Targeting development of incretin-producing cells increases insulin secretion

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Glucagon-like peptide-1–based (GLP-1–based) therapies improve glycemic control in patients with type 2 diabetes. While these agents augment insulin secretion, they do not mimic the physiological meal-related rise and fall of GLP-1 concentrations. Here, we tested the hypothesis that increasing the number of intestinal L cells, which produce GLP-1, is an alternative strategy to augment insulin responses and improve glucose tolerance. Blocking the NOTCH signaling pathway with the γ-secretase inhibitor dibenzazepine increased the number of L cells in intestinal organoid–based mouse and human culture systems and augmented glucose-stimulated GLP-1 secretion. In a high-fat diet–fed mouse model of impaired glucose tolerance and type 2 diabetes, dibenzazepine administration increased L cell numbers in the intestine, improved the early insulin response to glucose, and restored glucose tolerance. Dibenzazepine also increased K cell numbers, resulting in increased gastric inhibitory polypeptide (GIP) secretion. Using a GLP-1 receptor antagonist, we determined that the insulinotropic effect of dibenzazepine was mediated through an increase in GLP-1 signaling. Together, our data indicate that modulation of the development of incretin-producing cells in the intestine has potential as a therapeutic strategy to improve glycemic control.

Introduction

Glucagon-like peptide-1 (GLP-1) is a gut hormone with a powerful insulinotropic effect (1, 2). GLP-1 based therapies are widely used for the treatment of patients with type 2 diabetes (3). These treatments include GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors that decrease the breakdown of endogenously secreted GLP-1.

GLP-1–producing L cells in the intestinal lining originate from early secretory progenitors (4). The number of these progenitors is regulated by the γ-secretase/NOTCH pathway (5), and impairment of NOTCH signaling results in a relative increase in all types of secretory cells at the expense of enterocytes (6–9). Expression of neurogenin-3 (NGN-3) in differentiating secretory progenitors directs these cells toward an endocrine fate (10, 11). Late postmitotic precursors of L cells are believed to express the transcription factor neuronal differentiation 1 (NEUROD1) (12). Finally, the expression of preproglucagon defines the identity of mature L cells, which constitute only 0.5% of intestinal epithelial cells. We have recently shown that short-chain fatty acids (SCFAs) selectively increase the number of L cells in the intestinal epithelium in vitro, followed by a corresponding increase in GLP-1 secretion (13). SCFAs are likely to act through late endocrine precursors by increasing *Neurod1* expression (13). It is currently not clear how a change in the number of L cells relates to basal and stimulated GLP-1 concentrations in (patho-)physiological conditions and how it affects insulin secretion and glucose tolerance.

Here, we tested whether modulation of L cell development can increase the number of L cells, augment GLP-1 responses, and stimulate insulin secretion. The γ-secretase/NOTCH inhibitor dibenzazepine (DBZ) was used to induce L cell enrichment. We applied this model in vitro using the Matrigel-based intestinal organoid culture system with transgenic YFP expression in L cells (14). Subsequently, we translated the findings in vivo in a high-fat diet–fed (HFD-fed) mouse model.

Results

Effect of NOTCH inhibition on development of mouse and human L cells and GLP-1 secretion in vitro. In order to optimize L cell enrichment, we tested a range of DBZ concentrations added to the culture medium. We counted the number of L cells, identified by their expression of YFP, in Glu-Venus mouse organoids (14) after 96 hours of continuous exposure. DBZ concentrations of ≥1 nM were efficient at increasing L cell numbers (Figure 1A). However, consistent with the known effect of NOTCH inhibition on intestinal proliferation (6, 9), the rate of organoid growth diminished with increasing DBZ concentration, eventually resulting in a loss of crypt domains (15) at concentrations ≥1 μM (data not shown). Next, we tested a single-pulse regime and observed the greatest L cell enrichment when 5 μM DBZ was applied for 3 hours. This resulted in an 8-fold increase in L cell numbers after 96 hours, while maintaining the organoid domain structure (Figure 1, B–D). As NOTCH signaling might be involved in L cell maturation, as previously reported for Paneth cells (16), we tested whether NOTCH inhibition affects the function of

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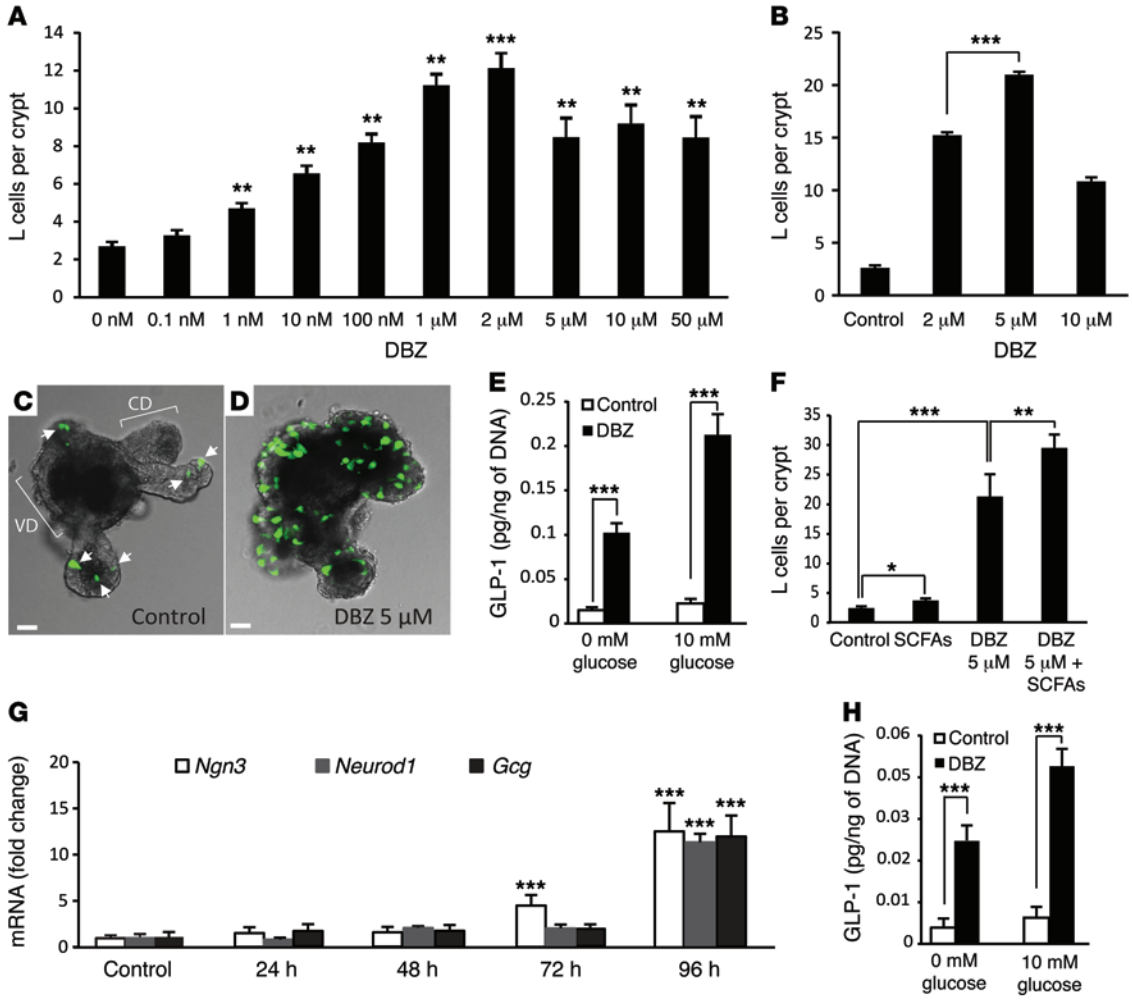

Figure 1. L cell enrichment in intestinal organoids by the NOTCH inhibitor DBZ. (**A**) L cell numbers in mouse ileum organoids after 96 hours of continuous exposure to different DBZ concentrations. (**B**) L cell numbers in mouse organoids 96 hours after a 3-hour DBZ pulse. (**C** and **D**) L cells (green) in a representative Glu-Venus mouse organoid before (**C**) and 96 hours after (**D**) a 3-hour pulse of 5 μM DBZ. Shown are maximum projections of a *z* stack through the organoid. VD, villus domain, CD, crypt domain. Arrows denote L cells. Scale bars: 20 μm. (**A**–**D**) *n* = 100 crypts (3 platings) per series. (**E**) Baseline and glucose-stimulated GLP-1 secretion in mouse ileum organoids 96 hours after a 3-hour pulse of 5 μM DBZ. *n* = 7 per series from 2 platings. (**F**) L cell numbers 96 hours after continuous treatment with SCFAs (combined 5 mM acetate, 1 mM propionate, and 1 mM butyrate), 5-μM DBZ pulse, or both. *n* = 100 crypts (3 platings) per series. (**G**) Gene expression of *Ngn3*, *Neurod1*, and *Gcg* in organoids at the indicated time points after a 5-μM DBZ pulse. *n* = 4–6 samples from 3 platings. (**H**) Baseline and glucose-stimulated GLP-1 secretion in human ileum organoids 96 hours after a 3-hour pulse of 5 μM DBZ. *n* = 6 per series from 2 platings. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. control or as indicated by brackets, 1-way ANOVA with Bonferroni test (**A**–**D** and **F**) or nonpaired 2-tailed Student's *t* test (**E**, **G**, and **H**).

L cells, measuring GLP-1 secretion from DBZ-treated organoids. Basal (0 mM glucose) and stimulated (10 mM glucose) GLP-1 secretion were 7- and 9.4-fold higher, respectively, in DBZ-treated versus control organoids. However, the relative increase in glucose-stimulated GLP-1 release over basal GLP-1 secretion after DBZ treatment was similar to the control group (Figure 1E). This indicates that the amplification of GLP-1 secretion was largely dependent on L cell mass and that DBZ treatment did not impair the glucose responsiveness of L cells. We next tested whether the effect of DBZ can be further amplified by SCFAs, which themselves increase L cell numbers in small intestinal organoids by approximately 2-fold (13). SCFAs increased the number of L cells in both DBZ-treated and control mouse organoids (Figure 1F).

We monitored markers of L cell development (10) by quantitative real-time PCR over time after the 5-μM DBZ pulse and found increased expression of *Ngn3* at 72 hours and of *Neurod1* and *Gcg* at 96 hours (Figure 1G). This suggests that, in accordance with its proposed mechanism of action (5, 8), DBZ increased numbers of early *Ngn3*-positive endocrine progenitors prior to the appearance of mature L cells. We then tested whether human intestinal tissue can respond to modulation of L cell development by NOTCH inhibition in a manner similar to that observed in mouse crypts. We applied a pulsed treatment of 5 μM DBZ to human ileal crypts in vitro and observed a 7.5-fold increase in the number of L cells identified by immunostaining (Supplemental Figure 1, A–C; supplemental material available online with this article; doi:10.1172/JCI75838DS1). Basal GLP-1 secretion increased 6-fold, and glucose-stimulated GLP-1 release increased 7.8-fold (Figure 1H). These results indicated that our findings in mouse intestinal tissue can be translated to human intestinal crypts.

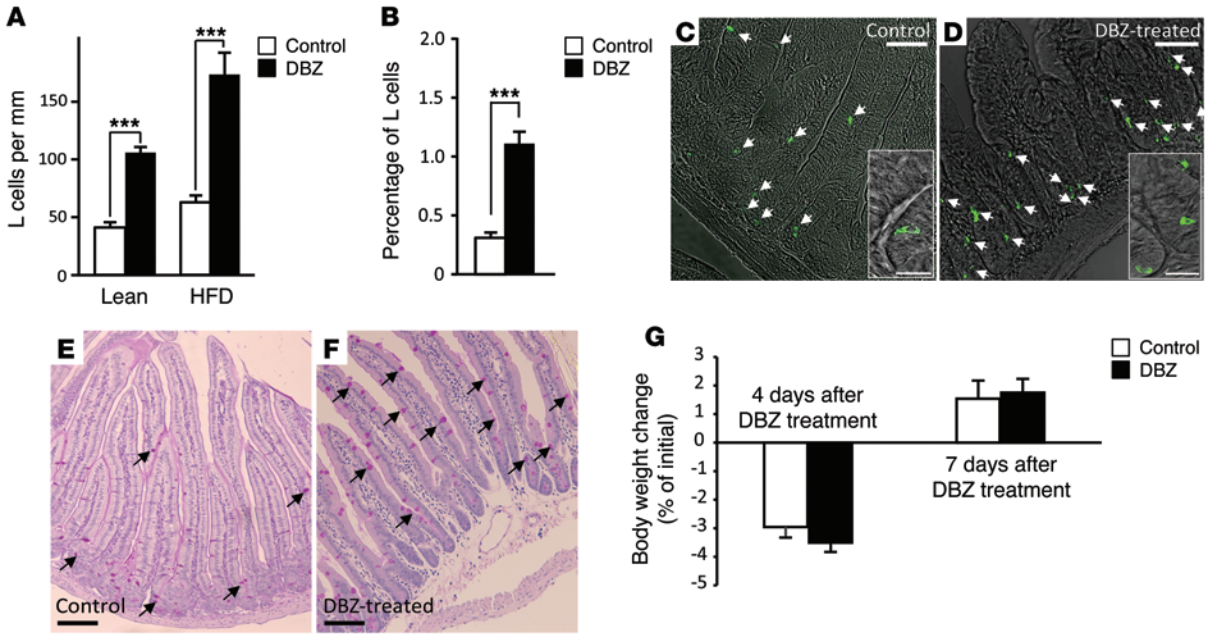

Figure 2. In vivo L cell enrichment by the 2× 10 mg/kg DBZ regimen. (**A**) L cell numbers in lean and HFD-fed mouse ileum. L cells were identified by GLP-1 staining and counted; results are expressed as number of cells per millimeter of mucosal lining. Data are from microscopy of 6 transverse sections per series from 3 lean and 4 HFD-fed mice. (**B**) L cell numbers, determined by FACS analysis, in combined lean mouse jejunum and ileum. $n = 3$ samples each (control and DBZ); each sample was pooled from 2 mice. (**A** and **B**) $^{***}P < 0.001$, nonpaired 2-tailed Student's t test. (**C** and **D**) Immunostaining for GLP-1 (green) in the small intestine of a lean mouse. Arrows denote L cells (also shown at higher magnification in the insets). (**E** and **F**) Periodic acid–Schiff (PAS) staining for goblet cells (arrows) in the small intestine. (**G**) Body weight change in control and DBZ-treated mice after overnight fasting for OGTT. Initial body weight in nonfasted mice before treatment was assigned as 0. $n = 12$ (control); 10 (DBZ). Nonpaired 2-tailed Student's t test. (**C**–**F**) Images are representative of 6 transverse sections per series (vehicle and DBZ). Scale bars: 100 μm; 20 μm (insets).

NOTCH inhibition increases L cell numbers in vivo. We next examined the physiological effects of L cell enrichment in vivo, using lean and HFD-fed mice. First, we tested the effects of several doses and dosing schemes of DBZ treatment on L cell numbers, plasma GLP-1 and insulin concentrations, and changes in intestinal morphology in lean mice (Supplemental Figure 2, A–F). The regimens tested were as follows: 50 mg/kg given once (referred to herein as 1× 50 mg/kg), 10 mg/kg given on 2 consecutive days (2× 10 mg/kg), and 5 mg/kg given on 4 consecutive days (4× 5 mg/kg). We found that an increase in L cell numbers, sufficient to produce a statistically significant elevation in plasma GLP-1 and insulin concentrations, could be achieved in vivo 96 hours after the 2× 10 mg/kg regimen (Supplemental Figure 2, B–D). The highest dose studied (1× 50 mg/kg regimen) also increased L cell numbers and GLP-1 secretion, but was toxic to the mice, causing severe alterations of the crypt and villus structure, malnutrition, and weight loss after 1 week (Supplemental Figure 2D and data not shown). This is consistent with the previously reported side effect profile of DBZ (17). Because of the side effects related to this dose could alter the metabolic state of the mice, we used the 2× 10 mg/kg regimen for further testing in HFD-fed mice. This dose was well tolerated and had no obvious effects on the animals' well-being within 2 weeks of the treatment. The increase in L cell numbers in the duodenum and jejunum was greater than that in the ileum (3.7- and 4.7-fold vs. 2.6-fold, Supplemental Figure 3E), but there were no changes in L cell density in the large intestine (Supplemental Figure 3, A and B).

DBZ increased L cell numbers in HFD-fed mouse ileum, to an extent similar to that in lean mice (Figure 2A). Using FACS sorting based on Venus fluorescence in the combined jejunum and ileum, we estimated a 3.6-fold enrichment of L cells (Figure 2, B–D). As shown by transmission electron microscopy (TEM), enteroendocrine cells in DBZ-treated mice maintained polarity and showed numerous large dense core granules (Supplemental Figure 3, C and D), indicative of preserved maturation of enteroendocrine cells. We also observed increased heterogeneity of granules in Paneth cells after DBZ treatment. This finding was in line with the previously reported involvement of NOTCH signaling in Paneth cell maturation (16). Overall, normal morphology was maintained with respect to villus length and epithelial cell appearance, but we observed an increase in the number of goblet cells (Figure 2, E and F). We next quantified populations of the major intestinal cell types — Paneth cells, goblet cells, enterocytes, all enteroendocrine cells, and enterochromaffin cells — in different intestinal segments. Similar to previous reports (7, 8), we found an increase in several types of secretory cells in the small intestine after DBZ treatment. In the colon, we observed an increase in the number of goblet cells only (Supplemental Figure 3, F–K). We observed a 26% reduction in enterocyte numbers in ileum, but not in other segments of the intestine (Supplemental Figure 3K).

A decrease in enterocyte numbers may reduce absorption of nutrients. Hypersecretion of GLP-1, and probably increased levels of other secretory products of L cells, including oxyntomodulin and peptide YY, may decrease appetite. A combination of these effects could result in loss of body weight and change in metabolism in HFD-fed mice. We therefore compared body weight changes in

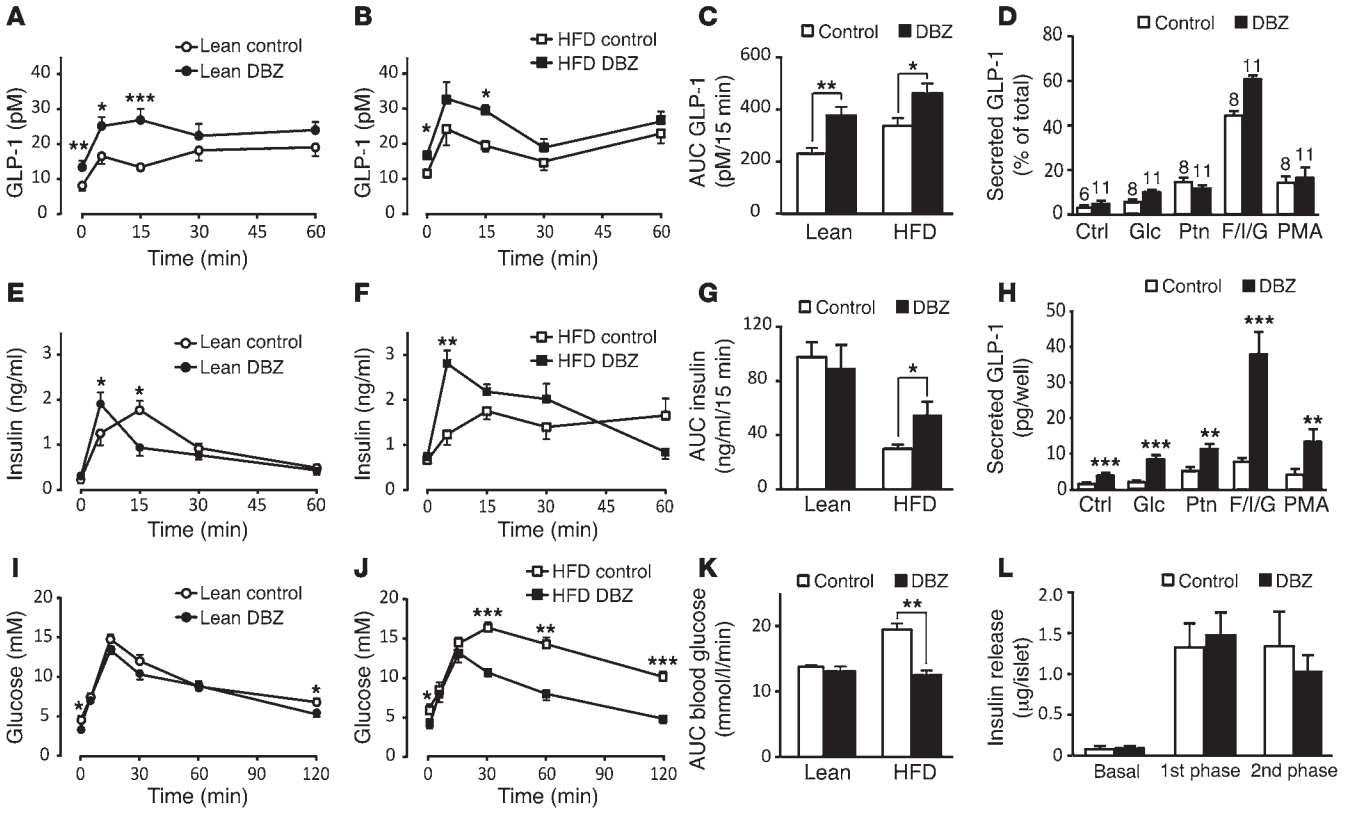

Figure 3. GLP-1 and insulin secretion and glucose tolerance after DBZ treatment. (**A**–**C**) Plasma GLP-1 concentration during OGTT (**A** and **B**) and area under curve (AUC) for GLP-1 release (0–15 minutes; **C**) in lean and HFD-fed mice. *n* = 12 (lean control); 10 (lean DBZ); 6 (HFD control); 8 (HFD DBZ). (**D**) GLP-1 release, expressed as percent total GLP-1 content, in response to vehicle control (Ctrl); 10 mM glucose (Glc); 0.5% peptone (Ptn); 10 μM forskolin, 10 μM IBMX, and 10 mM glucose combined (F/I/G); or 1 μM phorbol myristate acetate (PMA) in mouse intestinal cultures. *n* = 2 (control); 3 (DBZ). Number of cultures is indicated for each group. (**E**–**G**) Insulin response during OGTT (**E** and **F**) and AUC (0–15 minutes; **G**) in lean and HFD-fed mice. *n* = 10 (lean control); 8 (lean DBZ); 6 (HFD control and DBZ). (**H**) Absolute GLP-1 secretion per well in response to various stimuli in intestinal cultures (treatments and number of cultures as in **D**). *n* = 2 (control); 3 (DBZ). (**I**–**K**) Blood glucose concentrations during OGTT (**I** and **J**) and AUC (0–120 minutes; **K**) in lean and HFD-fed mice. *n* = 11 (lean control); 13 (lean DBZ); 6 (HFD control and DBZ). (**L**) Basal (3 mM glucose) or stimulated (20 mM glucose; 2 phases shown) insulin release in isolated mouse islets. *n* = 8 islet batches per group (2 mice per treatment). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, nonpaired 2-tailed Student's *t* test (**A**–**C**, **E**–**G**, and **I**–**L**) or 1-way ANOVA with Bonferroni test (**D** and **H**).

vehicle- and DBZ-treated mice, but found no difference between groups (Figure 2G).

L cell enrichment increases GLP-1 and insulin secretion and improves glucose tolerance in lean and HFD-fed mice. An oral glucose tolerance test (OGTT) was performed in vehicle- and DBZ-treated mice, and their plasma GLP-1, insulin, and glucose concentrations were measured. DBZ treatment (2× 10 mg/kg regimen) increased fasting GLP-1 concentrations in lean and HFD-fed mice and augmented the GLP-1 response to glucose during the first 15 minutes (Figure 3, A–C). We also measured GLP-1 release in response to a range of physiological stimuli in intestinal cultures from mice treated with the highest DBZ dose (1× 50 mg/kg regimen), which secreted 3–5 times more GLP-1 per well than vehicle control–treated cultures (mirroring their approximately 3-fold higher GLP-1 content) and exhibited a normal pattern of responsiveness (Figure 3, D and H).

Comparison of basal insulin concentrations showed no difference between vehicle- and DBZ-treated mice. Peak stimulated insulin concentrations were detected 5 minutes after the oral glucose intake in lean and HFD-fed mice treated with DBZ, while in vehicle-treated mice, the insulin concentration peaked at 15 minutes (Figure 3, E–G). To test whether DBZ has a direct stimulatory effect on β cells, we isolated islets from mice treated with vehicle or the highest dose of DBZ (1× 50 mg/kg regimen) and measured insulin release. DBZ treatment had no effect on basal insulin secretion (3 mM glucose) or on the first or second phase of stimulated insulin secretion (20 mM glucose) (Figure 3L).

DBZ treatment decreased fasting glucose concentrations in lean and HFD-fed mice ($P < 0.01$; Figure 3, I and J). However, whereas vehicle- and DBZ-treated lean mice exhibited similar glucose profiles after the oral glucose load, HFD-fed mice showed improved glucose tolerance after DBZ treatment (Figure 3, I–K).

Blockade of the GLP-1 receptor modulates the effect of DBZ on insulin secretion and glucose tolerance. To evaluate the relative contribution of the GLP-1 effect on insulin secretion, we performed an OGTT in DBZ-injected, HFD-fed mice in the presence of the GLP-1 receptor antagonist exendin 9-39. The increased insulin response after DBZ treatment was attenuated by exendin 9-39, and the improvement in glucose tolerance in DBZ-treated mice was blocked (Figure 4, A–D). These data indicate that improved glucose tolerance resulting from DBZ treatment was, to a large extent, attributable to increased GLP-1 action.

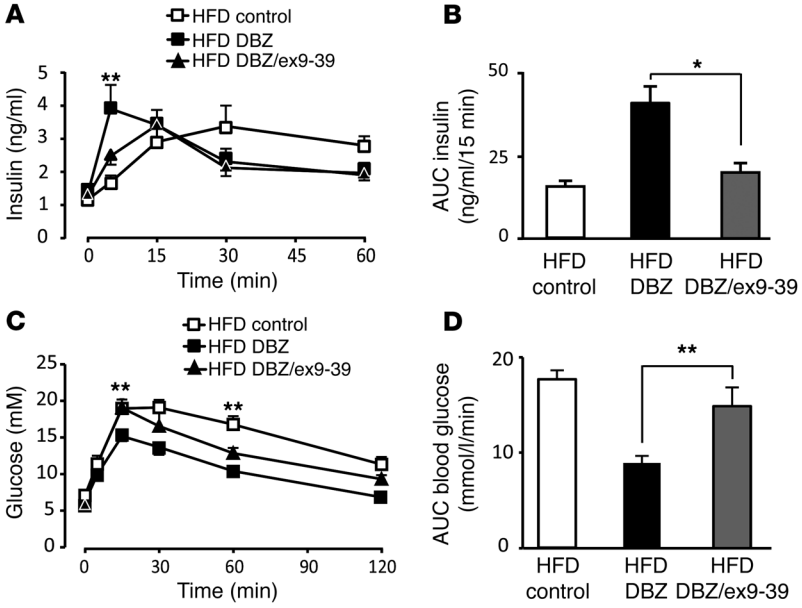

Figure 4. Blockade of the GLP-1 receptor modulates the effect of DBZ on insulin secretion and glucose tolerance. HFD-fed mice, treated or not with DBZ and/or exendin 9-39 (ex9-39), were subjected to OGTT. (**A**) Plasma insulin concentrations. (**B**) AUC insulin (0–15 minutes). (**C**) Blood glucose concentrations. (**D**) AUC glucose (0–120 minutes). $n = 6$ (HFD control and HFD DBZ); 7 (HFD DBZ plus exendin 9-39). $^*P < 0.05$, $^{**}P < 0.01$, 1-way ANOVA with Bonferroni test.

NOTCH inhibition increases K cell numbers and GIP secretion in HFD-fed mice. Next, the effect of DBZ on the number of K cells was assessed. We first tested duodenum organoids derived from GIP-Venus mice, in which K cells are labeled by YFP expression (Figure 5, A and B, and ref. 18), similar to L cells in the Glu-Venus mouse. Organoids treated with a 3-hour pulse of 5 μM DBZ showed an 8-fold increase in K cell numbers (control, 0.9 ± 0.1 cells/crypt; DBZ, 7.1 ± 0.49 cells/crypt). We next assessed the DBZ-treated organoids for GIP secretion and found elevated basal as well as glucose-stimulated GIP release compared with control organoids (Figure 5C). K cell numbers in control and DBZ-treated HFD-fed mice were also compared. In the duodenum, the most K cell–enriched region in the intestine, the number of K cells increased 2.8-fold after DBZ treatment (Figure 5D). GIP levels during an OGTT were correspondingly higher in DBZ-treated mice during the first 30 minutes after the glucose challenge (Figure 5, E and F).

Insulin sensitivity, assessed by insulin tolerance testing, is similar in control and DBZ- treated mice fed HFD. Non-L cell effects of NOTCH inhibition, such as increased hepatic insulin sensitivity (19), could contribute to the improved glucose tolerance observed in our model. Therefore, we compared insulin-mediated glucose uptake in DBZ- and vehicle-treated mice by an insulin tolerance test (ITT). No difference was found in the ability of insulin to lower glucose levels in lean or HFD-fed mice after DBZ treatment (Supplemental Figure 4).

Reduced insulin secretion after cessation of DBZ treatment. To find out how long the beneficial effects of DBZ treatment persist in vivo, the insulin response was assessed during an OGTT 8 days after DBZ treatment. This interval was chosen based on the observed decline in L cell numbers 5 days after the DBZ treatment (Supplemental Figure 2A), on the assumption that the turnover of L cells takes 4–5 days (4), and on the time needed for mice to recover from previous blood sampling in an OGTT. After 8 days, the elevated plasma GLP-1 and insulin responses were no longer evident, and L cell numbers were similar to those observed in vehicle-treated mice (Supplemental Figure 5, A–E).

In order to investigate the effect of repeated DBZ treatment, mice were repeatedly given the 2× 10 mg/kg DBZ regimen (i.e., on days 1 and 2, then again on days 4 and 5). These repeatedly treated mice were tested by OGTT on day 8 and showed improved glucose tolerance (Supplemental Figure 5F) and an increased number of L cells in the ileum compared with both vehicle-injected mice and mice receiving the single 2× 10 mg/kg regimen (Supplemental Figure 5A). However, the mice started to lose weight after the repeated treatment (data not shown) and developed skin lesions. We observed no increase in plasma GLP-1 concentrations after a single round of the 4× 5 mg/kg and 1× 50 mg/kg DBZ regimens (Supplemental Figure 2, B and C, and data not shown); therefore, we did not perform repetitive treatments using these regimens.

Discussion

In the present study, we showed that an increase in L cell numbers, mediated by NOTCH inhibition, translates into elevated GLP-1 secretion, which augments insulin secretion and improves glucose tolerance in HFD-fed mice. Our in vitro data showed that DBZ could be used in a pulsed manner to enhance the number of functional L cells in mouse and human intestinal organoids, which augmented GLP-1 secretion. This increase in L cell numbers was preceded by elevated expression of the transcription factors *Ngn3* and *Neurod1*, indicative of enhanced development of mitotic and postmitotic endocrine precursors (12). The L cell enrichment was further augmented by SCFAs, which are likely to act on *Neurod1*-expressing endocrine precursors (13, 20). Thus, the conversion of transit-amplifying cells to secretory cells by DBZ could hypothetically be combined with subsequent modulation of postmitotic endocrine precursors specifically toward the L cell fate. In vivo, DBZ treatment increased L cell numbers throughout the small intestine, with a stronger effect in jejunum than in ileum. This indicates a difference in plasticity of developing cells in the secretory compartment between intestinal segments, although the underlying mechanisms are not known. The potential implications of differentially increased L cell numbers for improving glucose homeostasis in the treatment of diabetes require further scientific investigation, as do the contributions of L cells from different parts of the intestine to glucose control. Although the numbers of other types of secretory cells were also increased by DBZ, including goblet, Paneth, and other enteroendocrine cells, we did not detect a significant deficit of enterocytes in the duodenum and jejunum, which suggests that nutrient absorption in these parts of the intestine was not severely affected.

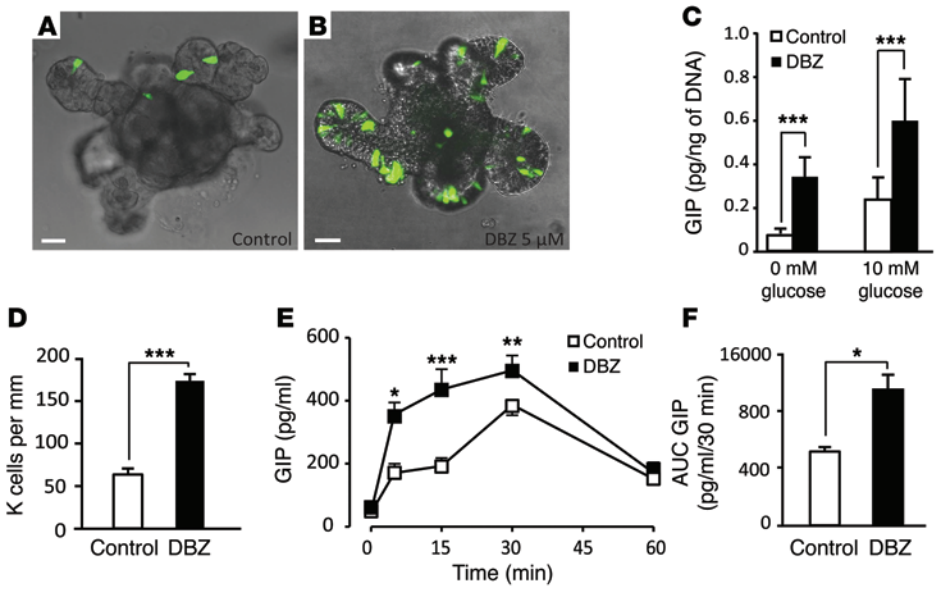

Figure 5. In vitro and in vivo K cell enrichment after DBZ treatment. (**A** and **B**) K cells (green) in a representative mouse duodenal organoid before (**A**) and 96 hours after (**B**) a 3-hour pulse of 5 μM DBZ. Shown are maximum projections of a *z* stack through the organoid. Images are representative of 100 organoids per series from 2 platings. Scale bars: 20 μm. (**C**) Basal and glucose-stimulated GIP secretion in control organoids and 96 hours after a 3-hour pulse of 5 μM DBZ. $n = 8$ per series from 2 platings. (**D**) K cell numbers in the duodenum of HFD-fed mice treated with vehicle or DBZ (2× 10 mg/kg regimen). Data were obtained from microscopy of 6 transverse sections from 3 HFD-fed mice per series. (**E**) GIP concentrations during OGTT in control and DBZ-treated HFD-fed mice. $n = 6$ (control); 7 (DBZ). (**F**) AUC for GIP release (0–30 minutes). (**C**–**F**) $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, nonpaired 2-tailed Student's *t* test.

The GLP-1 secretion tests and TEM of endocrine cells were suggestive of normal development of L cell structure and function in the presence of systemically administered DBZ. Importantly, DBZ-treated mice showed augmented GLP-1 and insulin responses to an OGTT, and the glucose tolerance in HFD-fed mice improved after DBZ treatment. The higher early insulin peak was particularly noticeable in HFD-fed mice treated with DBZ (Figure 3F). This rapid augmentation of insulin secretion is interesting because it addresses 2 key characteristics of the secretory defect associated with β cell dysfunction in type 2 diabetes: a reduction and a delay of the insulin response (21, 22). Thus, our present data strongly suggest that L cell enrichment in the intestinal epithelium can be beneficial for glucose control in type 2 diabetes. After cessation of treatment, L cell numbers fell to levels similar to those in untreated controls, and the improved glucose tolerance was no longer observed by day 8. Repeating the treatment regimen several days later was toxic for the mice. Our experiments in the presence of a GLP-1 receptor antagonist demonstrated that improved glucose tolerance and increased insulin secretion were in large part due to augmented GLP-1 release in DBZ-treated mice. In addition, DBZ treatment had no direct effect on insulin secretion, insulin-mediated glucose uptake, and body weight dynamics, which suggests that the improved glucose tolerance was mediated mostly through the increased insulin response after L cell enrichment.

Because the effect of NOTCH inhibition is not selective for L cells, we also observed increased K cell numbers and elevated GIP levels in DBZ-treated mice. The insulinotropic action of GIP alone is not very effective in β cells from patients with type 2 diabetes, in contrast to GLP-1–based therapy (23); however, it is possible that an increase in GIP release after modulation of K cell development by DBZ can act synergistically with augmented GLP-1 signaling, or that GIP sensitivity may be improved by augmented β cell function.

Whereas we here demonstrated the positive effect of L cell enrichment for glycemic control, NOTCH inhibitors are associated with several side effects, such as impaired development of absorptive cells and survival of stem cells (24). Ideally, compounds with a more specific action on L cell development would be a logical therapeutic strategy. Further investigation of factors directing development of L and K cells, combined with a search for selective modulators that can modulate this process, are necessary for the success of this approach.

In conclusion, we here provide proof of concept that enrichment of incretin-secreting cells by pharmacological agents can be a novel strategy by which to improve insulin secretion and glucose tolerance. This opens a unique window of opportunity to identify compounds that can modulate L cell development in order to improve glucose control in patients with diabetes mellitus.

Methods

Further information is available in Supplemental Methods.

C57BL/6 male mice (4–5 months old) were used for OGTT and ITT tests in HFD and lean groups. Intestinal fragments from 4-month-old Glu-Venus (14) and GIP-Venus male mice (18) were used for organoid culture and FACS experiments. Surgically resected human ileal tissues were obtained from the Diakonessen Hospital (Utrecht, The Netherlands). Mouse and human intestinal crypts were isolated, cultured, and grown into organoids as described previously (25, 26). DBZ [$C_{26}H_{23}F_2N_3O_3$; (S)-2-[2-(3,5-difluoro-phenyl)-acetylamino]-*N*-((S)-5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)-propionamide] was added to culture medium 48 hours after splitting in the concentration range of 0.1 nM to 50 μM, as a continuous 96-hour treatment. For pulsed treatment, organoids were treated with 2, 5, or 10 μM DBZ for 3 hours. SCFAs were applied continuously as a combination of 5 mM acetate, 1 mM propionate, and 1 mM butyrate.

For DBZ dose testing, all in vivo experiments and collection of intestine for L cell counts and histological analysis were done 48 hours after the 1× 50 mg/kg DBZ regimen, 96 hours after beginning the 2× 10 mg/kg DBZ regimen (except ITT), and 120 hours after beginning the 4× 5 mg/kg DBZ regimen. DBZ was given in saline solution with 0.1% hydroxypropylmethylcellulose i.p. Vehicle controls were injected with saline solution with 0.1% hydroxypropylmethylcellulose. HFD (60% fat) was fed to mice for 12–14 weeks. Lean and HFD-fed mice were given DBZ (2× 10 mg/kg regimen), and OGTT was performed

96 hours after beginning treatment (7). Exendin 9-39 (100 nmol/kg) was given i.p. ITT with 2 U/kg insulin was performed the day before OGTT. Intestinal samples for TEM, FACS sorting, and immunostaining for different cell types were taken immediately after the OGTT. Pancreatic islets and intestinal cultures from DBZ-treated and control mice were isolated 48 hours after the 1× 50 mg/kg DBZ regimen, cultured, and processed for insulin or GLP-1 secretion measurements as previously described (27).

Statistics. Data represent mean ± SEM. Comparison of 2 groups was done using nonpaired 2-tailed Student's *t* test. A *P* value less than 0.05 was considered significant. Data involving more than 2 groups were assessed by ANOVA.

Study approval. Animal experiments were approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences (permit no. HI 11.2503). The study on human intestinal tissues was approved by the ethical committee of the Diakonessen Hospital (Utrecht, The Netherlands). Informed consent was provided by all patients.

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