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Highlights

- α cell-derived glucagon-related peptides are critical for normal insulin secretion
- Normally, α cell-derived GLP-1 is not required for sufficient insulin secretion
- Paracrine GLP-1 in islets is needed for adaptation to aging and metabolic stress
- Deficiency in α cell-derived GLP-1 can be rescued by inhibition of DPP-4

Authors

Shuyang Traub, Daniel T. Meier, Friederike Schulze, ..., Pedro L. Herrera, Marianne Böni-Schnetzler, Marc Y. Donath

Correspondence
marc.donath@usb.ch

In Brief

Pancreatic α cells may process proglucagon to glucagon or GLP-1. Traub et al. find that α cell-derived GLP-1 is necessary for glucose homeostasis during aging and metabolic stress. Deficiency of α cell-derived glucagon-related peptides can be compensated by DPP-4 inhibition.
Pancreatic α Cell-Derived Glucagon-Related Peptides Are Required for β Cell Adaptation and Glucose Homeostasis

Shuyang Traub,1,2,6 Daniel T. Meier,1,2,6 Friederike Schulze,1,2 Erez Dror,1,2 Thierry M. Nordmann,1,2 Nicole Goetz,1,2 Norina Koch,1,2 Elise Dalmas,1,2 Marc Stawiski,1,2 Vaimir Makshana,1,2 Fabrizio Thorel,3,4,5 Pedro L. Herrera,3,4,5 Marianne Bönì-Schnetzler,1,2 and Marc Y. Donath1,2,7,*

1Endocrinology, Diabetes, and Metabolism, University Hospital Basel, 4031 Basel, Switzerland
2Department of Biomedicine, University of Basel, 4031 Basel, Switzerland
3Department of Genetic Medicine and Genomics in Geneva (iGE3), University of Geneva, 1211 Geneva, Switzerland
4Institute of Genetics and Genomics in Geneva (iGE3), University of Geneva, 1211 Geneva, Switzerland
5Centre facultaire du diabète, University of Geneva, 1211 Geneva, Switzerland
6Co-first author
7Lead Contact
*Correspondence: marc.donath@usb.ch
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SUMMARY

Pancreatic α cells may process proglucagon not only to glucagon but also to glucagon-like peptide-1 (GLP-1). However, the biological relevance of paracrine GLP-1 for β cell function remains unclear. We studied effects of locally derived insulin secretagogues on β cell function and glucose homeostasis using mice with α cell ablation and with α cell-specific GLP-1 deficiency. Normally, intestinal GLP-1 compensates for the lack of α cell-derived GLP-1. However, upon aging and metabolic stress, glucose tolerance is impaired. This was partly rescued with the DPP-4 inhibitor sitagliptin, but not with glucagon administration. In isolated islets from these mice, glucose-stimulated insulin secretion was heavily impaired and exogenous GLP-1 or glucagon rescued insulin secretion. These data highlight the importance of α cell-derived GLP-1 for glucose homeostasis during metabolic stress and may impact on the clinical use of systemic GLP-1 agonists versus stabilizing local α cell-derived GLP-1 by DPP-4 inhibitors in type 2 diabetes.

INTRODUCTION

The classical incretin concept views GLP-1 as a hormone produced in the intestinal L cells and acting via the circulation on satiety in the brain, gut motility, and insulin and glucagon secretion in the pancreatic islet (Campbell and Drucker, 2013; Drucker, 2006). However, in contrast to typical hormones, GLP-1 has a very short half-life of less than 2 min (Deacon, 2004; Hansen et al., 1999). This rapid degradation of GLP-1 raises questions about how its effects are mediated on distant target organs such as pancreatic β cells (Donath and Burcelin, 2013). It was shown that GLP-1 induces its metabolic actions by interacting with its receptor in extra-pancreatic locations such as the gut to activate the submucosal and myenteric nervous plexi (Waget et al., 2011; Washington et al., 2010) as well as the brain, which then signals to peripheral tissues (Baraboi et al., 2011; Burcelin et al., 2001; Cabou and Burcelin, 2011; Nakagawa et al., 2004; Shimizu et al., 1983). Furthermore, small doses of DPP-4 inhibitors improve glucose tolerance without increasing the blood concentration of GLP-1 in a GLP-1 receptor-dependent manner (Waget et al., 2011). Other observations suggest that circulating GLP-1 can also directly stimulate insulin secretion from pancreatic β cells without involvement of neuronal circuits (Lamont et al., 2012; Smith et al., 2014).

In intestinal L cells, GLP-1 is cleaved from its precursor proglucagon by the enzyme prohormone convertase 1/3 (PC1/3). In contrast, pancreatic α cells are viewed to process proglucagon to glucagon by PC2 (Furuta et al., 2001; Rouillé et al., 1995; Tucker et al., 1996), and it was believed that adult α cells do not produce GLP-1. However, adenovirus-mediated expression of PC1/3 in α cells may increase islet GLP-1 expression and improve insulin secretion (Wideman et al., 2006). Furthermore, several studies have shown that islet-derived GLP-1 can be stimulated by an increased demand of insulin secretion, suggesting that locally produced GLP-1 may play a role in long-term β cell adaptation (Ellingsgaard et al., 2011; Hansen et al., 2011; Kilimanik et al., 2010; Marchetti et al., 2012; Nie et al., 2000; Thyssen et al., 2006). Finally, we have shown that α cell hyperplasia occurs early in response to high-fat diet (HFD) feeding and precedes expansion of β cell mass required for β cell adaptation in response to increased secretory demand (Ellingsgaard et al., 2008, 2011). Therefore, the ability of α cells to produce GLP-1 is well documented.

However, the biological relevance of local α cell-derived GLP-1 on glucose homeostasis and β cell function in physiological...
settings and under metabolic stress remains to be demonstrated. For this purpose, we used a genetic mouse model of inducible α cell ablation and generated an α cell-specific PC1/3-deficient mouse to prohibit islet-derived GLP-1 production.

RESULTS

Diphtheria Toxin Injection into GluDTR Mice Induces Massive and Long-Lasting α Cell Ablation

α cells may impact β cell insulin secretion via GLP-1 and glucagon secretion (Huypens et al., 2000; Moens et al., 1998; Samols et al., 1965). Therefore, we first determined the impact of α cell ablation using GluDTR mice, which express the human diphtheria toxin (DT) receptor under the control of the rat glucagon promoter (Thorel et al., 2011). GluDTR mice were fully backcrossed to a C57BL/6N background, and positive mice as well as littermate controls were injected with 1.5 μg DT at the age of 6 weeks. 6 weeks later, the percentage of α cells in dispersed islet single cells decreased from 8.9% in controls to 0.4% in cells from GluDTR mice (Figure S1). Immunohistochemical staining for insulin and glucagon in pancreatic sections from GluDTR mice showed long-lasting α cell ablation without evidence for regeneration (Figure 1A). 6 weeks after DT injection, proglucagon mRNA expression in pancreatic islets decreased from 8.9 in controls to 0.4% in cells from GluDTR mice (Figure S1). Immunohistochemical staining for insulin and glucagon in pancreatic sections from GluDTR mice showed long-lasting α cell ablation without evidence for regeneration (Figure 1A). 6 weeks after DT injection, proglucagon mRNA expression in pancreatic islets...
was strongly reduced in GluDTR mice, while preproinsulin expression was similar to control animals (Figure 1B). Accordingly, islet protein content of glucagon and active GLP-1 was decreased (Figure 1C). The presence of intestinal L cells after DT injection was confirmed by immunostaining for GLP-1 in colon sections (Figure 1D), and active GLP-1 content in terminal ileum was similar in GluDTR and control mice (Figure 1E), confirming the specificity of α cell ablation. Body weight remained equal in GluDTR and control mice after DT injection (Figure 1F), while fasting plasma active GLP-1 concentrations were similar (Figure 1H).

Lack of α Cell-Derived GLP-1 Impairs Glucose Tolerance in Aged Mice

6 weeks after DT injection (at the age of 12 weeks), intraperitoneal (i.p.) glucose tolerance was comparable in GluDTR and control mice (Figure 2A), confirming previously published data (Pedersen et al., 2013; Thorel et al., 2011). Plasma insulin concentrations were also not different (Figure 2B). At the age of 28 weeks, i.p. glucose tolerance of GluDTR mice was impaired compared to controls (Figure 2C), fasting insulin secretion was decreased (0.8 ± 0.1 versus 1.2 ± 0.2 ng/mL, p < 0.05), and insulin levels were lower during the glucose tolerance test (Figure 2D). To stabilize systemic active GLP-1, we injected the DPP-4 inhibitor sitagliptin 30 min prior to i.p. glucose administration, as described previously (Timper et al., 2016). Glucose tolerance was completely restored in 30-week-old GluDTR mice upon DPP-4 inhibition, along with increased insulin secretion (Figures 2E and 2F). Glucagon administration 1 min prior to i.p. glucose tolerance testing resulted in a similar glucose tolerance in both GluDTR and control mice (Figures 2G and S2), whereas insulin levels remained significantly lower in GluDTR animals (Figure 2H). Therefore, prolonged α cell ablation leads to impaired insulin secretion, which can be rescued by increasing systemic GLP-1, but not glucagon levels.

Young α Cell-Ablated Mice Show Enhanced Oral Glucose Tolerance due to a Compensatory Increase in Systemic Active GLP-1 Secretion

To understand the relative role of systemic versus islet-derived GLP-1, we performed oral glucose tolerance tests, which lead to a stronger stimulation of intestinal GLP-1 compared to i.p. injection. As shown previously (Pedersen et al., 2013), 12-week-old GluDTR mice displayed enhanced oral glucose tolerance (Figure 3A). At this age, the content of active GLP-1 in the terminal ileum was higher in GluDTR mice than in controls (Figure 3B), suggesting compensatory upregulation of GLP-1 production in intestinal L cells. To stabilize systemic active GLP-1, we next injected sitagliptin prior to oral glucose tolerance testing. Active GLP-1 secretion was increased in GluDTR mice compared to Glucagon (20 μg/kg body weight) was i.p. injected 1 min prior to glucose injection. n indicates the number of animals, and error bars represent SEM. *p < 0.05, **p < 0.01, area under the curve (AUC). See also Figure S2.
control animals (Figure 3C), with concomitantly increased plasma insulin concentration (Figure 3D). To explore the role of glucagon, we administered exogenous glucagon to GluDTR mice prior to oral glucose tolerance testing. Glucagon did not increase glycemia compared to vehicle control (Figure 3E), probably due to increased insulin secretion (Figure 3F). This points to an insulin secretagogue effect of glucagon in GluDTR mice, similar to that of GLP-1 or to an effect of glucagon on GLP-1 secretion. However, active GLP-1 secretion was suppressed by glucagon (Figure 3G), thus pointing to a direct effect of glucagon on insulin secretion. In 28-week-old GluDTR mice, glycemia (Figure 3H) and plasma insulin concentration (Figure 3I) during glucose tolerance testing were comparable to control mice, suggesting that α cell-derived GLP-1 is not required for maintenance of oral glucose tolerance during aging. Accordingly, administration of the GLP-1 antagonist exendin(9–39) prior to oral glucose tolerance testing...
tolerance testing resulted in impairment of glycemia in both GluDTR and control mice (Figure 3J). Therefore, under normal conditions, lack of a cell-derived GLP-1 and glucagon is compensated by enhanced intestinal GLP-1 production.

**Glucagon Deficiency Protects against HFD-Induced Glucose Intolerance Despite Obesity**

After 22 weeks of HFD feeding, GluDTR mice showed improved i.p. (Figure 4A) and oral (Figure 4C) glucose tolerance, with similar insulin levels (Figures 4B and 4D). This can be explained by the lack of glucagon-mediated insulin resistance. Indeed, insulin sensitivity was increased in GluDTR mice (Figure 4E) despite similar body weight (Figure 4F). Finally, fasting systemic active GLP-1 concentrations were comparable (Figure 4G), while as expected, GluDTR mice displayed reduced fasting glucagon levels (Figure 4H). Therefore, the consequences of decreased systemic glucagon levels may mask the potential impairment in β cell function caused by the lack of a cell-derived GLP-1.

**a Cell-Derived Glucagon-Related Peptides Are Critical for Normal β Cell Function**

To substantiate the hypothesis that a cell-derived GLP-1 effects are required for normal β cell function, we performed ex vivo experiments in isolated islets, where GLP-1 action is limited to paracrine effects and signaling from peripheral GLP-1 target organs to the β cell (such as the nervous system) is missing. First, we performed glucose-stimulated insulin secretion (GSIS) with islets isolated from GluDTR mice. a cell-ablated islets showed a blunted insulin response to glucose (Figure 5A), suggesting that a cell-derived products are necessary for proper β cell function. To depict the precise role of GLP-1, we then confirmed the presence of bioactive GLP-1 in normal mouse islets by performing GSIS following incubation with a GLP-1 antagonist. Indeed, in the presence of exendin(9–39), GSIS was decreased by 57% (Figure 5B). Next, we used the GLP1 receptor activity reporter cell line HTLA (Kroeze et al., 2015) to test whether human islets secrete bioactive GLP-1. Addition of conditioned media from human islets or recombinant GLP-1 as positive control induced a luminescence signal in HTLA cells, suggesting GLP1 receptor signaling occurs (Figure 5C). Co-incubation with exendin(9–39) completely abolished GLP-1 receptor signaling-mediated luminescence. Administration of exogenous GLP-1 or glucagon during GSIS completely restored β cell secretory function (Figure 5D). Cumulative insulin release of cultured islets over 24 hr was significantly reduced in GluDTR islets (Figure 5E), whereas islet insulin content was increased compared to control islets (Figure 5F), as typically observed in situation of an insulin secretion defect. We have shown that the incretin
glucose-dependent insulinotropic polypeptide (GIP) is a strong inducer of active GLP-1 in islets (Timper et al., 2016). Exogenous GIP-induced insulin secretion was impaired in GluDTR versus control islets (Figure S3). Activation of both the GLP-1 and the glucagon receptor leads to an increase in intracellular cyclic AMP (cAMP) concentration (Seino and Shibasaki, 2005). Accordingly, the adenylyl cyclase activator forskolin was able to rescue the defective insulin secretion observed in GluDTR

Figure 5. α Cell-Derived Glucagon-Related Peptides Are Required for Normal GSIS and β Cell Function via cAMP-Mediated β Cell Potentiation

(A) Glucose-stimulated insulin secretion (GSIS) of isolated islets from GluDTR mice that had been injected with DT or saline. n = 3.
(B) GSIS of isolated C57BL/6N mouse islets with or without co-incubation of 100 nM exendin(9–39) during stimulation with 16.7 mM glucose. n = 3.
(C) Relative luminescence of the GLP1R activity luciferase reporter assay. 48 hr after transfection with the GLP1R-Tango vector, HTLA cells were treated with human islet conditioned medium (HI) with or without 100 nM exendin(9–39) or human islet medium containing 100 nM GLP-1 with or without 100 nM exendin(9–39), n = 3.

n indicates the number of independent experiments, and error bars represent SEM. **p < 0.01, ***p < 0.001. See also Figure S3.
islets (Figure 5G). Thus, a cell-derived glucagon-related peptides are indispensable for normal β cell function, which requires cAMP-mediated potentiation of the glucose effect.

α Cell-Specific PC1/3 Knockout Leads to Decreased Active GLP-1 Secretion and Cell Content

Islets from GluDTR mice lack both GLP-1 and glucagon. Thus, the lack of glucagon-induced insulin resistance (Figure 4) may mask the contribution of a cell-derived GLP-1 effects required for β cell function. To substantiate the relevance of a cell-derived GLP-1, we generated an a cell-specific PC1/3 knockout mouse. For this, we created a Pcsk1fl/fl mouse (Figure S4A) and crossed it with an a cell-specific Gcg-Cre-expressing mouse (Herrera, 2000) to obtain Pcsk1fl/fl.Gcg-Cre−/− ("aPcsk1−/−") and Pcsk1fl/fl.Gcg-Cre−/− ("Pcsk1−/−") littermate control mice. To confirm Cre recombinase-targeted recombination ofloxP sites in a tissue with abundant expression ofPC1/3, we crossed the Pcsk1fl/fl mice with PDxCreER mice to generate a tamoxifen-inducible β cell-specific PC1/3 knockout mouse. Following tamoxifen administration, effective knockdown of PC1/3 in aPcsk1−/− mice was confirmed by co-immunostaining for PC1/3 and insulin in pancreatic sections (data not shown) and RNA analysis of isolated islets (5.9% ± 0.8% Pcsk1 expression relative to control). Isolated islets from aPcsk1−/− mice showed decreased active GLP-1 secretion (65.9% of control; Figure 6A) and protein content (64.5% of control; Figure 6B). The extent of GLP-1 reduction is compatible to previously reported Cre recombinase activity in ~40%–45% of a cells in the Gcg-Cre model (Chow et al., 2014; Lu et al., 2010). Interestingly, glucagon secretion and content was increased in aPcsk1−/− compared to control mice, while insulin secretion and content were comparable (Figures 6A and 6B). GSIS was similar in aPcsk1−/− and control islets and was increased upon stimulation with the incretin hormone GIP (Figure 6C). aPcsk1−/− mice had normal body weight development (Figure 6D), and fasting plasma active GLP-1 levels were comparable to control mice (Figure 6E), suggesting intact systemic GLP-1 secretion. RNA analysis of isolated islets from aPcsk1−/− mice suggested that β cell identity is not altered compared to controls (Figure 6F). Thus, aPcsk1−/− mice show reduced levels of active GLP-1, while glucagon and insulin levels are not reduced.

α Cell-Derived GLP-1 Is Required for Adaptation to Metabolic Stress

I.p. glucose tolerance was slightly improved in 11-week-old aPcsk1−/− mice compared to controls (Figure 7A), and insulin
levels tended to be higher (Figure 7B), while oral glucose tolerance and insulin levels were similar between $\alpha Pcsk^{1/-}$ and control mice (Figures 7C and 7D). This somewhat paradoxical trend in i.p. glucose tolerance disappeared in 22-week-old chow-fed (Figures S5A and 5B) and 36-week-old HFD-fed (Figures S5E and S5F) $\alpha Pcsk^{1/-}$ mice, along with similar body weight and systemic glucagon and active GLP-1 levels as well as ileal active GLP-1 content compared to control animals (Figures S5C, S5D, and S5G–S5I). Next, we tested the adaptive capacity of the $\alpha Pcsk^{1/-}$ mice fed a HFD and injected with a single low dose of the $\beta$ cell toxin streptozotocin at 10 weeks of age. At the age of 15 weeks, body weight as well as active GLP-1 concentrations in plasma and ileal active GLP-1 content were similar in both groups (Figures 7E–7G). i.p. glucose tolerance was impaired in HFD/streptozotocin-treated $\alpha Pcsk^{1/-}$ mice compared to controls (Figure 4H), along with decreased insulin secretion (Figure 7I). Of note, while the difference between the genotype appears mild, it was consistently observed in four independent cohorts of six to ten animals each. In one cohort, the maximal impairment in $\alpha Pcsk^{1/-}$ mice was observed at 11 weeks of age, while in the other three, it was observed at 14 weeks. For simplicity, only the average of data of week 14 is shown. Sitagliptin injection prior to i.p. glucose tolerance testing partially reversed the impaired glucose tolerance (Figure 7J). However,
plasma insulin concentrations remained lower in αPcsk1−/− mice than in control mice (Figure 7K). To assess the potential relevance of these findings in humans, we compared GLP-1 content of human and mouse islets. Human islets showed an even higher active GLP-1 content than mouse islets (29.28 ± 3.48 versus 0.32 ± 0.04 pg/islet, n = 23–27, p < 0.001). To conclude, under metabolic stress and increased secretory demand, β cell adaptation requires α cell expression of PC1/3 and paracrine action of GLP-1. Finally, we tested whether there is increased α to β cell conversion in αPcsk1−/−, which was reported to occur after near-complete α cell destruction (Thorel et al., 2010). The level of proinsulin-related peptides (Figure S5J) and the number of insulin-negative but PC1/3-positive cells was identical in αPcsk1−/− and control mice, suggesting that α to β cell conversion is not increased in our mouse model.

DISCUSSION

In the present study, we show that α cell-derived glucagon-related peptides are mandatory for normal glucose-stimulated insulin secretion. While under normal conditions α cell-derived GLP-1 may be compensated by increased intestinal GLP-1 secretion, paracrine GLP-1 is needed for normal glucose homeostasis during aging and adaptation to metabolic stress.

Several in vitro and ex vivo studies have shown the ability of α cells to produce active GLP-1 (Ellingsgaard et al., 2011; Hansen et al., 2011; Klimkin et al., 2010; Marchetti et al., 2012; Nie et al., 2000; Thyssen et al., 2006). However, the biological role of local α cell-derived GPL-1 in islet function and glucose homeostasis remained unknown. We show that during aging α cell-ablated mice develop impaired insulin secretion and glucose homeostasis. Stabilization of systemic GLP-1 with the DPP-4 inhibitor sitagliptin completely rescued this phenotype, suggesting a role for α cell-derived GLP-1 in aging. In contrast, glucagon administration failed to normalize glycemia in α cell-ablated mice. To demonstrate the paracrine effect of local GLP-1 in islets where glucagon production is present, we generated a genetic mouse model of α cell-specific GLP-1 deficiency. The αPcsk1−/− mouse showed decreased active GLP-1 islet content and secretion into culture supernatant, while systemic active GLP-1 concentrations were comparable to control mice, which can be explained by intact GLP-1 secretion from intestinal L cells. In young, chow-fed αPcsk1−/− mice, i.p. glucose tolerance was slightly improved compared to control animals. However, this effect was lost upon aging. Metabolic stress in αPcsk1−/− mice impaired glycemic control, while administration of sitagliptin partially rescued this impairment in glucose tolerance.

One limitation of the αPcsk1−/− model is the low level of Cre recombinase activity in the Gcg-Cre mouse. Consistent with previous publications using the Ggc-Cre mouse (Chow et al., 2014; Lu et al., 2010), only a partial knockout of PC1/3 was achieved in α cells with remaining pancreatic active GLP-1 production. This could be the reason why aging did not affect glucose tolerance in αPcsk1−/− mice, while it did in the model of α cell ablation. Despite remaining intra-islet GLP-1 production, the uncovered impaired glucose disposal points to an important role of α cell-derived GLP-1 in adaptation to metabolic stress. α cells were shown to be able to convert to functional β cells after near-total and partial β cell ablation (Thorel et al., 2010). Thus, the present findings based on a model using HFD/streptozotocin could potentially be explained by conversion of PC1/3-deficient α cells to β cells. Such β cells would be expected to lack PC1/3 and thus would not be able to secrete fully processed insulin. However, this explanation is unlikely, as the levels of proinsulin (including des-64,65-proinsulin yielded by PC2, but not PC1/3 cleavage) were identical in plasma from αPcsk1−/− and control mice. In addition, the number of insulin-positive but PC1/3-negative cells in pancreata from both genotypes was identical. Altogether, this strongly suggests that, despite use of streptozotocin, α to β cell conversion is not increased in our mouse model.

Administration of the GLP-1 antagonist exendin9–39 during an oral glucose tolerance test resulted in impaired glucose tolerance in both α cell-ablated and control mice, indicating dependence on systemic GLP-1 signaling. In line with previously published data by Smith and co-workers (Smith et al., 2014), our data indicate that intra-islet GLP-1 is not required for oral glucose tolerance under normal conditions. Indeed, lack of GLP-1 receptors in β cells resulted in impaired i.p. glucose tolerance compared to controls, while oral glucose tolerance was normal. This suggests indirect action of systemic GLP-1 on β cells during oral glucose challenge (Smith et al., 2014). In young α cell-ablated mice, we observed enhanced oral glucose tolerance, resembling the phenotype of the double glucagon and GLP-1 receptor knockout mouse, which displays normal i.p. glucose tolerance and improved oral glucose tolerance (Ali et al., 2011). The reason for improved oral glucose tolerance in the double receptor knockout mouse is enhanced sensitivity toward other incretins. In our α cell-ablated islets, however, equimolar GLP-1 or GIP caused similar or lower fold increase of GSIS compared to control islets. Thus, we could not observe increased sensitivity of β cells toward these classical incretins. Instead, active GLP-1 content in the terminal ileum of α cell-ablated mice was increased compared to controls and active GLP-1 secretion was higher during oral glucose tolerance testing. This points to a compensatory increase of peripheral GLP-1, possibly to rescue the lack of local islet-derived GLP-1.

Glucagon administration in α cell-ablated mice prior to an oral glucose tolerance test suppressed GLP-1 secretion. In humans, fasting glucagon concentration is a strong determinant of mixed meal-induced GLP-1 secretion (Nauck et al., 2011). One study has reported decreased GLP-1 and GIP plasma levels in response to oral glucose loading in healthy human subjects when glucagon was infused concomitantly (Ranganath et al., 1999). However, highly supraphysiological concentrations of glucagon were infused, thus providing limited value for physiological processes. In another human study, where physiological plasma glucagon concentrations were maintained, no effect of exogenous glucagon on GLP-1 secretion after ingestion of a mixed meal was detectable (Meier et al., 2010). Interestingly, upon cessation of glucagon infusion, GLP-1 concentrations increased significantly. Even though our data show a clear decrease of GLP-1 secretion by glucagon, the effect might be also indirect via stimulated insulin secretion. Because glycemia did not change upon glucagon administration, the physiological
significance of this hormonal interaction remains to be elucidated.

We further show that glucagon deficiency protects against HFD-induced glucose intolerance in α cell-ablated mice. This phenotype has been described in glucagon-receptor-deficient mice (Conarello et al., 2007; Gelling et al., 2003). However, these studies remained inconclusive about the direct role of glucagon in HFD-induced glucose intolerance, because the mice had a lean phenotype and showed pronounced compensatory α cell hyperplasia along with highly elevated levels of systemic active GLP-1. Our HFD-fed α cell-ablated mice had body weights and systemic active GLP-1 concentrations comparable to their HFD-induced obese controls. Thus, the development of insulin resistance and glucose intolerance after HFD can be clearly attributed to the action of glucagon in our model of glucagon deficiency.

We (Ellingsgaard et al., 2011) and others have suggested that α cells may secrete GLP-1, but this is still debated. Using a bioactivity reporter assay, we now show that human islets secrete bioactive GLP-1. In isolated α cell-ablated mouse islets, GSIS was dramatically reduced. This phenotype was fully rescued upon addition of exogenous GLP-1 or glucagon. The adenylyl cyclase activator forskolin also increased GSIS in α cell-ablated islets. Consistent with our findings, isolated islets from glucagon and GLP-1 receptor knockout mice also demonstrated decreased GSIS (Ali et al., 2011). These data show that glucose alone is not sufficient to induce normal insulin secretion from pancreatic β cells and that cAMP-mediated β cell potentiation by α cell-produced glucagon-related peptides is mandatory for stimulus-secretion coupling in β cells.

Taken together, our data show that the paracrine action of α cell-derived glucagon-related peptides are important for normal glucose homeostasis and β cell function. α cell-derived GLP-1 is required for β cell adaptation to aging and metabolic stress. It follows that the clinical use of systemic GLP-1 agonists in the treatment of type 2 diabetes may be less effective to impact on β cell function compared to stabilizing local α cell-derived GLP-1 by DPP-4 inhibitors.

**EXPERIMENTAL PROCEDURES**

**Experimental Animals**

All animal experiments were performed in accordance with the federal laws of Switzerland and approved by the cantonal and institutional authorities. C57BL/6N were obtained from Charles River Laboratories. GluDTR mice, which have been described before (Thorel et al., 2010), were backcrossed to a C57BL/6N background using microarray-based speed congenics (Biolytix). Both GluDTR and littermate control mice (“WT”) received DT injections, except when noted otherwise. To generate conditional Pcsk1 knockout mice (“αPcsk1”−/−), C57BL/6N embryonic stem cells carrying a transgene encoding a FRT-flanked lacZ and neo cassette and IoxP sites around exon 5 of the Pcsk1 gene (EUCCOMM program) were injected into blastocysts. Offspring of chimera with germline transmission were crossed with a ROSA26:FLPe mouse (Farley et al., 2000) to excise the FRT-flankase sequence. The resulting Pcsk1fox/fox mice (B6-Pcsk1<1 tm1Boe > N) were crossed with Ggc-Cre mice (Herrera, 2000), which were previously backcrossed to the C57BL/6N genetic background, to obtain α cell-specific Pcsk1 knockout mice (B6-Tg(Ggc-Cre)1Herki/N x B6-Pcsk1<1 tm1Boe > N) (Figure 5D). Male mice were used for all studies. See figure legends for specific ages. Animals were housed in a pathogen-free animal facility with a 12-hr light/12-hr dark cycle and were allowed free access to food and water.

**DT Injections**

DT (D0564; Sigma) dissolved in 0.9% NaCl was administered to 5- to 6-week-old mice in three i.p. injections of 500 ng each on days 1, 3, and 5 as reported before (Thorel et al., 2010). The total amount of injected DT was 1.5 μg per mouse.

**HFD/Streptozotocin Model**

αPcsk1−/− and Pcsk1fox/fox littermate mice were fed a HFD (D12331, 58 kcal % fat, Research Diets) starting at 4 weeks of age. At 10 weeks of age, animals received an i.p. injection of 130 mg/kg body weight streptozotocin (Sigma). At 14 weeks of age, an i.p. glucose tolerance test was performed. The glucose tolerance test was repeated at 15 weeks of age with the addition of an injection of 25 mg/kg body weight sitagliptin (S8576, Sigma) 30 min prior to glucose administration.

**Glucose and Insulin Tolerance Tests**

Glucose (2 g/kg body weight) was either administered by i.p. injection or oral gavage after 6 hr of fasting (8:00–14:00). Blood samples were obtained by tail tip bleeding and assayed using a glucometer (Freestyle; Abbott) or mixed with EDTA, aprotinin (A1153; Sigma), or diprotin A (H9758; Sigma) and spun down, and plasma was stored for further analysis. To stabilize systemic active GLP-1, 25 mg/kg body weight sitagliptin dissolved in 0.9% NaCl was i.p. injected 30 min prior to glucose administration. Synthetic glucagon dissolved in PBS (20 μg/kg body weight; G2044; Sigma) was i.p. injected 1 min prior to glucose administration. Synthetic exendin-9–39 (25 μg/kg body weight; H-8740; Bachem) was i.p. injected 30 min prior to glucose administration. For insulin tolerance tests, mice were fasted 3 hr (8:00–11:00) and injected with 1 U/kg body weight insulin (Novorapid, Novo Nordisk), and measurement of blood glucose was done as described above.

**Human Pancreatic Islets**

Human islets isolated from pancreata of cadaver organ donors in accordance with the local institutional ethical committee were provided by the islets for research distribution program through the European Consortium for Islet Transplantation, under the supervision of the Juvenile Diabetes Research Foundation (31-2012-783). Islets were cultured in CMRL-1066 medium containing 5 mM streptomycin, 2 mL penicillin, 10 μg/mL dexamethasone, and 10% fetal calf serum (FCS) (Invitrogen) on extracellular-matrix-coated 24-well plates (Novamed) in humid environment containing 5% CO2. Islets were cultured for 48 hr with 1 mM diprotin A to stabilize GLP-1 secretion. Conditioned media were collected in tubes containing diprotin A (end concentration 100 μM) and stored at −80°C for the GLP-1R activity luciferase reporter assay.

**Mouse Pancreatic Islets**

Mouse islets were isolated by collagenase digestion (4189; Worthington) and cleaned by filtration trouch a mesh and hand picking. They were either lysed directly after isolation for mRNA extraction or cultured on extracellular-matrix-coated 24-well plates (Novamed) in humid environment containing 5% CO2. Islets were cultured for 48 hr with 1 mM diprotin A to stabilize GLP-1. Conditioned media were collected in tubes containing diprotin A (end concentration 100 μM) and stored at −80°C for the GLP-1R activity luciferase reporter assay.

**GSIS Assay**

For ex vivo GSIS experiments, mouse islets were plated in quadruplicate and allowed to attach for 2 days. Islets were pre-incubated for 30 min in modified Krebs-Ringer bicarbonate buffer [KRB; 115 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl2, 2 mM KH2PO4, 1.2 mM MgSO4, 7 mM HCO3, 10 mM HEPES, and 0.5% BSA (pH 7.4)] containing 2.8 mM glucose. KRB was then replaced by KRB containing 0 mM glucose, 25 mg/kg body weight sitagliptin (S8576, Sigma), 25 mg/kg body weight dapagliflozin (C-23662, Novo Nordisk), and measurement of blood glucose was done as described above.

**Ileal Hormone Content Extraction**

To determine active GLP-1 content of the terminal ileum, 3 cm of ileum was excised proximal from the caecum, rinsed with PBS, and extracted with

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0.18 N HCl in 70% EtOH. Samples were spun down and supernatants stored for further analysis.

**Hormone Measurements**

Insulin and active GLP-1 concentrations in plasma and islet supernatants as well as glucagon concentrations in islet supernatants were determined using commercially available kits (Meso Scale Discovery). Plasma glucagon levels were determined using a glucagon ELISA (Mercodia).

**RNA Extraction and qPCR**

Total RNA of isolated mouse islets was extracted using the NucleoSpin RNA II Kit (Macherey Nagel). cDNA was prepared with random hexamers and SuperScript II reverse transcriptase (Invitrogen). For qPCR, the real-time PCR system 7500 (Applied Biosystems) and the following TaqMan assays were used: Gcg - Mm00480171_m1; Ins2 - Mm00731595_gH; Pcsk1 – Mm01345254_m1; Pcsk2 – Mm00500974_m1; Gcgr - Mm00435565_m1; Glp1r – Mm00445292_m1; Pdx1 - Mm01329193_m1. Gene expression was analyzed with the comparative 2^ΔΔCT method.

**Immunohistochemical Staining**

For tissue sections, formalin-fixed pancreata were embedded in paraffin and cut into 4-μm-thick sections. Tissue sections were deparaffinized, rehydrated, and stained with the indicated antibodies. For staining of single mouse islet cells, mouse islets were dispersed with 0.02% trypsin-EDTA (Invitrogen) followed by cytospin onto glass slides for 3 min at 1,000 rpm. Islet cells were fixed for 30 min with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 for 4 min, and stained with the indicated antibodies. The following antibodies were used: guinea pig anti-insulin (1:100; Dako), mouse anti-glucagon (1:1,000; Sigma), goat anti-GLP1 (1:50; Santa Cruz Biotechnology), and rabbit anti-PC1/3 (1:100; Millipore). Secondary antibodies were coupled to Alexa Fluor 647 dyes (Molecular Probes) and fluorescein isothiocyanate (FITC) (Jackson ImmunoResearch Laboratories). Nuclei were labeled with DAPI (Sigma). Sections were analyzed with Olympus BX61 and BX63 using CellSens software and imageJ (Fiji edition). For determination of insulin+/PC1/3- cells in pancreata from HFD/streptozotocin-treated mice, a GluDTR reporter and a Roth (University of North Carolina). Cells were plated at 2.2 × 10^4 cells per well into 96-well plates containing 180 μL DMEM medium per well. On day 3, transfected cells were transfected at 30,000 cells per well into 96-well plates containing 100 μL DMEM medium per well. On day 4, the medium was removed and replaced with human-islet-derived conditioned media containing 1 mM diphorin A. On day 5, medium was removed and 100 μL Bright-Glo solution (Promega) diluted 1:1 in RPMI buffer was added. Luminescence was measured 5 min later.

**Supplemental Information**

Supplemental Information includes five figures and can be found with this article online at [http://dx.doi.org/10.1016/j.celrep.2017.03.005](http://dx.doi.org/10.1016/j.celrep.2017.03.005).

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**AUTHOR CONTRIBUTIONS**

S.T., D.T.M., M.B.-S., and M.Y.D. designed the study and wrote the manuscript. S.T., D.T.M., E. Dror, and M.B.-S. performed and analyzed the experiments. F.S., T.M.N., N.G., N.K., E. Dalmass, M.S., and V.M. helped with the experiments. F.T. and P.L.H. provided the GluDTR mice.


