515
36. Boushey RP, Abadir A, Flamez D, et al. Hypoglycemia, defective islet gluca
