Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice

Highlights
- The mouse and human pancreas contain low levels of active GLP-1
- The gut enteroendocrine system is responsible for 95% of circulating active GLP-1
- The proximal gut sustains normal plasma GLP-1 levels in response to enteral glucose
- Gut Gcg expression controls glucose tolerance and gastric emptying

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In Brief
The gut-derived hormone GLP-1 also functions as an islet-derived insulinotropic peptide. Song and Koehler et al. now show that whereas the pancreas contains active GLP-1, deletion of the Gcg gene from the gut lowers circulating GLP-1, impairs glucose tolerance, and accelerates gastric emptying, highlighting essential metabolic roles of the gut GLP-1 system.
Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice

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SUMMARY

The importance of pancreatic versus intestinal-derived GLP-1 for glucose homeostasis is controversial. We detected active GLP-1 in the mouse and human pancreas, albeit at extremely low levels relative to glucagon. Accordingly, to elucidate the metabolic importance of intestinal proglucagon-derived peptides (PGDPs), we generated mice with reduction of Gcg expression within the distal (GcgDistalGut−/−) or entire (GcgGut−/−) gut. Substantial reduction of gut Gcg expression markedly reduced circulating levels of GLP-1, and impaired glucose homeostasis, associated with increased levels of GIP, and accelerated gastric emptying. GcgDistalGut−/− mice similarly exhibited lower circulating GLP-1 and impaired oral glucose tolerance. Nevertheless, plasma levels of insulin remained normal following glucose administration in the absence of gut-derived GLP-1. Collectively, our findings identify the essential importance of gut-derived PGDPs for maintaining levels of circulating GLP-1, control of gastric emptying, and glucose homeostasis.

INTRODUCTION

Glucagon, a 29-amino-acid peptide, was originally identified as a hormone isolated from the pancreas with hyperglycemic activity. The molecular cloning of cDNAs and genes encoding proglucagon expanded the complexity of the proglucagon-derived peptides (PGDPs) (Drucker, 2006). Mammalian species transcribe a single proglucagon mRNA transcript yet generate a different profile of PGDPs in brain, pancreas, and intestine through tissue-specific proteolytic cleavage, mediated by members of the prohormone convertase enzyme family (Sandoval and D’Alessio, 2015). Within the pancreas, glucagon is the major liberated PGDP, and the glucagon-like peptides are contained within a larger, unprocessed major proglucagon fragment (Patzelt and Schiltz, 1984). In contrast, enteroendocrine L cells process proglucagon to liberate glicentin, oxyntomodulin, and two glucagon-like peptides: glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) (Mojsov et al., 1986).

Within the gut, two bioactive GLP-1 moieties, GLP-1(7-37), and GLP-1(7-36) amide are derived from proglucagon (Orskov et al., 1994). Both peptides circulate at low basal levels in the fasting state, are secreted rapidly following meal ingestion, and are equipotent stimulators of insulin secretion (Vahl et al., 2003). Indeed, pharmacological blockade of GLP-1 action in animals and humans, or genetic interruption of GLP-1 receptor

Context and Significance

Glucagon-like peptide 1 (GLP-1)-based drugs are effective anti-diabetic therapies. How and where GLP-1 and its related proteins are naturally produced in the body has been the topic of some debate. Investigators at the University of Michigan and University of Toronto re-examined some of their previous conclusions that the pancreas (which also produces insulin) is the main source of GLP-1 in mice. Although small amounts of active GLP-1 can be detected in both the mouse and human pancreas, disruption of the GLP-1-producing gene in the mouse gut had pronounced adverse effects on glucose control. The results reinforce the importance of gut GLP-1 secretion, physiologically and potentially pharmacologically, for control of metabolism.
signaling in mice, impairs the insulinotropic action of GLP-1, establishing the physiological importance of GLP-1 as an incretin hormone (Drucker, 2007).

Despite the preponderance of evidence demonstrating the synthesis and secretion of bioactive GLP-1 in the small and large intestine (Kreymann et al., 1988; Mjoqvist et al., 1986; Orskov et al., 1994), studies have also implicated pancreatic islet \( \alpha \) cells as a source of bioactive GLP-1. Rodent and human islet cells cultured ex vivo secrete GLP-1 with incretin activity (Ellingsgaard et al., 2011; Hansen et al., 2011; Heller and Aponte, 1995; Marchetti et al., 2012; Timper et al., 2016). Moreover, experimental pancreatic injury, including surgical manipulation, chemical damage, aging, or the prolonged nutritional stress of energy-dense diets, is associated with increased pancreatic production of GLP-1 (Kilimnik et al., 2010; Nie et al., 2000; Vasu et al., 2014).

We recently used mouse genetics and the GLP-1R antagonist exendin(9-39) to examine the consequences of selective re-induction of endogenous Gcg gene expression in the intestine versus the pancreas of Gcg \(-/-\) mice (Chambers et al., 2017). Reactivation of intestinal Gcg expression using the Villin-Cre system (GcgRA\textsuperscript{AVLICre}) restored intestinal Gcg and GLP-1 production, in the setting of complete loss of pancreatic Gcg expression (Chambers et al., 2017). Surprisingly, the GLP-1R antagonist exendin(9-39) failed to impair glycemic excursions to both oral and intraperitoneal (i.p) GLP-1R antagonist exendin(9-39) failed to impair glycemic excursions to both oral and intraperitoneal (i.p) GLP-1R antagonist exendin(9-39). Since the publication of these findings required careful consideration of the experimental context, namely reactivation of Gcg expression in Gcg \(-/-\) mice known to exhibit \( \alpha \) cell hyperplasia (Hayashi et al., 2009). These conditions favor the generation of pancreatic islet GLP-1 production in dedifferentiated \( \alpha \) cells following the reintroduction of pancreatic Gcg expression (Hayashi et al., 2009). Moreover, Gcg \(-/-\) mice may exhibit compensatory induction of islet \( \beta \) cell GIP expression (Fukami et al., 2013), further complicating interpretation of incretin action. Critically, the importance of pancreatic versus intestinal GLP-1 activity was inferred indirectly via the glycemic response to exendin(9-39) (Chambers et al., 2017). Since the publication of these provocative findings, several studies have demonstrated that exendin(9-39) also blocks the insulinotropic actions of glucagon at the islet GLP-1 receptor (Capozzi et al., 2019a; Svendsen et al., 2018; Zhu et al., 2019), complicating precise mechanistic attribution of glycemic responses following use of exendin(9-39).

Accordingly, we have now re-examined the metabolic importance of endogenous gut-derived PGPDs in the absence of exendin(9-39) using two new lines of mice with the elimination of Gcg expression in the distal gut, or throughout the small and large bowel. Our findings re-establish gut-derived PGPDs as important metabolically active peptides, highlighting the potential for both enteroendocrine cells and pancreatic islet cells to serve as sources of PGPDs with glucoregulatory action.

RESULTS

Assessment of GLP-1 and Glucagon Content in Mouse and Human Pancreas

Although GLP-1 may be detected in the pancreas in the context of pancreatic injury and inflammation (Drucker, 2013; Habener and Stanojevic, 2017), active GLP-1 was not detected in mouse pancreatic perfusate (Svendsen et al., 2018) and no extractable active GLP-1 was detectable in the normal mouse pancreas (Galsgaard et al., 2018). Hence, we re-examined levels of active GLP-1 in mouse and human pancreas. Active GLP-1 content in the pancreas of wild-type (WT) Gcg \(^{+/+}\) mice, maintained on normal chow (NC) or fed a 60% high-fat diet (HFD), was extremely low but detectable (Figure 1A), several orders of magnitude lower than active GLP-1 content in the colon (Figure 1A). Moreover, the levels of active GLP-1 (liberated from a common proglucagon precursor; Figure S1) in the mouse pancreas were 20- to 30-fold lower than that of glucagon and were not different after 7 weeks of HFD feeding (Figures 1B and 1C). Similar findings were observed in Cre control and mice with Gcg reactivation in the pancreas (GcgRA\textsuperscript{APDX1Cre})(Chambers et al., 2017) (Figure 1D), where glucagon content greatly exceeded that of active GLP-1 in pancreas-tissue extracts (Figure 1E). In contrast, neither glucagon nor active GLP-1 was detectable in the pancreas of Gcg-null or intestinal Gcg-reactivated (GcgRA\textsuperscript{AVLICre}) mice despite abundant levels of insulin (Figure 1D).

To assess the potential for species-specific differences in pancreatic GLP-1 production, we examined the human pancreas. Active GLP-1 was also detectable in normal human pancreas extracts at levels higher than in the mouse pancreas (Figure 1F, middle panel). Levels were somewhat variable, as were both glucagon and insulin content (Figure 1F, left and right panels, respectively), most likely reflecting different numbers of islets in each sample and/or age of the respective donor (Table S1). Nevertheless, glucagon content greatly exceeded that of active GLP-1 in the human pancreas (Figures 1F and 1G). Taken together, active GLP-1 is very low, but detectable, in tissue extracts from both mouse and human pancreas, albeit at much lower levels relative to glucagon.

Generation and Characterization of Gcg\(^{Gut-/-}\) and Gcg\(^{DistalGut-/-}\) Mice

Our previous studies addressed the glucoregulatory activity of GLP-1 in mice with reactivation of intestinal or pancreatic proglucagon (Gcg) expression in Gcg \(-/-\) mice (Chambers et al., 2017). Interpretation of the biological importance of GLP-1 in these studies relied on exendin(9-39), a GLP-1 receptor antagonist subsequently shown to block the activity of both GLP-1 and glucagon at the pancreatic GLP-1 receptor (Capozzi et al., 2019a; Svendsen et al., 2018; Zhu et al., 2019). Accordingly, to elucidate the importance of intestinal PGDP action in the absence of exendin(9-39), we generated two new lines of mice enabling the selective loss of Gcg expression in the intestine. Gcg\(^{flow/flox}\) mice were crossed with Vill-Cre mice to generate Gcg\(^{Gut-/-}\) mice (Figure 2A). Gcg\(^{Gut-/-}\) mice exhibited a >95% reduction in Gcg mRNA levels throughout the entire small intestine and colon, whereas Gcg mRNA transcripts in the brainstem were normal (Figure 2A). Islet histology was similar (Figure S2A) and pancreatic glucagon mRNA transcripts levels were higher.
yet pancreatic levels of active GLP-1 were very low and not different in Gcg<sup>−/−</sup> mice (Figure 2B). Moreover, plasma levels of glucagon were similar in Gcg<sup>−/−</sup> and Gcg<sup>+/+</sup> mice (Figure S2B). Consistent with the marked reduction of Gcg mRNA transcripts, active and total GLP-1 content was markedly reduced in all regions of the small intestine and colon from Gcg<sup>−/−</sup> mice (Figures 2B and S2C), whereas intestinal GIP levels were not different (Figure 2C).

Despite detection of some active GLP-1 in the pancreas of both Gcg<sup>−/−</sup> and Gcg<sup>+/+</sup> mice, levels were markedly lower than in the small intestine or colon of Gcg<sup>+/+</sup> mice (pancreas, ~6.7 pg/mg protein versus jejunum, 1,389 pg/mg protein; colon, 9,470 pg/mg protein) (Figure 2B). Similarly, relative levels of active GLP-1 remained lower in the pancreas versus the small intestine and colon of Gcg<sup>−/−</sup> mice (pancreas, ~9.4 pg/mg protein versus jejunum, 35.5 pg/mg protein; colon, 57.7 pg/mg protein) (Figure 2B), whereas active GLP-1 levels in the pancreas of Gcg<sup>+/+</sup> versus Gcg<sup>−/−</sup> mice were not different (6.7 ± 1.8 pg/mg protein versus 9.4 ± 3.2 pg/mg protein, p = 0.09).

Although increased pancreatic GLP-1 production and activity have been demonstrated in the presence of metabolic stress and experimental diabetes (Klimnink et al., 2010; Traub et al.,...
Figure 2. Characterization of Gcg<sup>Gut<–/–</sup> and Gcg<sup>DistalGut<–/–</sup> Mice

(A) Proglucagon (Gcg) mRNA abundance normalized to levels of mRNA for ribosomal protein L32 (Rpl32) in different regions of the small intestine, colon, and pancreas (left panel) or brainstem (right panel) of 16- to 18-week-old female mice by qPCR using a primer probe set against exons 1–2 of the Gcg gene (n = 6–14/group). Gene expression was expressed relative to values for Gcg mRNA transcripts in proximal ileum (prox ileum; left panel), or brainstem (right panel) of control Gcg<sup>+/+</sup> mice.

(B and C) Active GLP-1 (B) and total GIP levels (C), normalized to total protein content, in whole-tissue extracts from different regions of the intestine and pancreas of 16- to 18-week-old female mice (n = 6–8/group for GLP-1, n = 6–10 for GIP). Duo, duodenum; Jej, jejunum; Prox ileum, proximal ileum; Panc, pancreas. Gcg<sup>+/+</sup> mice represent combined data from WT, Gcg<sup>flox/flox</sup>, and Vil-Cre control mice.

(D) Gcg mRNA abundance normalized to cyclophilin (Ppia) in different regions of the small intestine and colon (left panel), pancreas (middle panel), or brainstem (right panel) of 20- to 23-week-old male mice by qPCR (n = 6–9/group). Gene expression was expressed relative to Gcg mRNA levels in control (Cdx2<sup>-Cre</sup>) mice.
2017), active GLP-1 levels from pancreatic extracts of Vil-Cre and Gcg<sup>Gut</sup>−/− mice remained exceptionally low and did not increase after 7 weeks of HFD feeding (Figure S2D). In contrast to the low pancreatic levels of active GLP-1, assessment of total immunoreactive GLP-1 in the pancreas using an assay that recognizes all molecular forms of GLP-1 (Figure S1) revealed much higher levels (Figure S2C), consistent with the immunodetection of the GLP-1 sequence contained within the major proglucagon fragment (MPGF) (Patzeit and Schiltz, 1984).

We next established a second mouse model of intestinal PGDP deficiency within the distal, but not proximal gut, by crossing Gcg<sup>lox/lox</sup> mice with Cdx2-Cre mice to generate Gcg<sup>DistalGut</sup>−/− mice. Gcg mRNA transcripts were markedly reduced in the ileum and colon of Gcg<sup>DistalGut</sup>−/− mice, without perturbation of Gcg mRNA levels in the proximal small intestine, pancreas, or brainstem (Figure 2D). Both active and total GLP-1 levels were markedly reduced in the ileum and colon of Gcg<sup>DistalGut</sup>−/− mice; however, GLP-1 content was not reduced in the jejunum (Figures 2E and S3A). Similarly, Gip mRNA levels and tissue GIP content were not different in Gcg<sup>DistalGut</sup>−/− mice (Figures 2F and 2G).

**Gcg<sup>Gut</sup>−/− Mice Exhibit Impaired Oral but Normal i.p. Glucose Tolerance**

Despite the preservation of pancreatic Gcg expression in Gcg<sup>Gut</sup>−/− mice (Figure 2A), fasting plasma levels of total immunoreactive GLP-1 were lower in Gcg<sup>Gut</sup>−/− mice (Figure 3A). Moreover, fasting plasma active GLP-1 levels were markedly reduced in Gcg<sup>Gut</sup>−/− mice (Figure 3B), highlighting the predominant contribution of the intestine to the circulating pool of bioactive GLP-1. Conversely, although intestinal GIP levels were not different in Gcg<sup>Gut</sup>−/− versus Gcg<sup+/+</sup> mice (Figure 2C), fasting plasma levels of GIP were higher in Gcg<sup>Gut</sup>−/− mice (Figure S2B) without differences in fasting levels of insulin and glucagon (Figure S2B). Remarkably, despite the importance of basal GLP-1R signaling for control of food intake and body weight (Campbell and Drucker, 2013; Patterson et al., 2011; Sandoval and D’Alessio, 2015), both incremental and cumulative food intake and body weight on regular chow and HFD were similar in Gcg<sup>Gut</sup>−/− versus control mice (Figures S2E and S2F). Thus, marked reductions in GLP-1 of intestinal origin have no meaningful impact on food intake and body weight.

Following an oral glucose challenge, Gcg<sup>Gut</sup>−/− mice exhibited higher glycemic excursions compared to Vil-Cre controls without differences in fasting glucose (Figures 3C and 3D). Consistent with the marked reduction of Gcg mRNA transcripts in the intestine, both total and active plasma GLP-1 levels did not increase following enteral glucose loading in Gcg<sup>Gut</sup>−/− mice but increased in Gcg<sup>Gut</sup>+/− mice (Figures 3E and 3F). In contrast, plasma GIP levels were higher following glucose challenge in Gcg<sup>Gut</sup>−/− mice than Vil-Cre controls (Figure 3G). Surprisingly, glucose-stimulated insulin levels and reductions in plasma glucagon were similar between Gcg<sup>Gut</sup>−/− and Vil-Cre control mice (Figures 3H and 3I). However, gastric emptying was accelerated in Gcg<sup>Gut</sup>−/− mice (Figure 3J). Accordingly, we bypassed the gut and administered i.p. glucose. Gcg<sup>Gut</sup>−/− mice exhibited glycemic excursions comparable to controls following i.p. glucose challenge (Figure 3K), without differences in plasma insulin levels (Figure 3L).

**Gcg<sup>DistalGut</sup>−/− Mice Exhibit Impaired Oral and i.p. Glucose Tolerance**

Although levels of Gcg mRNA transcripts and tissue content of PGDPs are higher in the distal gut (Roberts et al., 2019; van der Wielen et al., 2016), the relative importance and contribution of the proximal versus distal gut to GLP-1 secretion and action remains uncertain and controversial (Swendsen et al., 2015). In contrast to findings in Gcg<sup>DistalGut</sup>−/− mice, fasting plasma total GLP-1 levels were comparable between Gcg<sup>DistalGut</sup>−/− mice and controls (Figure 4A), suggesting that proximal gut Gcg expression contributes to circulating immunoreactive GLP-1. Strikingly, plasma levels of active GLP-1 were markedly reduced in fasted Gcg<sup>DistalGut</sup>−/− mice (Figure 4B), revealing the predominant contribution of distal gut GLP-1 expression to circulating bioactive GLP-1. In contrast, fasting plasma GIP, insulin, and glucagon were similar between Gcg<sup>DistalGut</sup>−/− mice and controls (Figure S3B), and food intake and body weight were not different in Gcg<sup>DistalGut</sup>−/− mice on regular chow or after several weeks of HFD feeding (Figures S3C and S3D).

Consistent with the reductions in plasma active GLP-1, Gcg<sup>DistalGut</sup>−/− mice exhibited oral glucose intolerance, associated with slightly higher fasting glucose levels (Figures 4C and 4D). Surprisingly, both total and active GLP-1 plasma levels increased equivalently following oral glucose challenge in Gcg<sup>DistalGut</sup>−/− versus Cdx2-Cre control mice, despite the elimination of distal gut Gcg expression (Figures 4E and 4F), unmasking the importance of proximal gut Gcg expression for the rapid rise in plasma GLP-1 levels following oral glucose. Consistent with findings in Gcg<sup>DistalGut</sup>−/− mice, GIP levels were higher after oral glucose challenge in Gcg<sup>DistalGut</sup>−/− versus Cdx2-Cre mice (Figure 4G). Despite elevated glucose excursions, glucose-stimulated insulin levels and reductions in plasma glucagon were similar between Gcg<sup>DistalGut</sup>−/− and Cdx2-Cre control mice after oral glucose challenge (Figures 4H and 4I). Gcg<sup>DistalGut</sup>−/− mice also exhibited modestly higher glucose excursions following an intraperitoneal glucose load; however, this was partially driven by higher fasting glucose levels (Figures 4J and 4K), and glucose-stimulated insulin levels were not different in Gcg<sup>DistalGut</sup>−/− versus Cdx2-Cre control mice (Figure 4L)

**DISCUSSION**

Initial concepts of GLP-1 action as a gut-derived incretin hormone stemmed from its isolation from the small and large bowel, whereas pancreatic extracts contained little bioactive intact GLP-1 (Orskov et al., 1987), and studies with perfused pancreas preparations did not detect GLP-1 in pancreatic effluents.
Figure 3. Loss of Gut Gcg Expression Produces Glucose Intolerance Following Oral, but not i.p., Glucose Administration

(A and B) Fasting plasma levels of (A) total GLP-1 or (B) active GLP-1 in overnight fasted 10- to 17-week-old male Gcg\textsuperscript{Gut}\textsuperscript{-/-} and control mice (n = 11–19/group).

(C and D) Fasting blood glucose levels (C) and blood glucose levels (D) 0–120 min after oral glucose (OGTT, 1.5g/kg) and area under the curve (AUC, inset) for glucose excursions in overnight fasted 12- to 17-week-old male Gcg\textsuperscript{Gut}\textsuperscript{-/-} and Vil-Cre control mice (n = 29–34/group).

(E–I) Plasma total GLP-1 (E), active GLP-1 (F), total GIP (G), insulin (H), and glucagon (I) measured before and 5–60 min after oral glucose challenge, as indicated (n = 4–15/group).

(J) Plasma acetaminophen levels, as a measurement of gastric emptying, 0–60 min after co-administration of oral acetaminophen and glucose (n = 8–10/group).

(K) Blood glucose levels before and after i.p. administration of glucose (IPGTT, 1.5 g/kg) and AUC (inset) for i.p. glucose excursions in overnight fasted 14- to 19-week-old male Gcg\textsuperscript{Gut}\textsuperscript{-/-} and Vil-Cre control mice (n = 19–18/group).

(L) Plasma insulin measured before and 10 min after an intraperitoneal glucose challenge (n = 14/group).

Data are presented as the mean ± SEM. (A and B) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, control versus Gcg\textsuperscript{Gut}\textsuperscript{-/-} mice. (D–L) *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, Gcg\textsuperscript{Gut}\textsuperscript{-/-} versus Vil-Cre mice. See also Figures S2 and S4.
Figure 4. Gcg<sup>DistalGut</sup>−/− Mice Exhibit Impaired Glucose Tolerance

(A and B) Fasting plasma levels of (A) total GLP-1 or (B) active GLP-1 in overnight fasted 10- to 14-week-old male Gcg<sup>DistalGut</sup>−/− and control mice (n = 7–17/group).

(C and D) Fasting glucose (C) blood glucose levels (D) after oral glucose (OGTT, 1.5 g/kg) and area under the curve (AUC, inset) for glucose excursions in overnight fasted 12- to 15-week-old male Gcg<sup>DistalGut</sup>−/− and Cdx2-Cre control mice (n = 19–22/group).

(E–I) Plasma total GLP-1 (E), active GLP-1 (F), total GIP (G), insulin (H), and glucagon (I) measured before and 5–60 min after oral glucose challenge as indicated (n = 5–9/group).

(legend continued on next page)
(Orskov et al., 1986; Svendsen et al., 2018). Moreover, circu-
lating GLP-1 levels increased briskly following oral glucose or
meat ingestion, consistent with a gut-derived source for the pep-
tide. Nevertheless, some (Mojsov et al., 1990), but not all (Gals-
gaard et al., 2018; Laurantie et al., 2019), studies report small
amounts of GLP-1(7-36)amide in the normal rat or mouse
pancreas and both pancreatic GLP-1 and Pcsk1 expression
were increased in the rat pancreas following induction of exper-
imental diabetes with streptozotocin (Nie et al., 2000). A consid-
erable body of subsequent evidence is now consistent with the
production of islet GLP-1 in the context of experimental pancre-
atric inflammation, diabetes, and aging (Chen et al., 2018; Ellings-
gaard et al., 2011; Habener and Stanoevic, 2017). Our current
findings further support the concept of pancreatic GLP-1 pro-
duction by demonstrating low but detectable levels of active
GLP-1 in the mouse and human pancreas.

The studies described herein stemmed from our provocative
findings revealing the importance of pancreatic PGDPs and
GLP-1 (Chambers et al., 2017), motivated in part by new data
confirming exendin(9-39) as a dual glucagon and GLP-1 antago-
nist at the GLP-1R (Capozzi et al., 2019a; Svendsen et al., 2018;
Zhu et al., 2019). Using a different experimental design, Cham-
bers et al. (2017) assessed the consequences of reactivating
pancreatic or intestinal Gcg expression in Gcg
−−/− mice using
the glycemic response to exendin(9-39) to infer the relative
importance of pancreatic versus intestinal GLP-1 production.
Nevertheless, multiple studies (Capozzi et al., 2019a, 2019b;
Svendsen et al., 2018; Zhu et al., 2019) have recently demon-
strated that (1) glucagon acts as a potential insulinotropic factor
via the islet GLP-1 receptor and (2) the insulinotropic actions of
glucagon are blocked by exendin(9-39).

Notably, Gcg
−−/− mice exhibit resistance to the development of
glucose intolerance (Fukami et al., 2013), reflecting the absence
of pancreatic glucagon, which in turn lowers hepatic glucose
production. Moreover, Gcg
−−/− mice exhibit substantial pancre-
aric enlargement and both islet and x cell hyperplasia, further
magnifying the potential contribution of islet-derived GLP-1 or
glucagon produced following the re-establishment of Gcg
expression within hyperplastic islets (Chambers et al., 2017;
Hayashi et al., 2009). Furthermore, some lines of Gcg
−−/− mice
exhibit upregulation of islet GIP expression (Fukami et al.,
2013), additionally complicating interpretation of incretin action,
insulin levels, and islet function in vivo.

Given these limitations, we re-examined the importance of in-
testinal PGDPs and GLP-1 for glucose homeostasis without the
concomitant use of exendin(9-39). We generated two new mouse
models using Cre driver lines targeting the epithelium,
including enteroendocrine cells, in the distal or the entire gut.
These two new mouse lines enabled physiological analysis of
the metabolic consequences of distal (Gcg
DistalGut
−−/− ) or virtually
complete (Gcg
DistalGut
−−/− mice) elimination of intestinal Gcg expres-
sion, while preserving normal pancreatic islet structure, function,
and PGDP expression. Our current studies reveal the importance
of gut Gcg expression for normal circulating levels of active
GLP-1 and glucoregulation. First, plasma active GLP-1 levels
were clearly reduced in both Gcg
DistalGut
−−/− and in Gcg
DistalGut
−−/−
mice. Second, plasma GLP-1 levels failed to increase following
oral glucose challenge in Gcg
DistalGut
−−/− mice, providing clear proof
that the gut is a key source of circulating GLP-1 following enteral
nutrient ingestion. Third, loss of gut-derived PGDPs was associ-
ated with impaired oral glucose tolerance in Gcg
DistalGut
−−/− mice and
Gcg
DistalGut
−−/− mice, highlighting the importance of gut PGDPs
for glucose homeostasis. Collectively, these findings are consis-
tent with substantial evidence that intestinal-derived GLP-1 acts
as a circulating hormone, or locally through a gut-brain axis
(Grasset et al., 2017), to control gut motility, islet hormone secre-
tion, and glucose homeostasis.

Nevertheless, despite the loss of gut GLP-1 production and
reduced glucose-stimulated levels of GLP-1 in Gcg
DistalGut
−−/− mice, we did not detect any changes in food intake and body weight
or any reduction of glucose-stimulated insulin levels following
the oral glucose challenge. Notably, however, Gcg
DistalGut
−−/− mice and Gcg
DistalGut
−−/− mice are unable to fully augment insulin
secretion to prevent hyperglycemia in the face of an enteral
glucose challenge. Another explanation for the relatively normal
insulin response resides in the increased levels of GIP following
oral glucose challenge in both Gcg
DistalGut
−−/− and Gcg
DistalGut
−−/−
mice. It also seems likely that both pancreatic GLP-1 and
glucagon may act as local glucocinetics to enhance insulin
secretion in the face of reduced intestinal GLP-1 production (Ca-
pozzi et al., 2019a; Svendsen et al., 2018; Zhu et al., 2019). A
notable phenotype of gut GLP-1 deficiency demonstrated herein
is the acceleration of gastric emptying. This observation is
consistent with an essential role for GLP-1 in the inhibitory con-
trol of basal and meal-regulated antroduodenal motility (Schirra
et al., 2006; Witte et al., 2011). Collectively, these findings
emphasize the essential importance of gut-derived PGDPs for
control of gastric emptying and glucose homeostasis.

Considerable historical debate and controversy surround the
relative abundance and importance of proximal versus distal
gut L cells for the control of glucose homeostasis. Some studies
have highlighted a key role for proximal-distal gut communica-
tion, wherein distal gut L cells are rapidly activated via neural
and hormonal amplification signals (Lim and Brubaker, 2006).
Alternatively, direct perfusion of the proximal versus distal hu-
mans gut with glucose revealed a relatively greater capacity of
the distal small bowel for GLP-1 secretion and acute glucose
disposal (Zhang et al., 2019). Recent studies have suggested
that the proximal gut may be a more important source of func-
tionally competent L cells than originally postulated (Glass
et al., 2017; Jorsal et al., 2018; Svendsen et al., 2015). Surpris-
ingly, levels of active GLP-1 rose briskly following oral glucose
administration and were not different in control versus
Gcg
DistalGut
−−/− mice. Hence, the proximal gut is indeed capable
of mounting a brisk GLP-1 response to oral glucose despite the
marked reduction of distal gut Gcg expression. Nevertheless,
Gcg\textsuperscript{DistalGut} /− /− mice exhibit reduced fasting levels of circulating active GLP-1, together with impaired oral glucose tolerance. These findings buttress the importance of the distal gut as a critical source of circulating PGDPs with glucoregulatory activity.

In normal healthy mice, the relevance of pancreatic production of GLP-1 for the control of glucose homeostasis remains uncertain, as the extent of activation of the GLP-1 receptor arising from pancreatic GLP-1 is substantially higher than those of GLP-1, and recent studies highlight the activity of islet glucagon, acting predominantly through the GLP-1 receptor, as a local regulator of insulin secretion (Capozzi et al., 2019a; Svendsen et al., 2018; Zhu et al., 2019). Taken together, our findings integrated together with previous studies of pancreatic PGDP activity (Capozzi et al., 2019a; Chambers et al., 2017; Svendsen et al., 2018; Traub et al., 2017) emphasize the potential contributions of both the pancreas and the intestine to the generation of glucoregulatory PGDPs, and the regulation of GLP-1R-dependent insulin secretion and glucose homeostasis.

Limitations of Study

Our experimental design and findings have a number of limitations. We studied predominantly healthy mice and did not examine mice with experimental diabetes or prolonged HFD feeding and severe obesity. The new mouse lines studied here exhibit germline elimination of the Gcg gene; hence, we cannot rule out the possibility that one or both lines have adapted physiologically to compensate for developmental loss of gut PGDPs. Indeed, plasma levels of GIP were increased in both Gcg\textsuperscript{DistalGut} /− /− and Gcg\textsuperscript{DistalGut} /− /− mice. Hence, the absence of gut GLP-1 and its impact on the incretin effect were potentially modified by compensatory upregulation of GIP action, as described previously for GIP\textsuperscript{−−} and Gcg\textsuperscript{−−} mice (Fukami et al., 2013; Pederson et al., 1998). Notably, we detected a very small amount of active GLP-1 in mouse pancreatic extracts. Hence, we cannot rule out a role for local islet GLP-1 or glucagon action in the context of the distal or complete elimination of intestinal Gcg expression. Importantly, we also report variable yet consistently detectable levels of active GLP-1 in the human pancreas. Furthermore, the current study was not designed to distinguish the extent of pancreatic GLP-1 receptor activation arising from the locally produced glucagon and GLP-1, versus circulating GLP-1 or oxyntomodulin produced in the gut. Nevertheless, our new findings highlight the unequivocal importance of intestinal PGDPs in the control of gut motility and the regulation of glucose homeostasis.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- LEAD CONTACT AND MATERIALS AVAILABILITY
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Animals
- METHOD DETAILS
  - Assessment of Gcg Knockdown
  - Glucose Tolerance Testing

- Metabolic Measurements
- Pancreas Samples
- Tissue Peptide Content
- Gastric Emptying
- Food Intake
- Immunohistochemistry

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cmet.2019.08.009.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Y.S., J.A.K., L.L.B., and D.J.D. designed experiments. Y.S., J.A.K., and L.L.B. carried out experiments and analyzed data. D.A.S. provided mouse lines and reviewed and edited the paper. A.C.P. provided human pancreas samples and edited the manuscript. Y.S., J.A.K., L.L.B., and D.J.D. wrote and edited the paper. D.J.D. is the guarantor for this work and the Lead Contact.

DECLARATION OF INTERESTS

D.J.D. has served as an advisor, consultant, or speaker in the past 12 months to Forkhead Biotherapeutics, Helioine, Inc., Intarcia Therapeutics, Kalylope, Eli Lilly, Merck Research Laboratories, Novo Nordisk, Inc., Pfizer, Inc., and Sanofi, Inc. Neither D.J.D. nor his family members hold stock directly or indirectly in any of these companies.

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REFERENCES

Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1


derived glucagon-related peptides are required for beta cell adaptation and glucose homeostasis. Cell Rep 18, 3192–3203.
**STAR METHODS**

**KEY RESOURCES TABLE**

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**Experimental Models: Organisms/Strains**

- B6.Cg-Tg(Vil1-cre)997Gum/J (Vil-Cre) Jackson Laboratories Cat# JAX:004586; RRID: IMSR_JAX:004586
- B6.Cg-Tg(CDX2-cre)101Erf/J (Cdx2-Cre) Jackson Laboratories Cat# JAX009350; RRID: IMSR_JAX:009350
- Gcg<sup>tm1Rsy</sup> (Gcg<sup>flox/flox</sup>) Darleen Sandoval, University of Michigan N/A

**Oligonucleotides**

- Proglucagon (Gcg) Applied Biosystems Cat# Mm01269053_m1
- Gastric Inhibitory Polypeptide (Gip) Applied Biosystems Cat# Mm00433601_m1
- Cyclophilin (Ppia) Applied Biosystems Cat# Mm02342430_g1
- Ribosomal protein L32 (Rpl32) Applied Biosystems Cat# Mm02528467_g1

**Software and Algorithms**

- Graph Pad Prism 7 Graphpad Software https://www.graphpad.com

**Other**

- Regular Chow Diet (RC) Harlan Teklad Cat# 2018
- High Fat Diet (HFD) Research Diets Cat# D12492i

**LEAD CONTACT AND MATERIALS AVAILABILITY**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Daniel Drucker. Drucker@lunenfeld.ca

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Animals**

All mouse experiments were approved by the Animal Care and Use Subcommittee at the Toronto Centre for Phenogenomics (TCP), Mt. Sinai Hospital and the University of Michigan animal care committee. Mice were housed up to five per cage, kept under a 12-h light/12-h-dark cycle in the TCP facility, and maintained on regular chow (RC; 18% kcal from fat, 2018, Harlan Teklad, Mississauga, ON), or High Fat Diet (HFD; 60% kcal from fat, D12492i, Research Diets, New Brunswick, NJ). All mice were given free access to food and water unless otherwise indicated. All experiments were performed using age- and sex-matched littermates. B6.Cg-Tg(Vil1-cre) 997Gum/J (Vil-Cre) (Cat No: 004586) and B6.Cg-Tg(CDX2-cre)101Erf/J (Cdx2-Cre) (Cat No: 009350) mice (Hinoi et al., 2007) were obtained from Jackson Laboratories (Bar Harbor ME). Gcg<sup>tm1Rsy</sup> (Gcg<sup>flox/flox</sup>) mice, in which exon 2 of the Gcg gene is flanked by loxP sites, was provided by Darleen Sandoval (Chambers et al., 2017). To generate Gcg<sup>flout/+</sup> and Gcg<sup>distaltgut</sup> mice, Vil-Cre and Cdx2-Cre mice were bred with Gcg<sup>tm1Rsy</sup> mice (Chambers et al., 2017), respectively. Germline deletion was minimized by restricting Cre expression to female breeders for the Vil-Cre line, and male breeders for the Cdx2-Cre line, and genotypes were verified by...
isolating RNA and determining length of \( Gcg \) transcripts in relevant tissues. To control for gene dosage, breeders were heterozygous for the \( Cre \) gene. Intercrossing \( Cre \)-positive and \( Cre \)-negative \( Gcg \) \( loxP \) heterozygotes from these 2 lines resulted in 6 genotypes: wildtype mice with no \( Cre \) (WT), mice homozygous for the \( LoxP \) \( Gcg \) gene (\( Gcg^{loxP/loxP} \))(Floxed), wildtype mice expressing \( Cre \) recombinase (\( Vil-Cre \) or \( Cdax2-Cre \)) and \( Gcg^{loxP/loxP} \) mice expressing \( Cre \) recombinase (\( Gcg^{Gut-Cre} \) and \( Gcg^{DistalGut-Cre} \) mice). All mice were born at the expected Mendelian ratios and appeared healthy. Whenever possible, we carried out experiments in all groups of mice; no major phenotypic differences in glucose homeostasis were observed between any of the control lines (Figure S4). For the purposes of clarity, data was presented as comparisons between \( Vil-Cre \) or \( Cdax2-Cre \) control mice to \( Gcg^{Gut-Cre} \) and \( Gcg^{DistalGut-Cre} \) mice, respectively.

**METHOD DETAILS**

**Assessment of \( Gcg \) Knockdown**

Following euthanasia, the small intestine was removed, flushed with PBS and divided into four equal sections: the first quarter was defined as duodenum, pancreas and colon, the second quarter, jejunum and the final quarter defined as ileum. \( Gcg \) expression was also assessed in brainstem, pancreas and colon. Total RNA was isolated from the tail of the pancreas, the first 1 cm piece of each intestinal section (for duodenum, jejunum and proximal ileum), the last 1 cm piece (ileum/distal ileum), or from the proximal half of the colon. Total RNA was extracted from tissues using Tri Reagent (Molecular Research Center, Cincinnati, OH). cDNA was synthesized from DNase I-treated (Thermo-Fisher Scientific, Markham, ON) total RNA (2 \( \mu \)g) using random hexamers and Superscript III (Thermo-Fisher Scientific, Markham, ON). Real-time PCR was carried out using a QuantStudio 5 System and TaqMan Gene Expression Assays (Thermo-Fisher Scientific, Markham, ON). Primer-probe sets were manufactured by Taqman Assays-on-Demand (Applied Biosystems) to measure Exons 1-2 of proglucagon (\( Gcg \) Mm01269053_m1), Exons 3-4 of Gastric Inhibitory Peptide (\( Ppia \) mRNA)

**Glucose Tolerance Testing**

Mice were fasted overnight (~16 h) and oral or intraperitoneal glucose tolerance tests (OGTT or IPGTT, respectively) were carried out using 1.5 g/kg body weight of glucose (15% solution). Blood glucose levels were assessed in tail vein blood using a hand-held glucometer (Contour glucometer, Bayer Healthcare, Toronto, ON). In accordance with animal protocol guidelines regarding blood volumes collected per mouse in a given experiment, blood was collected at either 0, and 5, or 10 minutes (for GLP-1, insulin and GIP), or 0, 15, 30 and 60 minutes (for insulin and glucagon) after glucose administration in heparin-coated capillary microvette tubes. For measurement of total or active GLP-1, insulin, total GIP, and glucagon, blood was mixed with 10% TED (vol/vol) (5,000 KIU/ml Trasyol, 1.2 mg/ml EDTA and 0.1 nmol/l Diprotin A) and plasma was isolated after centrifugation (13,000 rpm 5 min 4°C) and stored at -80°C until further analysis.

**Metabolic Measurements**

Insulin (#80-INSMSU-E01, Alpco, Salem, NH), glucagon (#10-1281-01, Mercodia, Winston Salem, NC), total GIP (#81517, Crystal Chem, Elk Grove Village, IL), active and total GLP-1 (#K150JWC-1 and #K150JVC-1, Mesoscale, Rockville, MD) were measured in plasma samples obtained before and 5-60 minutes after glucose administration.

**Pancreas Samples**

Pancreata samples from six non-diabetic human donors (male donors: 18, 18, 20, 35 years of age; female donors: 45, 55 years of age) were obtained through a partnership of Vanderbilt University Medical Center and Dr. Rita Bottino (Institute of Cellular Therapeutics, Allegheny Health Network, Pittsburgh, PA, USA) working with the International Institute for Advancement of Medicine (IIAM) and the National Disease Research Interchange (NDRI), as previously described (Brissova et al., 2018). Small (< 1 cm) human pancreatic samples were snap-frozen and stored at -80°C until analysis. The Vanderbilt University Institutional Review Board declared that studies on de-identified human pancreatic specimens do not qualify as human subject research. Pancreas-reactivated (\( Gcg^{ΔPDX1-Cre} \)), intestinal-reactivated (\( Gcg^{ΔVil-Cre} \), \( Gcg \)-null, \( Pdx-1-Cre \) and \( Vil-Cre \) control pancreas samples from 28-week-old male mice were kindly provided by Dr. Darleen Sandoval (University of Michigan).

**Tissue Peptide Content**

To measure tissue GLP-1, glucagon, insulin or GIP content, a 0.5 cm segment of intestine, 20-35 mg murine pancreas tissue, or 6-37 mg human pancreas tissue were homogenized in 500 \( \mu \)l (or 250 \( \mu \)l for human pancreas tissue) of lysis buffer (50 mM Tris HCl (pH 8 at 4°C), 1 mM EDTA, 10% glycerol (wt/vol), 0.02% Brij-35 (wt/vol) supplemented with protease inhibitors. Peptide levels were measured in homogenates using the above mentioned assays and normalized to protein content (Bradford assay), or tissue weight.

**Gastric Emptying**

Male mice were subjected to an acetaminophen absorption test to assess the rate of gastric emptying. Following an overnight fast (~16 h), oral administration of a glucose solution (1.5 g/kg BW) containing 1% (w/v) acetaminophen (#A7085, Sigma, Oakville, ON).
was administered at a dose of 100 mg/kg. Blood was collected from the tail vein into heparin-coated tubes before and 15, 30 and 60 minutes after acetaminophen administration as described (Maida et al., 2008). Acetaminophen levels were measured in plasma using an enzymatic-spectrophotometric assay (Acetaminophen-L3K, #506-30, Sekisui Diagnostics, Lexington, MA).

**Food Intake**
After an overnight fast (~16 h), mice were weighed, singly housed and given a pre-weighed amount of food (RC; 18% kcal from fat, 2018, Harlan Teklad, Mississauga, ON) with free access to water. Food was then weighed after 2, 4, 8 and 24 hours.

**Immunohistochemistry**
The pancreas was fixed in 10% neutral-buffered formalin and paraffin embedded. Immunohistochemistry was done on 5-μm histological sections, and serial sections were stained for insulin (#ab181547, Abcam, Toronto, ON), or glucagon (#2760S, Cell Signaling, Danvers, MA).

**QUANTIFICATION AND STATISTICAL ANALYSIS**
Results are expressed as the mean ± SEM. Statistical comparisons were made by ANOVA, followed by a Dunnet or Tukey post-hoc, or by a 2-way ANOVA followed by a Sidak post-hoc, or by Student t test (when only 2 conditions) using GraphPad Prism 7. A p value <0.05 was considered to be statistically significant.
Supplemental Information

Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice

Youngmi Song, Jacqueline A. Koehler, Laurie L. Baggio, Alvin C. Powers, Darleen A. Sandoval, and Daniel J. Drucker
Figure S1

Proglucagon

Pancreas (α cell)

Intestine (L cell)

Brain

PC 1/3

PC 2

N-terminally
Extended GLP-1
(1-37; 1-36NH₂)

Active GLP-1
(7-37; 7-36NH₂)

DPP-IV cleaved GLP-1
(9-37; 9-36NH₂)

Glicentin

OXM

Active GLP-1
(7-37; 7-36NH₂)

DPP-IV cleaved GLP-1
(9-37; 9-36NH₂)

Mesoscale Total GLP-1
ELISA (ver2)
(K150JVC-1)

Mesoscale Active GLP-1
ELISA (ver2)
(K150JWC-1)
Figure S1 Related to Figures 1-4: Schematic Representation of Tissue-specific Processing of Proglucagon. In pancreatic α cells, proglucagon is processed by PC 2 into glicentin-related pancreatic polypeptide (GRPP), glucagon (Gluc), the major proglucagon fragment (MPGF), and to some degree to an N-terminally extended form of GLP-1 (1-36NH₂/1-37). In intestinal L cells, proglucagon is cleaved by prohormone convertase 1/3 (PC 1/3) to produce glicentin, oxyntomodulin (OXM), glucagon-like peptide-1 (GLP-1) and GLP-2. Also depicted are the potential products recognized by the two GLP-1 ELISAs utilized in this study. DPP-IV: Dipeptidyl peptidase-4. IP: Intervening peptide.
Figure S2 Related to Figures 2 and 3: Glucoregulatory Hormones, Food Intake and Body Weight Gain in Gcg<sup>Gut-/-</sup> mice.

(A) Representative pancreas sections from 22-24-week-old male Gcg<sup>Gut-/-</sup> and Vil-Cre control mice stained for insulin or glucagon. (B) Fasting plasma levels of total GIP, insulin and glucagon in 14-17-week-old male Gcg<sup>Gut-/-</sup> and control mice. (n=7-19/group). (C) Total GLP-1 levels, normalized to protein content, in tissue extracts from different regions of the intestine and pancreas of 16-18-week-old female mice. (n=6-8/group).

(D) Active GLP-1 levels, normalized to protein content (left panel), or tissue weight (right panel), in extracts from pancreas of male Gcg<sup>Gut-/-</sup> and Vil-Cre mice fed normal chow (NC), or a 60% high-fat diet (HFD) for 7 weeks. (n=8-11/group). (E) Incremental (left panel) and cumulative (right panel) food intake over 24 hours in overnight fasted 11-14-week-old male Gcg<sup>Gut-/-</sup> and control mice.

(F) Weekly body weight gain of male Gcg<sup>Gut-/-</sup> and control mice fed normal chow (NC, left panel), or a 60% high-fat diet (HFD, right panel). (n=3-15/group). Data are presented as the mean ± SEM. **p<0.01, ***p<0.001 Gcg<sup>Gut-/-</sup> vs. control mice.
**Figure S3 Related to Figure 2 and 4. Glucoregulatory Hormones, Food Intake and Body Weight Gain in GcgDistalGut-/- mice.** (A) Total GLP-1 levels, normalized to protein content, in whole tissue extracts from different regions of the intestine and pancreas of 20-23-week-old male GcgDistalGut-/- and control mice. (n=7-15/group). (B) Fasting plasma levels of total GIP, insulin and glucagon in 10-14-week-old male GcgDistalGut-/- and control mice. (n=5-25/group). (C) Incremental (left panel) and cumulative (right panel) food intake in overnight fasted 8-week-old male GcgDistalGut-/- and control mice over 24 hours. (n=4-10/group). (D) Weekly body weight gain of GcgDistalGut-/- and control mice fed normal chow (NC, left panel), or a 60% high-fat-diet (HFD, right panel). (n=3-15/group). Data are presented as the mean ± SEM.
Figure S4

**Gcg^Gut^-/- control mice**

A. Fasting Glucose (mmol/L)  
B. OGTT  
C. Plasma Total GLP-1 (pg/mL)  
D. Plasma Active GLP-1 (pg/mL)  
E. Plasma Total GIP (pmol/L)  
F. Plasma Insulin (ng/mL)  
G. Plasma Glucagon (pmol/L)  
H. Gastric Emptying

**Gcg^DistalGut^-/- control mice**

I. IPGTT  
J. Plasma Total GLP-1 (pg/mL)  
K. Plasma Total GIP (pmol/L)  
L. OGGT  
M. Plasma Active GLP-1 (pg/mL)  
N. Plasma Insulin (ng/mL)  
O. Plasma Glucagon (pmol/L)  
P. **WT**  
Q. **Floxed**  
R. **Vil-Cre**  
S. **WT**  
T. **Floxed**  
U. **Cdx2-Cre**  
V. **Gcg^DistalGut^-/-**
Figure S4 Related to Figures 3 and 4: Oral and Intraperitoneal Glucose Tolerance in WT, Gcg^{flox/flox}, Vil-Cre and Cdx2-Cre Control Mice. (A-B) Fasting glucose and blood glucose levels after oral glucose (1.5g/kg) in overnight fasted 12-17-week-old male Gcg^{Gut-/-} control mice. (n=34-43/group). (C) Plasma total GLP-1, (D) active GLP-1, (E) total GIP, (F) insulin, and (G) glucagon measured before and 5-60 min after oral glucose challenge as indicated. (n=4-20/group). (H) Plasma acetaminophen levels, as a measurement of gastric emptying, 0-60 min after co-administration of oral acetaminophen and glucose in overnight fasted 14-17-week-old male Gcg^{Gut-/-} control mice. (n=7-11/group). (I) Blood glucose levels taken after intraperitoneal (i.p.) administration of glucose (1.5g/kg), and (J) plasma insulin 10 min after i.p. glucose challenge in overnight fasted 14-19-week-old male Gcg^{Gut-/-} control mice. For (I) n=19-30/group, and (J) n=12-19/group. (K-L) Fasting glucose and blood glucose levels after oral glucose (1.5g/kg) in overnight fasted 12-15-week-old male Gcg^{DistalGut-/-} control mice. (n=16-26/group). (M) Plasma total GLP-1, (N) active GLP-1, (O) total GIP, (P) insulin, and (Q) glucagon measured before and 5-60 min after oral glucose challenge as indicated. (R) Blood glucose levels taken after intraperitoneal (i.p.) administration of glucose (1.5g/kg). (S) Plasma insulin, and (T) active GLP-1 10 min after i.p. glucose challenge in overnight fasted 14-17-week-old male control mice (S and T), and Gcg^{DistalGut-/-} mice (T). For (R) n=11-21/group and (S) n=6-10/group, (T) n=4-6/group. Data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 basal vs. 5-60 min, #p<0.05 Gcg^{DistalGut-/-} vs. control mice as indicated.
### Table S1

**Information related to the human pancreatic samples analyzed in Figure 1. N/A – not available**

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