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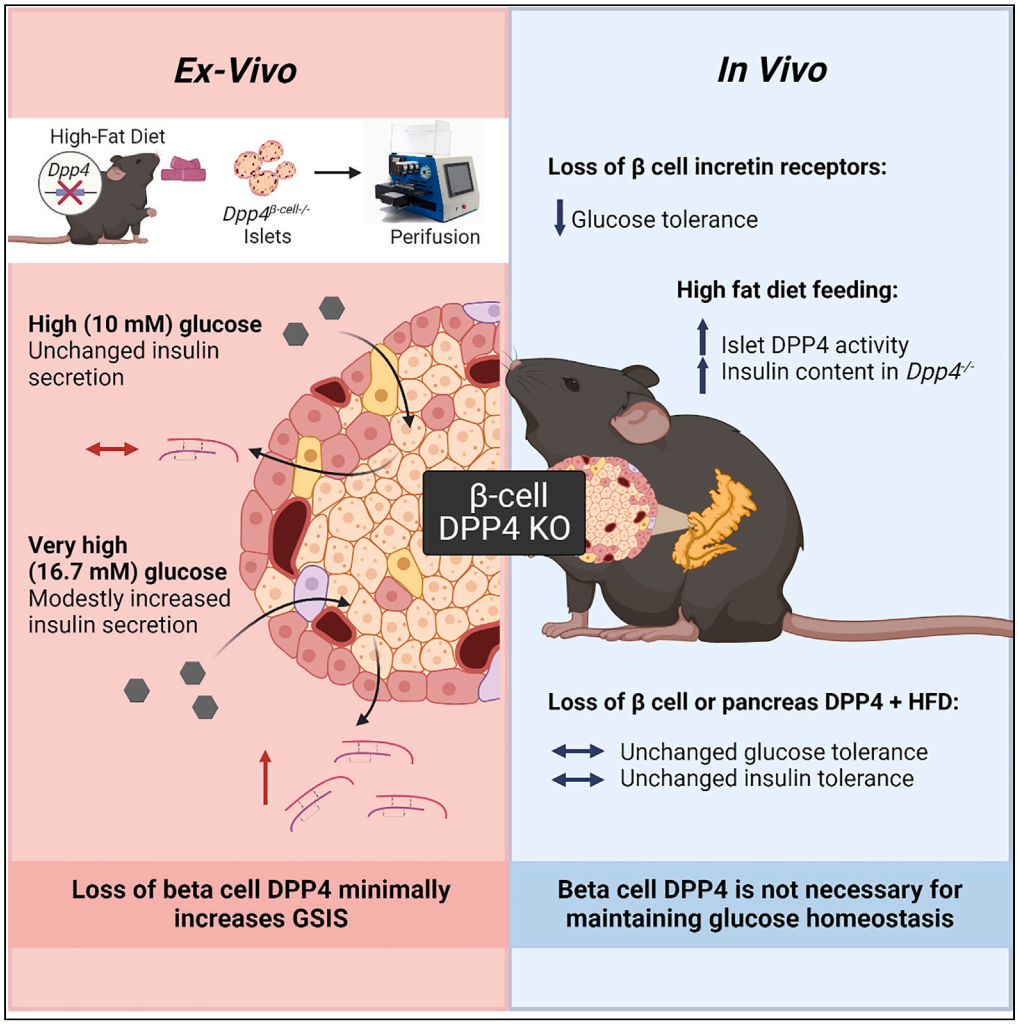

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Highlights

β cell incretin receptors are required for DPP4i-mediated glucose lowering

$Dpp4^{\beta\text{-cell}-/-}$ increases glucose-stimulated (16.7 mM) insulin secretion in isolated islets

Glucose tolerance is not improved in HFD-fed $Dpp4^{\beta\text{-cell}-/-}$ or $Dpp4^{Pan-/-}$ mice

Insulin secretion is unchanged in HFD-fed $Dpp4^{\beta\text{-cell}-/-}$ or $Dpp4^{Pan-/-}$ mice

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Pancreas-derived DPP4 is not essential for glucose homeostasis under metabolic stress

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SUMMARY

Mice systemically lacking dipeptidyl peptidase-4 (DPP4) have improved islet health, glucoregulation, and reduced obesity with high-fat diet (HFD) feeding compared to wild-type mice. Some, but not all, of this improvement can be linked to the loss of DPP4 in endothelial cells (ECs), pointing to the contribution of non-EC types. The importance of intra-islet signaling mediated by α to β cell communication is becoming increasingly clear; thus, our objective was to determine if β cell DPP4 regulates insulin secretion and glucose tolerance in HFD-fed mice by regulating the local concentrations of insulinotropic peptides. Using β cell double incretin receptor knockout mice, β cell- and pancreas-specific *Dpp4*$^{-/-}$ mice, we reveal that β cell incretin receptors are necessary for DPP4 inhibitor effects. However, although β cell DPP4 modestly contributes to high glucose (16.7 mM)-stimulated insulin secretion in isolated islets, it does not regulate whole-body glucose homeostasis.

INTRODUCTION

Hormone secretion from the islets in the pancreas is tightly linked to nutrient ingestion through the action of the gut-derived incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).[1] Biologically active incretins regulate glucagon secretion and potentiate insulin secretion to tailor postprandial glucose uptake and utilization.[2,3] Together, GLP-1 and GIP action account for 50–70% of the postprandial rise in insulin in healthy individuals,[4] but their collective effect is greatly diminished in patients with type 2 diabetes (T2D), even during early disease pathogenesis where reliable glycemic control is maintained.[5,6] Active incretins are rapidly cleaved by dipeptidyl peptidase-4 (DPP4), a widely expressed serine protease, which limits the half-life of GLP-1 and GIP to minutes and eliminates their glucoregulatory action.[7,8] Three classes of drugs based on enhancing incretin signaling are current treatments for T2D: DPP4 inhibitors (DPP4i) that selectively inhibit the catalytic action of DPP4, GLP-1 receptor agonists (GLP-1RAs) and structural variants of endogenous human GLP-1 or lizard exendin-4 that are resistant to degradation by DPP4, as well as the recently approved GLP-1R/GIPR (GIP receptor) dual agonist, tirzepatide.[9–11]

Although the role of DPP4 in the regulation of incretin hormone action and glucose homeostasis is well documented, the cell and tissue types essential for incretin degradation have remained elusive. Mice with whole-body *Dpp4* knockout are protected against HFD feeding-induced obesity and insulin resistance; they display improved glycemic excursions, elevated plasma GLP-1 and insulin, reduced islet hypertrophy, and protection from streptozotocin-induced β cell death.[12] Ablation of *Dpp4* in endothelial cells (ECs), but not in adipocytes, or enterocytes, leads to a significant increase in glucose-induced active GLP-1 and GIP levels and partially recapitulates the improved glucose tolerance seen in global *Dpp4* knockout.[13,14] Hepatocyte-derived DPP4 regulates portal GLP-1 bioactivity and hepatic glucose production.[15] Still, glucose control is improved further with systemic DPP4 inhibition,[16] revealing that ECs contribute most significantly to systemic DPP4 activity and that additional unknown cell types also robustly contribute to DPP4-mediated postprandial glucoregulation.

There is now increasing evidence of local production and paracrine action of GLP-1 within the islet due to the presence of prohormone convertase (PC) $^{1}/_{3}$, an enzyme that cleaves islet-derived preproglucagon into

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GLP-1 and several other peptides.[17–22] The expression of intra-islet PC1/3 is increased under the conditions of hyperglycemia, hyperlipidemia, or metabolic stress[17,19,23] and can be induced pharmacologically by activation of the β cell GLP-1R with the GLP-1RA, liraglutide.[24] GLP-1RA treatment has been shown to increase the expression of prohormone convertase subtilisin/kexin type 1 (*PCSK1*), as well as the abundance of GLP-1^{+} signal in a subset of human α cells,[24] raising the possibility that the pool of intra-islet GLP-1 can be pharmacologically manipulated. While colonic L cells are a bona fide source of glucoregulatory GLP-1,[25] several studies have supported that pancreatic islets synthesize a distinct pool of glucoregulatory GLP-1.[26,27] Indeed, the hyperglycemic effects of the GLP-1R antagonist, exendin 9-39, cease to exist in mice with loss of glucagon (*Gcg*) expression in α cells, but not L cells,[26] suggesting the glucoregulatory actions of proglucagon peptides originate from α cells. Consistent with this, the islet-derived proglucagon pool is required for maintaining glucose homeostasis during metabolic stress induced by high-fat feeding.[26–28] However, these studies must be interpreted in the context of the ability of glucagon to act as a GLP-1RA, inducing an insulin response from β cells in a paracrine manner,[29] even in the absence of a functional *Gcg* receptor[30] through mechanisms that primarily rely on activation of the GLP-1R. Still, the emerging evidence that α cells are a meaningful source of active GLP-1 and insulin secretion forces the consideration of local DPP4 expression within the islet as a key regulator of this local paracrine system. In support of this, intra-islet DPP4 has been suggested to modulate islet function via a paracrine mechanism due to its direct co-localization with β cells in the mouse islet.[31] However, this has yet to be empirically tested. Therefore, we aimed to understand intra-islet DPP4 in the paracrine regulation of insulin secretion and glucose metabolism by studying mice with selective deletion of *Dpp4* in β cells.

RESULTS

GLP-1R and GIPR signaling within the β cell is required for regulation of glucose by systemic DPP4 inhibition

Previous studies in whole-body double incretin receptor knockout (DIRKO) and β cell-specific *Glp1r* knockout mice have demonstrated the importance of GLP-1R and GIPR signaling for proper glucose homeostasis, both of which are required for the glucoregulatory effects of the DPP4i, sitagliptin.[32–34] GLP-1R and GIPR action within the portal circulation and enteric or central nervous system has also been proposed to contribute to their net glucoregulatory effect.[35–40] To determine if incretin receptor activation on the β cell alone underlies the entirety of their glucose-lowering effects and eliminates the ability for one incretin receptor to compensate for lack of the other,[32,34] we generated a mouse model with selected elimination of both *Glp1r* and *Gipr* within β cells (β cell double incretin receptor knockout; DIRKO$^{\beta\text{-cell}-/-}$ mice). *Gipr* and *Glp1r* mRNA transcripts were significantly reduced in islets from DIRKO$^{\beta\text{-cell}-/-}$ mice compared to controls (mixed group of mouse insulin (*Ins1*) promoter with mouse estrogen receptor (ERT), MIP-Cre and *Gipr/Glp1r*$^{fl/fl}$) (Figure 1A), with no differences detected in other tissues (Figures 1B and 1C), strongly suggesting the specific deletion of the incretin hormone receptors in the most abundant islet cell type, the β cell. We then investigated if the β cell incretin receptors were required for the DPP4i-dependent glucose-lowering in response to either a dose of sitagliptin that provides selective enteral inhibition (14 μg) and systemic inhibition (10 mg/kg).[14,32] Both doses of sitagliptin improved oral glucose tolerance in control mice (Figures 1D and 1E) but failed to elicit a glucose-lowering effect in DIRKO$^{\beta\text{-cell}-/-}$ mice (Figures 1F and 1G). As anticipated, the area under the curve (AUC) from the glucose tolerance test (GTT) was greater in DIRKO$^{\beta\text{-cell}-/-}$ compared to control mice (Figure 1H). Together, these findings demonstrate that β cell incretin receptors are essential for DPP4i-mediated regulation of glucose control.

Insulin secretion is enhanced in islets lacking DPP4 in the β cell

Next, we investigated the role of endogenous DPP4 in islet composition and function. First, we found that islet *Dpp4* mRNA levels trended higher in wild-type (WT) C57BL6/J mice fed an HFD compared with those fed a standard laboratory chow diet (chow) ($p = 0.0754$; Figure 2A). Additionally, DPP4 activity was not detectable in islets isolated from female mice fed a chow diet; however, three out of four age-matched female mice on HFD for 5 weeks had islet DPP4 activity for the same amount of islet protein (Figure 2B). We then evaluated GLP-1 secretion in islets during dynamic perifusion from WT male and female mice fed chow or a high-fat high-cholesterol (HFHC) diet. Islets from most mice fed both diets had higher active GLP-1 secretion at 16.7 mM glucose than 10 mM glucose which was associated with significantly higher insulin secretion in both diets (Figures 2C and 2D). *Dpp4* knockout mice (*Dpp4*$^{-/-}$) had marginally, but insignificantly, higher pancreatic insulin content compared to WT controls ($p = 0.114$) when fed chow (Figure 2E).

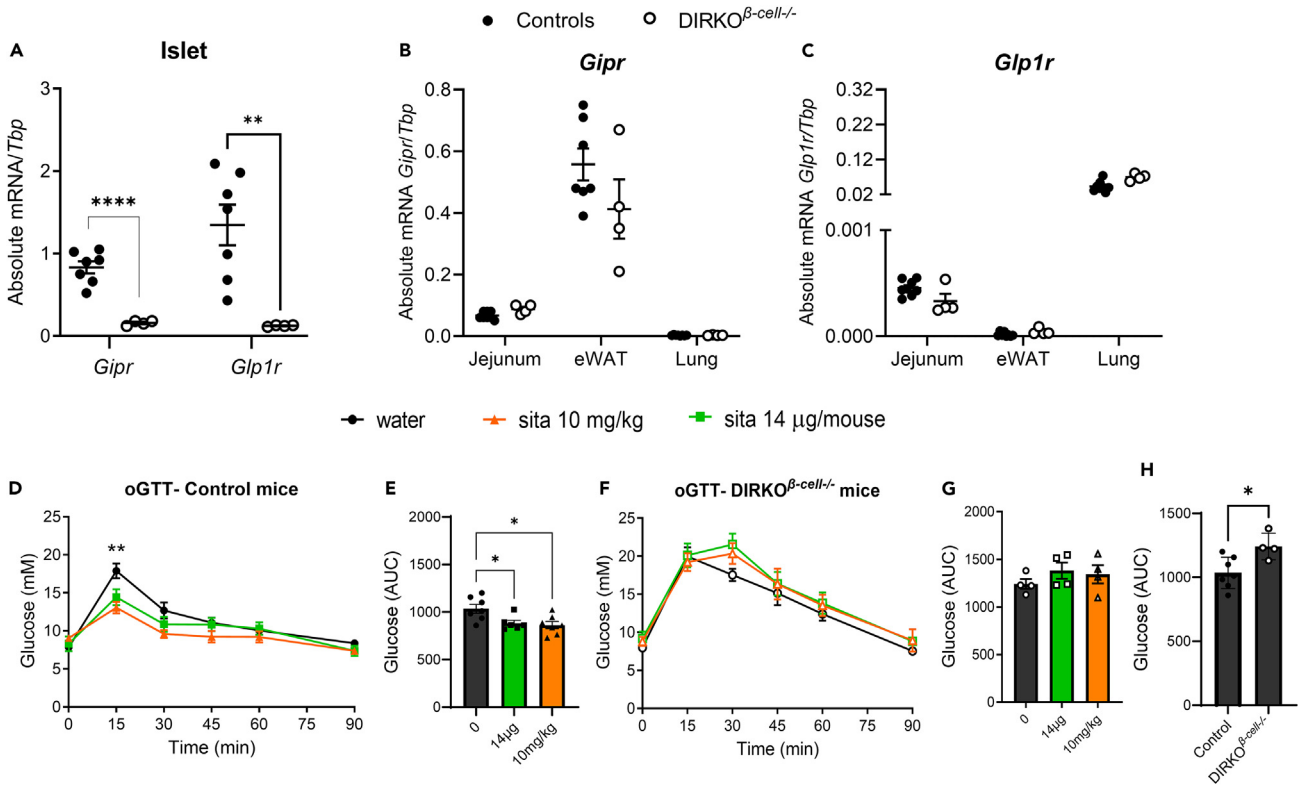

Figure 1. Activation of the GLP-1R and GIPR within the β cell is required for regulation of glucose by DPP4i

(A–E) Abundance of *Gipr* and *Glp1r* mRNA in islets of male and female control and DIRKO$^{\beta\text{-cell}-/-}$ mice normalized to *Tbp*. (B and C) Expression of *Gipr* (B) and *Glp1r* (C) mRNA transcripts in whole extracts of jejunum, epididymal white adipose tissue (eWAT), and lung tissue normalized to *Tbp*. (D-E) Oral glucose tolerance test (2 g/kg) glycemia (D) and AUC (E) in male and female control (MIP-Cre and *Dpp4*$^{fl/fl}$) mice administered sitagliptin (14 µg/mouse or 10 mg/kg) by oral gavage 30 min prior to gavage of glucose.

(F and G) Oral glucose tolerance test (2 g/kg) glycemia (F) and AUC (G) in DIRKO$^{\beta\text{-cell}-/-}$ mice administered sitagliptin (14 µg/mouse or 10 mg/kg) by oral gavage 30 min prior to gavage of glucose.

(H) Oral glucose tolerance test AUC in male and female control compared to DIRKO$^{\beta\text{-cell}-/-}$ mice without sitagliptin. Statistical differences between groups were analyzed by an unpaired t test (A–C, H) or ANOVA (1 way or mixed-effects analysis) with post-hoc Tukey test (D–G). Data are represented as mean ± SEM, *p < 0.05, **p < 0.01 (control compared to sita 10 mg/kg), ***p < 0.001, ****p < 0.0001.

However, insulin content was significantly greater in *Dpp4*$^{-/-}$ mice following 4 weeks of HFD feeding compared to WT controls and chow-fed *Dpp4*$^{-/-}$ mice (Figure 2E).

Based on previous findings showing that HFD-fed *Dpp4*$^{-/-}$ mice have stronger glycemic control than *Dpp4* EC knockout (*Dpp4*$^{EC-/-}$) mice[14] and that dysregulated glucose is associated with upregulation of DPP4 activity,[41] we hypothesized that the increased intra-islet DPP4 may contribute to whole-body glucose dysregulation in response to the high metabolic demand induced by HFD feeding. To determine this, we generated a β cell-specific *Dpp4* knockout model (*Dpp4*$^{\beta\text{-cell}-/-}$) and compared it to controls (*Dpp4*$^{+/+}$ MIP-Cre+, denoted as MIP-Cre). We confirmed that *Dpp4*$^{\beta\text{-cell}-/-}$ mice had a 40% reduction in DPP4 activity within islets compared to controls with no difference in other tissues including the liver (Figure 2F). Other dipeptidyl peptidases in the DPP4 family, fibroblast activation protein (FAP), DPP8, and DPP9, may contribute to the remaining protease activity in *Dpp4*$^{\beta\text{-cell}-/-}$ islets. Plasma DPP4 activity was not different between *Dpp4*$^{\beta\text{-cell}-/-}$ mice and controls, and DPP4 protein in plasma was insignificantly increased in *Dpp4*$^{\beta\text{-cell}-/-}$ mice (Figures 2G and 2H). β cell-derived DPP4 protein was detected by fluorescence microscopy using a protocol previously described[42] whereby DPP4 was co-stained with insulin or glucagon. While HFD-fed WT mouse islets had uniform DPP4 signal, no detectable DPP4 was found in the islets or liver of the *Dpp4*$^{-/-}$ group used as a negative control (Figure 2I). In the *Dpp4*$^{\beta\text{-cell}-/-}$ liver, DPP4 signal was similar to WT controls, confirming the specificity of the knockout (Figure 2I). Consistent with reduced DPP4 activity, islets of HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ demonstrated a nearly complete loss of DPP4 signal

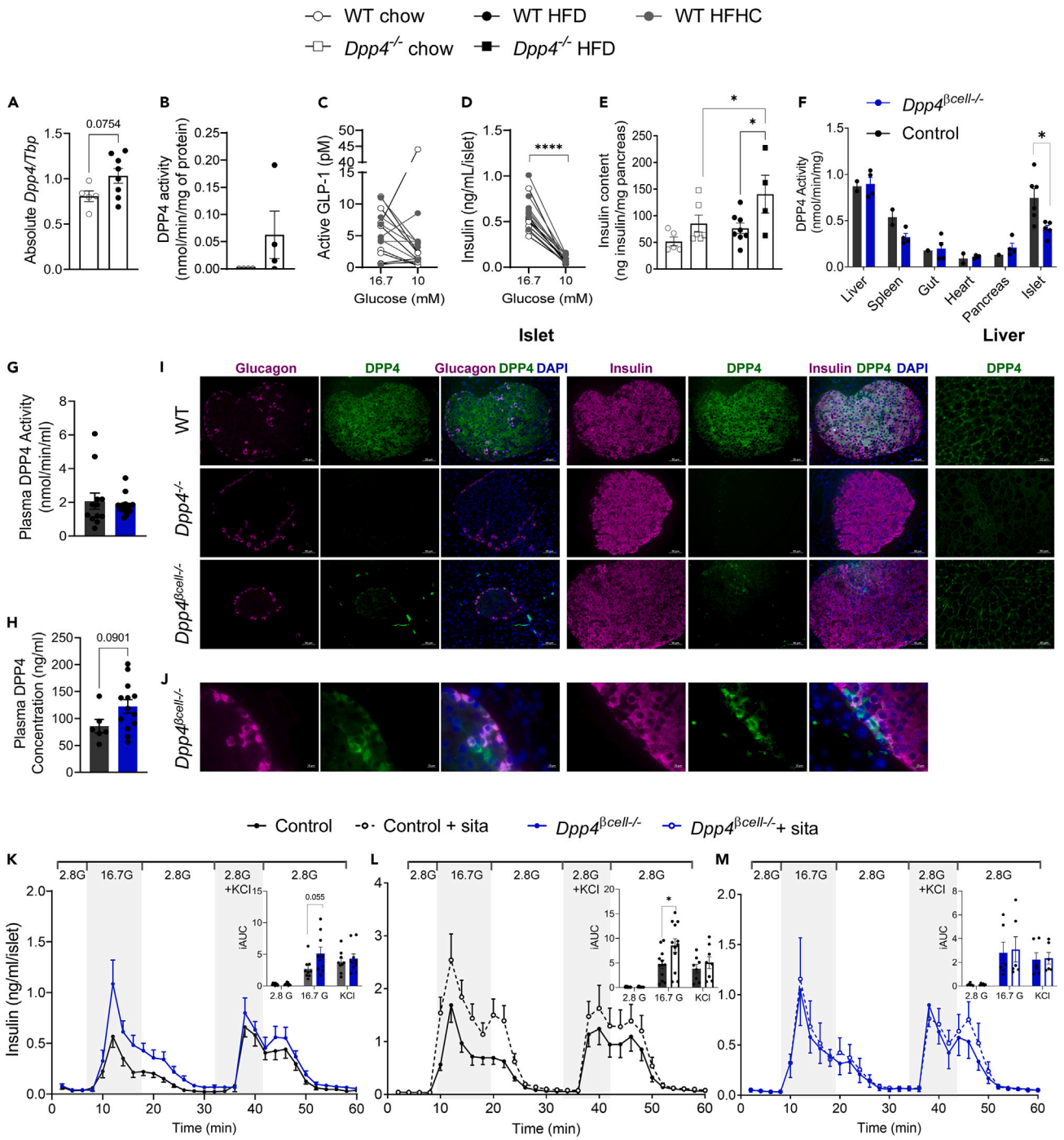

Figure 2. Islets from HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ mice have significantly reduced islet DPP4 activity and expression

(A) mRNA abundance of *Dpp4* in islets isolated from 52-week-old wild-type C57BL/6J mice fed chow or a high-fat diet.

(B) DPP4 activity normalized to total protein in isolated islets from young female mice fed chow or high-fat diet (5 weeks).

(C and D) Active GLP-1 and insulin in perfusate from islets of WT male and female mice fed chow or HFHC (12 weeks) diets exposed to 16.7 mM and 10 mM glucose in dynamic perifusion.

(E) Insulin content measured in the pancreas of 12-week-old *Dpp4*$^{+/+}$ or *Dpp4*$^{-/-}$ littermate controls fed chow or HFD (4 weeks).

(F) DPP4 activity normalized to total protein measured in whole extracts of tissues (liver, spleen, gut, heart, pancreas, islets) control (WT, MIP-Cre, *Dpp4*$^{fl/fl}$) and *Dpp4*$^{\beta\text{-cell}-/-}$ male mice.

(G and H) Plasma DPP4 activity (G) and concentration (H) in plasma of HFD-fed control and *Dpp4*$^{\beta\text{-cell}-/-}$ male mice.

Figure 2. *Continued*

(I) Immunofluorescence staining of glucagon (purple) and DPP4 (green) and insulin (purple) and DPP4 (green) in HFD-fed male and female mouse pancreatic sections (20X) (WT – top, *Dpp4*$^{-/-}$ – middle, *Dpp4*$^{\beta\text{-cell}-/-}$ – bottom). DAPI staining (blue) was used to identify nuclei. DPP4 staining in liver sections of WT – top, *Dpp4*$^{-/-}$ – middle, *Dpp4*$^{\beta\text{-cell}-/-}$ – bottom mice.

(J) Magnified *Dpp4*$^{\beta\text{-cell}-/-}$ islet cells showing presence of lack of double glucagon and DPP4 or insulin and DPP4 stain.

(K–M) Glucose-stimulated insulin secretion (GSIS) and area under the curve (AUC) measured in islets isolated from control (WT, *Dpp4*$^{fl/fl}$, and MIP-Cre) and *Dpp4*$^{\beta\text{-cell}-/-}$ (n = 3–5) 40-week-old HFD-fed male mice in response to perifusion with buffer containing 2.8 mM glucose, 16.7 mM glucose, or 2.8 mM glucose + KCl (30 mM). (L,M) GSIS measured in islets isolated from 40-week-old HFD-fed control (n = 9) (L) or *Dpp4*$^{\beta\text{-cell}-/-}$ (n = 6) (M) male mice in response to perifusion with and without DPP4 inhibitor sitagliptin. Graphs were analyzed using mixed-effects analysis and Sidak's multiple comparisons (C-E, K-M) or unpaired t test (A, B, F, G, H and AUCs). Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Scale bars represent 50 μm (I) and 10 μm (J).

(Figure 2I), of which the remaining signal colocalizes with glucagon^{+} and insulin^{-} islet cells (Figure 2J). Additionally, we performed flow cytometry in dissociated islets, defined α, β, and δ cells as previously described,[43] and quantified DPP4 levels in these populations. In accordance with the histology, DPP4 expression was reduced by 79% in the β cell population (Figure S1A). Alpha (21%) and delta (3.1%) cell populations had much less reduction in DPP4 expression by comparison (Figure S1A). However, the overall recovery of β cells was lower than expected in both control and *Dpp4*$^{\beta\text{-cell}-/-}$ mice; this may be due to the effects of MIP-Cre expression or tamoxifen treatment (Figure S1B).[44,45]

We then evaluated the role of intra-islet DPP4 on islet secretory function *ex vivo*. Consistent with relatively low immuno-detectable DPP4 in chow-fed mice, DPP4 inhibition in perifusion of islets isolated from WT mice fed chow had similar glucose-stimulated insulin secretion (GSIS) as control treated islets (Figures S2A and S2B). While basal glucose and KCl-induced insulin release remained similar between all groups, higher GSIS was observed in *Dpp4*$^{\beta\text{-cell}-/-}$ islets isolated from mice fed an HFD for 15 weeks and sacrificed at 40 weeks of age compared to controls after perifusion with 16.7 mM glucose (incremental AUC (iAUC), p = 0.055) (Figure 2K). To further investigate these differences, we compared GSIS in islets from another set of mice from these two groups with and without the addition of 200 nM sitagliptin. Here, sitagliptin significantly increased insulin response to 16.7 mM glucose in islets from control mice (Figure 2L) but had no effect in islets from *Dpp4*$^{\beta\text{-cell}-/-}$ mice (Figure 2M). Taken together, the increase in GSIS in *Dpp4*$^{\beta\text{-cell}-/-}$ islets vs. controls (Figure 2K) and the marked increase in GSIS following the sitagliptin treatment in control islets (Figure 2L), but not *Dpp4*$^{\beta\text{-cell}-/-}$ (Figure 2M), suggest that the catalytic action of β cell DPP4 is a regulator of GSIS *ex vivo*. Although not detectable in all perifusion samples, similar to our studies in WT mice (Figure 2C), we were able to measure GLP-1 from a subset of high glucose-stimulated effluents (16.7 mM), with *Dpp4*$^{\beta\text{-cell}-/-}$ islets releasing ~4-fold more active GLP-1 than control islets (*Dpp4*$^{\beta\text{-cell}-/-}$ islets [n = 8 mice], 0.692 ± 0.119 pM vs. MIP-Cre islets [n = 4 mice], 0.164 ± 0.065 pM, p = 0.014), consistent with increased glucose-stimulated local GLP-1 (data not shown). To determine if the increase in GSIS was the result of enhanced signaling through the GLP-1R, islets from MIP-Cre and *Dpp4*$^{\beta\text{-cell}-/-}$ mice were treated with 100 nM of the GLP-1R antagonist exendin 9-39. Blockade of the GLP-1R resulted in a 25% reduction in GSIS in islets isolated from both MIP-Cre (Figure S3A) and *Dpp4*$^{\beta\text{-cell}-/-}$ mice (Figure S3B) indicating a similar reliance in both groups on GLP-1R signaling.

Elimination of β cell-derived *Dpp4* fails to improve glucose tolerance or prevent incretin degradation in male mice on an HFD

Mice were fed an HFD to assess the impact of DPP4 in the context of metabolic stress. After five weeks of HFD feeding, both *Dpp4*$^{\beta\text{-cell}-/-}$ and control mice exhibited similar glucose tolerance in both oral and intraperitoneal (i.p.) glucose administration (Figures 3A and 3E). Sitagliptin reduced glucose excursion with oral, but not i.p. glucose challenge, in both groups (Figures 3A and 3E). Consistent with previous findings,[14] systemic inhibition with sitagliptin prior to GTTs increased the concentration of active circulating GIP (Figures 3B and 3F) and active GLP-1 (Figures 3C) 15 min post-glucose challenge; however, no increase in plasma insulin was observed in *Dpp4*$^{\beta\text{-cell}-/-}$ compared to MIP-Cre HFD-fed mice (Figures 3D and 3G). Insulin response to glucose was insignificantly increased with sitagliptin, likely due to HFD-induced metabolic dysfunction (Figures 3D and 3G). *Dpp4*$^{\beta\text{-cell}-/-}$ mice demonstrated similar insulin sensitivity compared to control mice regardless of glycemia being represented in raw units (mmol/L) or expressed as a percentage of the fasting glucose (Figures 3H and 3I).

Finally, to assess islet function *ex vivo*, male mice were sacrificed at 63–65 weeks of age after HFD feeding for 38–40 weeks. Islets were sequentially perifused with Krebs-Ringer Bicarbonate HEPES (KRBH) buffer

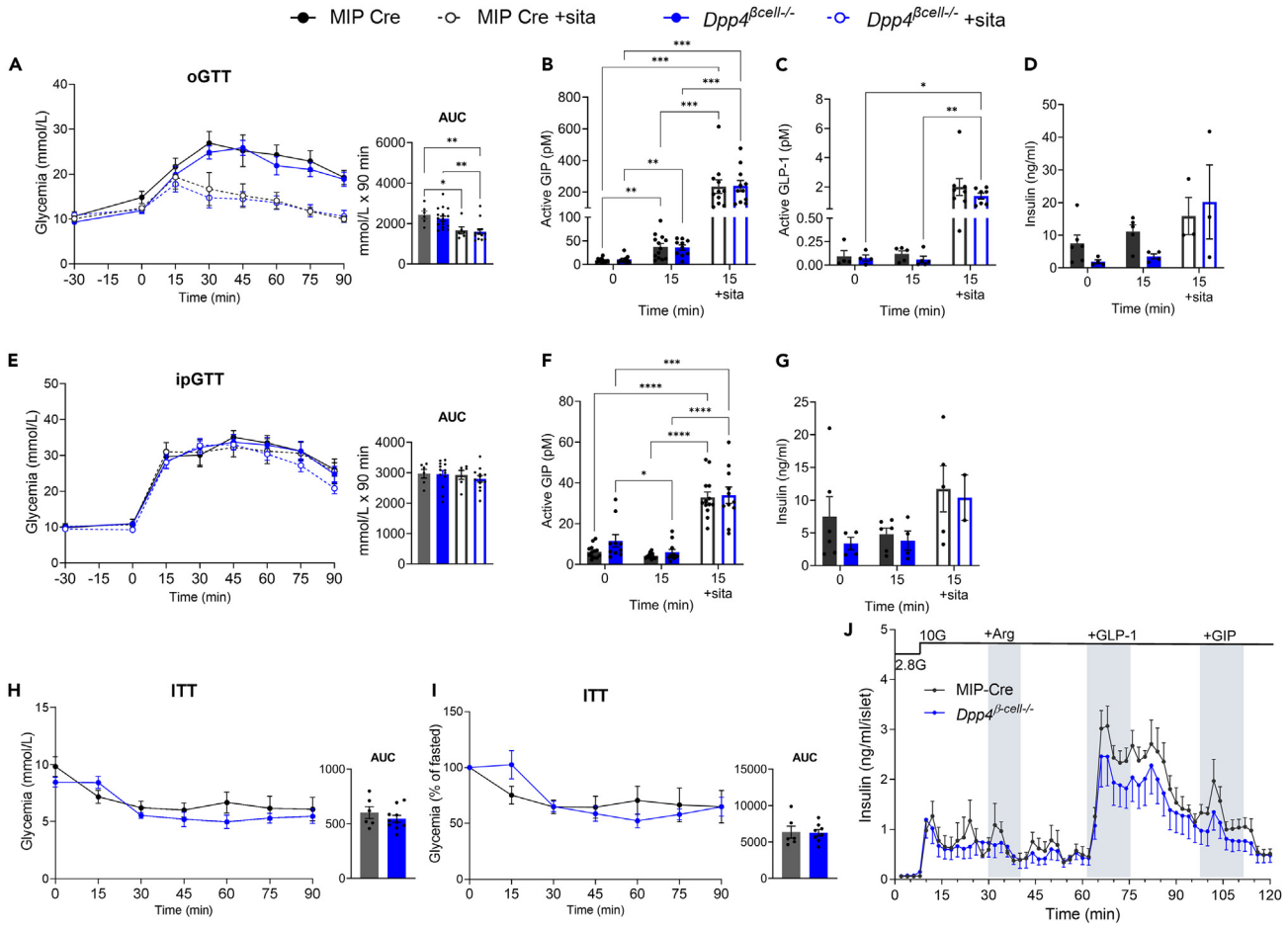

Figure 3. Elimination of β cell-derived DPP4 does not improve glucose tolerance and insulin tolerance or prevent incretin degradation in male HFD-fed mice

(A–D) Oral glucose tolerance (A) and plasma active GIP (B), active GLP-1 (C), and insulin (D) in male HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ and MIP-Cre control mice in response to oral glucose gavage ± sitagliptin.

(E–G) Intraperitoneal (*i.p.*) glucose tolerance (E) and plasma active GIP (F), and insulin (G) secreted in response to *i.p.* glucose injection. Sitagliptin was given by oral gavage 30 min prior to glucose tolerance tests (2 g/kg body weight).

(H) Glucose levels and AUC during an insulin (0.6 IU/kg) tolerance test in male, HFD-fed mice.

(I) Insulin tolerance test glycemia as a percentage of fasted glucose and AUC.

(J) Insulin secretion measured in perifusion of islets from 65-week-old HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ (n = 4) and MIP-Cre control (n = 4) mice with arginine (1 mM), GLP-1 (0.3 μm) and GIP (100 nM). Graphs were analyzed by one-way ANOVA (A, E AUCs), unpaired t test (H, I AUCs), or mixed-effects analysis with Tukey's multiple comparisons. All data are represented as the mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

containing (in order) 2.8 mM glucose and 10 mM glucose with arginine (1 mM), GLP-1 (0.3 μM), and GIP (100 nM). Unlike when given 16.7 mM glucose (Figure 2K) no trends or differences in GSIS were observed in islets from *Dpp4*$^{\beta\text{-cell}-/-}$ (n = 4) or MIP-Cre (n = 4) mice at 10 mM glucose (Figure 3J). This may be due to higher islet GLP-1 contribution at 16.7 mM versus 10 mM glucose exposure as we have seen in islet from WT male and female, chow- and HFHC-fed mice (Figure 2C). Taken together, these data demonstrate that, in male mice, elimination of DPP4 from pancreatic β cells is not sufficient to improve glucose tolerance achieved by the whole-body *Dpp4*$^{-/-}$ mice or systemic DPP4 inhibition with sitagliptin.[14]

Dpp4 deletion in β cells does not affect glucose tolerance, incretin levels, or GSIS in HFD-fed female mice

Following HFD feeding, female *Dpp4*$^{\beta\text{-cell}-/-}$ mice demonstrated an overall glycemic response similar to the MIP-Cre controls in response to an oral glucose tolerance test (oGTT) (Figure 4A), including levels of plasma active GIP and insulin (Figures 4B and 4C). We also observed no change in *i.p.* glucose tolerance

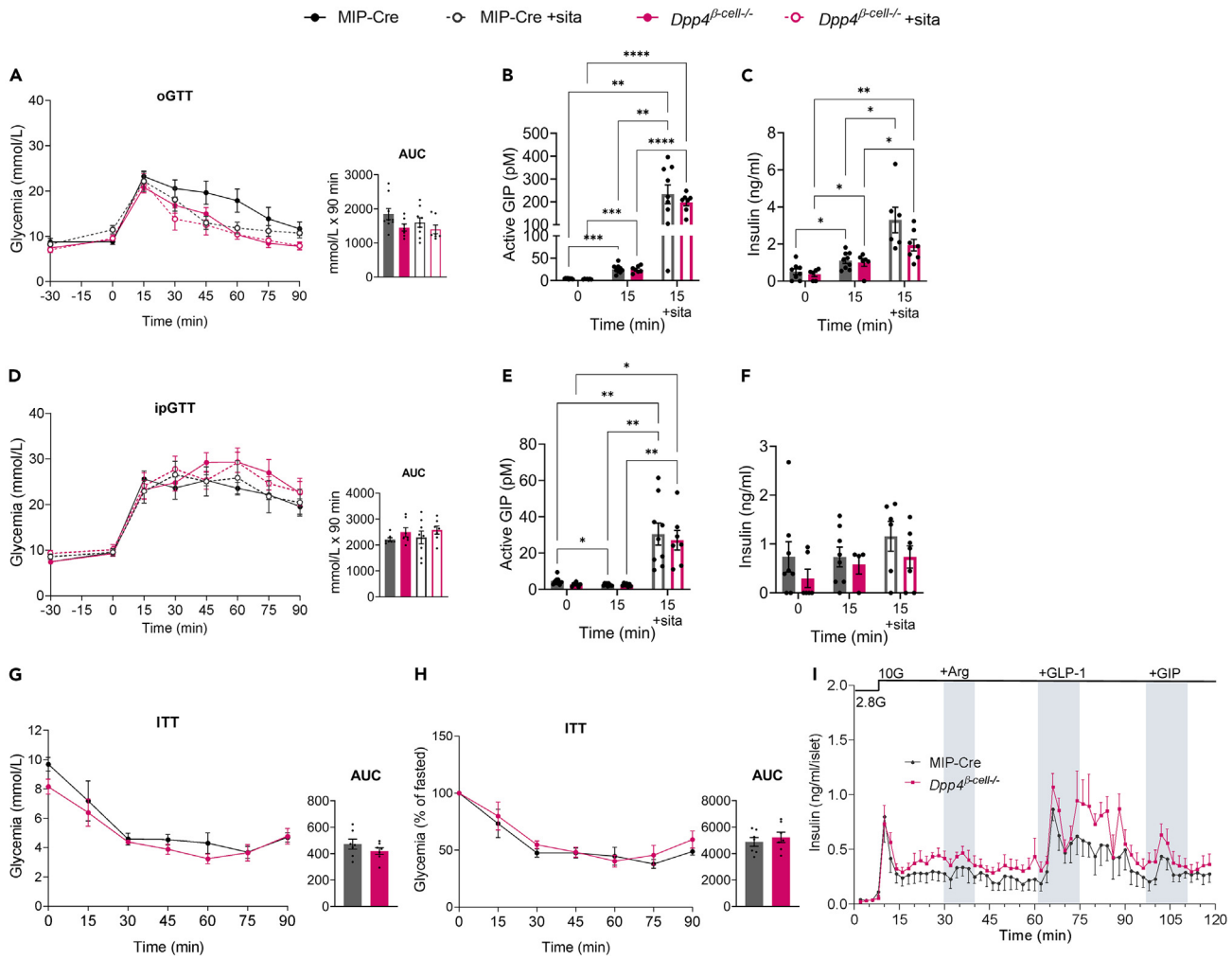

Figure 4. Elimination of β cell-derived DPP4 does not improve glucose tolerance, incretin levels, or GSIS in HFD-fed female mice

(A–C) Oral glucose tolerance (A), active GIP (B), and insulin secreted (C) in female HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ and MIP-Cre control mice in response to oral glucose gavage ± sitagliptin.

(D–F) Intraperitoneal (*i.p.*) glucose tolerance (D), active GIP (E), and insulin secreted (F) in female HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ and MIP-Cre control mice in response to *i.p.* glucose injection. Sitagliptin was given by oral gavage 30 min prior to glucose tolerance tests (2 g/kg body weight for oGTT and at 1.2 g/kg for ipGTT) (n = 7–10 per group).

(G) Glucose levels and AUC during an insulin (0.6 IU/kg) tolerance test in female HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ and MIP-Cre control mice.

(H) Insulin tolerance test glycemia as a percentage of fasted glucose and AUC.

(I) GSIS measured during perifusion of islets isolated from 65-week-old HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ (n = 4) and MIP-Cre control (n = 4) mice with arginine (1 mM), GLP-1 (0.3 μm) and GIP (100 nM). AUC graphs represent area under the curve, analyzed by ANOVA with post-hoc Tukey test (A, D) or unpaired t test (G, H). For non-AUC graphs we used mixed-effects analysis with Tukey's multiple comparisons. All data are represented as the mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

with and without sitagliptin (Figure 4D), plasma active GIP levels (Figure 4E), or insulin levels (Figure 4F) following glucose administration between control and *Dpp4*$^{\beta\text{-cell}-/-}$ mice. Insulin tolerance was also unchanged (Figures 4G and 4H). Finally, islets isolated from female *Dpp4*$^{\beta\text{-cell}-/-}$ and MIP-Cre control mice exhibited similar GSIS in perifusion, with no significant differences observed after stimulation with arginine, GLP-1, or GIP (Figure 4I), indicating that β cell-specific DPP4 plays an insignificant role in physiological glycemic regulation in HFD-fed male and female mice.

Loss of whole pancreas *Dpp4* does not improve glucose tolerance or GSIS in HFD-fed mice

Since we found no differences in glucose tolerance *in vivo* with *Dpp4*$^{\beta\text{-cell}-/-}$ mice but did detect DPP4 signal in other islet and pancreas cells (Figures 2I and 2J), we decided to probe if a more global deletion

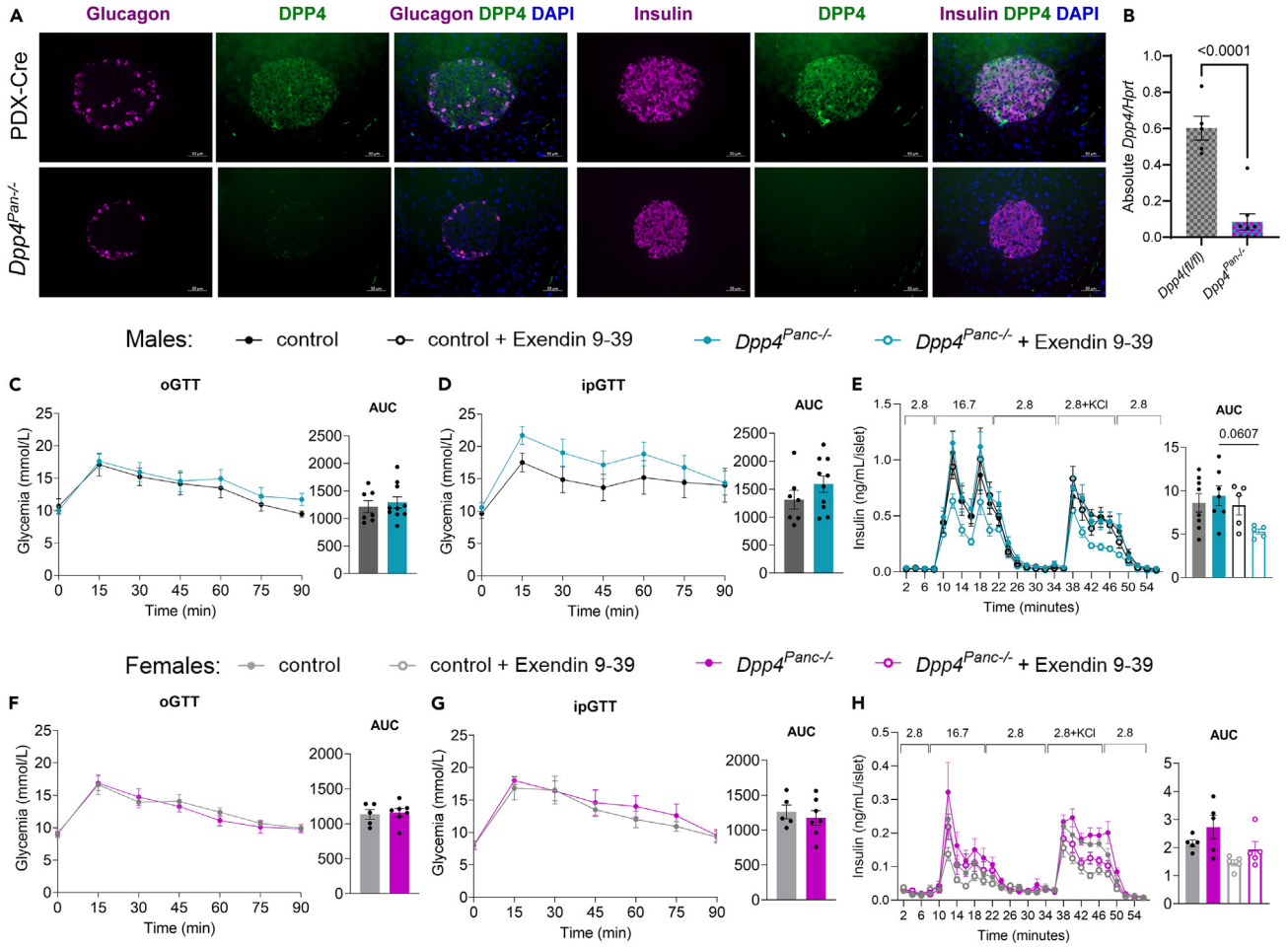

Figure 5. Ablation of whole pancreas *Dpp4* does not improve glucose tolerance or GSIS

(A) Immunofluorescence staining of glucagon (purple) and DPP4 (green) and insulin (purple) and DPP4 (green) in HFD-fed mouse pancreatic sections of PDX-Cre and $Dpp4^{Pan-/-}$. DAPI staining (blue) was used to identify nuclei.

(B) Absolute mRNA abundance of *Dpp4* in islets isolated from one-year-old control (*Dpp4(fl/fl)*) and $Dpp4^{Pan-/-}$ male and female mice.

(C and D) Glycemia and AUC glucose during (C) oral and (D) *i.p.* glucose tolerance tests in HFD-fed control and $Dpp4^{Panl-/-}$ male mice.

(E) Perifusion GSIS in male mice fed HFD for 25–30 weeks, with and without Exendin 9-39.

(F and G) Glycemia and AUC glucose during (F) oral and (G) *i.p.* glucose tolerance tests in HFD-fed control and $Dpp4^{Pan-/-}$ female mice.

(H) Perifusion GSIS in female mice fed HFD for 25–30 weeks, with and without Exendin 9-39 (100 nM). Statistical analysis was done with mixed-effects analysis and Tukey's multiple comparisons (C–H), one-way ANOVA (AUC E and H) or unpaired t test (B, AUC C, D, F and G). All data are represented as the mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Scale bars represent 50 μm.

of DPP4 within the pancreas would impact whole-body glucose homeostasis. We generated $Dpp4^{Pan-/-}$ mice and pancreatic and duodenal homeobox 1 (PDX)-Cre littermate controls and fed them an HFD. We confirmed DPP4 elimination with flow cytometry (Figures S4A and S4B), immunostaining of pancreatic sections, and RT-qPCR in islets (Figures 5A and 5B). Male and female HFD-fed control and $Dpp4^{Pan-/-}$ mice were challenged with oral and *i.p.* GTTs, and their islets were isolated for perifusion. There was no difference in oral and *i.p.* glucose tolerance between male HFD-fed $Dpp4^{Pan-/-}$ and control mice (Figures 5C and 5D). In isolated islets from male $Dpp4^{Pan-/-}$ and control mice, GSIS was not different (Figure 5E). Interestingly, treatment with GLP1-R antagonist exendin 9-39 only reduced GSIS in the $Dpp4^{Pan-/-}$ male mice (trend, p = 0.0607), indicating that consistent with the $Dpp4^{\beta\text{-}cell-/-}$ mice they are more reliant on GLP-1R signaling for maximal insulin secretion in response to high glucose (Figure 5E). In females, no differences in glucose tolerance were detected in control and $Dpp4^{Pan-/-}$ HFD-fed mice (Figures 5F and 5G). In islets isolated from female mice, loss of pancreas *Dpp4* did not impact GSIS with and without exendin 9-39 treatment (Figure 5H).

GLP-1R signaling has been proposed to be an important link between the α and β cell.[28,46] To probe this relationship more closely, we performed arginine tolerance tests and meal tolerance tests in male and female *Dpp4*$^{Pan-/-}$ and control mice fed a long-term HFD. When challenged with an *i.p.* arginine injection, glycemia was not different between *Dpp4*$^{Pan-/-}$ and controls, neither in males (Figure S4C) nor in females (Figure S4D). Post-arginine injection glucagon (Figure S4E-F) levels were not different between *Dpp4*$^{Pan-/-}$ and controls in males or females. To evaluate the response to a mixed meal, mice were fasted and gavaged with Ensure meal replacement. *Dpp4*$^{Pan-/-}$, and control mice had similar glucose before and up to 90 min after gavage in both sexes (Figures S4G–S4J). Similarly, active GLP-1 was not different between groups in either sex (Figures S4K and S4L).

DISCUSSION

Previous work has identified vascular ECs as a principal site of action for the acute glucoregulatory actions of DPP4 inhibitors.[14,32] However, despite a significant improvement in glucoregulation of HFD-fed *Dpp4*$^{EC-/-}$ mice, they did not achieve the same degree of improvement in glucose tolerance than observed in *Dpp4*$^{-/-}$ mice, which indicated that inhibition of DPP4 in other cell types is contributing to these beneficial glycemic effects.[14] In this report, we evaluated the role of intra-islet DPP4 by deleting *Dpp4* in MIP+ or PDX+ cells in the regulation of glycemia, insulin resistance, and islet hormone release.

Whole-body deletion of the incretin receptors impairs glucose tolerance through suppressed insulin response to oral glucose in mice.[32,47] Mice in which only the β cell *Glp1r* is deleted still respond to DPP4i pointing to the importance of GIPR signaling and compensation.[34] Our study found that limiting the genetic deletion of *Gipr* and *Glp1r* to the islet β cell compartment also leads to elevated glucose excursion during an oGTT. Moreover, this increase could not be improved with a systemic dose of sitagliptin, demonstrating that the activation of the incretin receptors on the β cell is essential for the glucose-lowering action of sitagliptin.

To further explore the link between metabolic dysregulation, islet-derived DPP4, and insulin secretion, we generated a line of β cell-specific *Dpp4* knockout mice (*Dpp4*$^{\beta\text{-cell}-/-}$). In human islets, direct inhibition of islet DPP4 with the DPP4 inhibitors MK-0626[48] or sitagliptin[22] has been shown to improve β cell function, survival, and insulin secretion. Consistent with this, we show that *Dpp4*$^{-/-}$ mice have a significant increase in pancreatic insulin content with HFD feeding. We also found that islets from HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ mice secreted more insulin in perifusion experiments with very high glucose (16.7 mM) compared to controls. However, KCl-stimulated membrane depolarization resulted in comparable insulin release in both groups, suggesting the difference is dependent on augmented signaling in response to glucose and not just improved islet function. Further studies would be needed to determine if loss of islet DPP4 improves islet health or survival through prolonged α cell GLP-1 activity. Furthermore, pharmacological inhibition of DPP4 significantly increased GSIS in control HFD-fed DPP4-positive islets but had no further effects on the *Dpp4*$^{\beta\text{-cell}-/-}$ islets. Our data are consistent with previous findings in human islets treated with a DPP4i[48] and the increase in glucose-stimulated insulin granule exocytosis observed with the DPP4i (MK-0626).[49]

The production of active GLP-1 has been observed in both mouse and human islet α cells, but whether it is essential for glucose regulation *in vivo* remains controversial.[20,22,23,26,27,50–52] The importance of intra-islet communication between α cell-derived products and β cell signaling to regulate insulin secretion has been recently established.[28–30] Deciphering the contribution of glucagon from other proglucagon-derived peptides is challenging because it is a more abundant[53] but weaker GLP-1RA[28] than other proglucagon-derived peptides. Studies in α cell-ablated mice have identified pancreatic GLP-1 as required for glucose homeostasis during metabolic stress and shown that a deficiency in α cell-derived GLP-1 could be partially rescued by inhibition of DPP4i with sitagliptin.[27] While GLP-1 levels in mouse pancreas effluents are frequently undetectable,[30] we were able to detect increased GLP-1 secreted from islet perifusion chambers from HFD-fed *Dpp4*$^{\beta\text{-cell}-/-}$ mice at higher levels than controls. This is consistent with recent detection of low levels of GLP-1 (7–36 amide or 7–37) in mouse islets using product ion monitoring approaches.[53] Together with the 25% reduction in GSIS with exendin 9-39 following *Dpp4* deletion, our data indicate that islet-derived GLP-1R signaling contributes to the increase in GSIS[50] but only in response to very high-glucose treatment (16.7 mM). Studies incubating islets from healthy human donors or donors with T2D in more intermediate concentrations of glucose (11.1 mM) reported sitagliptin to increase active GLP-1 levels with no meaningful effect on GSIS.[23] Our findings also suggest that glucose concentrations must be sufficiently high to stimulate robust paracrine effects from the α cell to the β cells and that

enzymatic cleavage by DPP4 and GLP-1R signaling is at best a very modest regulator of GSIS in islets from HFD-fed mice *in vitro*.

Although GIP is also a DPP4 substrate and regulator of GSIS, recent proteomic analysis performed in islets from mice on a chow or HFD did not detect the presence of intra-islet GIP,[53] and our *in vivo* studies did not demonstrate any differences in GIP bioactivity in the circulation of $Dpp4^{\beta\text{-cell}-/-}$ mice. However, our study could not rule out intra-islet contributions from other DPP4 substrates, including but not limited to protein YY (PYY), stromal-derived growth factor 1 (SDF-1), and insulin growth factor 1 (IGF-1).[54–56] While SDF-1 may increase GLP-1 secretion from α cells after stress and IGF-1 may be important in maintaining post-streptozotocin (STZ) insulin secretion, the impact of truncation by DPP4 on these effects is unknown.[55,56] On the other hand, PYY may differentially impact GSIS in its cleaved and uncleaved forms.[54,57] Additionally, the effects of paracrine signaling in mouse islets may contribute less than in human islets because of reduced contact between cell types with mouse islets having a mostly β cell core and an α and δ cell mantle.[23]

Diet-induced weight gain and metabolic stress may induce increased islet PC1/3 expression,[51,52] which has been proposed to increase intra-islet GLP-1 production, amplifying the potential differences in glucose metabolism between groups fed an HFD. However, no differences in oral glucose, *i.p.* glucose, or insulin tolerance were observed between male or female $Dpp4^{\beta\text{-cell}-/-}$ and HFD-fed control mice. Our data demonstrate that intra-islet DPP4 does not significantly contribute to circulating DPP4 concentration or activity, incretin secretion, incretin cleavage, or insulin secretion following glucose gavage or injection in HFD-fed mice. DPP4 inhibition with sitagliptin improved glycemic excursion to an oral glucose gavage of both groups but had no effects during an *i.p.* injection in either group regardless of the diet, providing further evidence for a gut-dominant incretin response in mice.

Taken together, our data indicate that regulation of GLP-1 bioactivity in the mouse islet by DPP4 is dispensable for regulation of whole-body glucose homeostasis but under conditions of metabolic stress may have some control of intra-islet insulin release.

Limitations of the study

A major limitation of the findings presented in this report is the use of CreERT2/*loxP* system to induce genetic recombination. The use of the MIP-Cre model for genetic deletion has established caveats,[58] including the presence of human growth hormone that becomes apparent with STZ treatment in addition to HFD feeding.[45,59] However, multiple metabolic comparisons between WT and MIP-Cre have been performed, and no significant differences are observed with the presence of the transgene or tamoxifen treatment in the absence of STZ.[60] Also, PDX-Cre lines also have established limitations with regards to cell specificity.[58] Additionally, $Dpp4^{\beta\text{-cell}-/-}$ mice may have adapted following gene deletion to compensate for the loss of pancreatic DPP4, influencing the impact of the deletion, although measurements of related family member fibroblast activation protein within the pancreas were very low.

STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Experimental animals
- METHOD DETAILS
  - Glucose, insulin, meal, and arginine tolerance tests
  - Gene Expression
  - Islet isolation and perifusion
  - DPP4 activity and protein measurements
  - Immunofluorescent analysis
  - Flow cytometry
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

Study concept and design: E.F., J.E.C., and E.E.M.; Acquisition of data: E.F., C.A.A.L., N.A.T., M.A.N, M.E.C., B.V., N.M.M., I.LS., A.A,H., P.G., E.M.V., M.A.D., R.S., J.E.C., and E.E.M.; Analysis and interpretation of data: E.F., C.A.A.L., M.D.F., J.E.C., and E.E.M.; Drafting of the manuscript E.F., C.A.A.L., and E.E.M.; Obtained funding: E.E.M. All authors provided critical revision of the manuscript for important intellectual content.

DECLARATION OF INTERESTS

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INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Polyclonal goat anti-mouse DPP4	R&D Systems	Cat#AF954; RRID:AB_355739
Alexa Fluor488 donkey anti-goat	Thermo Fisher	Cat#A32814; RRID:AB_2762838
Recombinant rabbit monoclonal anti-insulin	Abcam	Cat#ab181547; RRID:AB_2716761
Recombinant anti-glucagon	Abcam	Cat#ab92517; RRID:AB_10561971
Alexa Fluor Plus 647 donkey anti-rabbit	Thermo Fisher	Cat#A32795; RRID:AB_2762835
TER-119/Erythroid Cells	BioLegend	Cat#133307; RRID:AB_11124348
Biotin anti-mouse CD45 Antibody	BioLegend	Cat#103103; RRID:AB_312968
Biotin anti-mouse CD31 Antibody	BioLegend	Cat#102503; RRID:AB_312910
APC/Fire750 anti-mouse CD24 Antibody	BioLegend	Cat#101839; RRID:AB_2650875
FITC anti-mouse CD71 Antibody	BioLegend	Cat#113805; RRID:AB_313566
PerCP/Cyanine5.5 anti-mouse CD326 (Ep-CAM) Antibody	BioLegend	Cat#118219; RRID:AB_2098647
PE anti-human/Mouse CD49f Antibody	BioLegend	Cat#313611; RRID:AB_893374
Anti-CD26 Rat Monoclonal Antibody	BioLegend	Cat#137807; RRID:AB_10663403
Streptavidin-BV421	BioLegend	Cat#405226
Chemicals, peptides, and recombinant proteins		
Sitagliptin (Januvia 100mg tablets)	Merck Laboratories	Not Applicable
Sitagliptin Phosphate Monohydrate (for perifusion)	BioVision	Cat#1757
Humalog (insulin lispro injection)	Lilly	DIN#02229704
GIP-(D-Ala^{2})-GIP	Bachem	Cat#4054476
GLP-1 7-36	Bachem	Cat#4030663
Exendin 9-39	Bachem	Cat#4017799
Collagenase from *Clostridium histolyticum*	Sigma	Cat# C7657
Histopaque(R)-1077,sterile-filtered, density: 1.077 g/mL	Sigma-Aldrich	Cat#10771
Tamoxifen	Sigma-Aldrich	Cat#T5648
Critical commercial assays		
Ultrasensitive Mouse Insulin ELISA	ALPCO	Cat#80-INSMSU-E01
High Range Mouse Insulin ELISA	ALPCO	Cat#80-INSMSH
U-PLEX Mouse GLP-1 (active) Assay	Mesoscale	Cat#K1526LK
Mouse Active GIP Elisa Kit	CrystalChem	Cat#81511
Mouse Glucagon Elisa Kit	CrystalChem	Cat#81518
High-Capacity cDNA Reverse Transcription Kit	Applied Biosystems	Cat#4368814
Pierce Rapid Gold BCA Protein Assay Kit	Thermo Fisher	Cat#A53225
Mouse DPPIV/CD26 DuoSet ELISA	R&D Systems	Cat#DY954
H-Gly-Pro-AMC HBr (for DPP4 activity assay)	Bachem	Cat#I-1225
AMC (for DPP4 activity assay)	Bachem	Cat#Q-1025
Experimental models: Organisms/strains		
MIP-Cre mice (B6.Cg-Tg(Ins1-cre/ERT)1Lphi/J)	The Jackson Laboratory	Stock #024709; RRID:IMSR_JAX:024709
Glp1r$^{fl/fl}$ mice	R. Seeley lab	Not applicable

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Gipr$^{fl/fl}$ mice	D. Drucker lab	Not applicable
Dpp4$^{fl/fl}$ mice (C57BL/6-*Dpp4*$^{tm1.1Mrl}$)	Merck Laboratories; (Distributed by Taconic)	Model#10935
Dpp4$^{-/-}$ mice	Generously provided by Didier Marguet	Not applicable
PDX-Cre mice (B6.FVB-Tg(Pdx1-cre)6Tuv/J)	The Jackson Laboratory	Stock #014647; RRID:IMSR_JAX:014647
Oligonucleotides		
Tbp	Applied Biosystems	Mm01277042_m1
Hprt	Applied Biosystems	Mm03024075_m1
Glp1r	Applied Biosystems	Mm00445292_m1
Gipr	Applied Biosystems	Mm01316344_m1
Dpp4	Applied Biosystems	Mm00494538_m1
Software and algorithms		
Graph Pad Prism 9	Graphpad Software	https://www.graphpad.com
Other		
Regular Chow Diet	Harlan Teklad	Cat#2018
High Fat Diet	Research Diets	Cat#D12451
High Fat High Cholesterol Diet	Envigo	Cat#TD88137

RESOURCE AVAILABILITY

Lead contact

Requests for related resources or information will be addressed by the lead contact, Dr. Erin Mulvihill (emulvihi@uottawa.ca).

Materials availability

No unique reagents were generated in this study.

Data and code availability

- All data will be shared upon request to the lead contact. No standardized datatype data were generated in this study.
- This study did not generate new code.
- Any additional analysis information for this work is available by request to the lead contact.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experimental animals

Experiments were performed in adult, male and female mice, aged 12 to 65 weeks old as described in figure legends. Male and female mice were analyzed separately apart from the verification of the knockout models (Figures 1, 3I, 3, and 5B). Gender as a social construct was not applicable to our study.

Dpp4$^{-/-}$ mice were re-derived from a colony described previously.[61] *Dpp4*$^{fl/fl}$ mice were obtained from Merck Research Laboratories.[14] B6.Cg-Tg(Ins1-cre/ERT)1Lphi/J (MIP-Cre) (stock # 024709) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Animals were cared for in accordance with the Canadian Guide for the Care and Use of Laboratory animals (CCAC). All experimental procedures were approved under AUP#2909 and AUP#2029 by the University of Ottawa Animal Care and Veterinary Service. Dr. Daniel Drucker (Lunenfeld Tanenbaum Research Institute, Toronto Centre for Phenogenomics) kindly provided *Glp1r*$^{fl/fl}$/*Gipr*$^{fl/fl}$ MIP-Cre (β cell Double Incretin Knockout; DIRKO) mice and littermate controls. *Dpp4*$^{+/+}$ MIP-Cre^{+} mice were crossed with *Dpp4*$^{fl/fl}$ mice, producing *Dpp4*$^{fl/+}$MIP-Cre$^{+/-}$ offspring. Intercrossing *Dpp4*$^{fl/+}$MIP-Cre$^{+/-}$ with *Dpp4*$^{fl/+}$ generated four experimental groups: *Dpp4*$^{+/+}$ (WT), *Dpp4*$^{+/+}$ WT with MIP-Cre expression (MIP-Cre), *Dpp4* floxed without Cre (*Dpp4*$^{fl/fl}$), and *Dpp4*$^{fl/fl}$ MIP-Cre^{+} (*Dpp4*$^{\beta\text{-cell-}/-}$). Controls for *Dpp4*$^{\beta\text{-cell-}/-}$ experiments are combined WT, MIP-Cre control and

Dpp4$^{fl/fl}$ as no significant differences were observed among groups. In our hands, MIP-Cre and WT mice have identical glucose tolerance and islet function under high-fat fed conditions.[60] For pancreas specific *Dpp4* knockout mice (*Dpp4*$^{Pan-/-}$), PDX-Cre-expressing mice (JAX stock #014647)[62] were crossed with *Dpp4*$^{fl/fl}$ mice and resulting *Dpp4*$^{fl/+}$ offspring with and without PDX-Cre were then crossed. This generated four genotypes: *Dpp4*$^{+/+}$ (WT), *Dpp4* WT with Cre expression (PDX-Cre), Dpp4 floxed without PDX-Cre (*Dpp4*$^{fl/fl}$), and *Dpp4* floxed with Cre expression (*Dpp4*$^{Pan-/-}$). Controls for *Dpp4*$^{Pan-/-}$ experiments are combined WT, PDX-Cre, and *Dpp4*$^{fl/f}$ unless indicated as Cre control where only PDX-Cre mice are used.

Mice were housed under a 12-hour light/dark cycle at the University of Ottawa Heart Institute, and maintained on regular chow (Harlan Teklad, 2018, 18% kcal from fat) prior to HFD (Research Diets #D12451, 45% kcal from fat) with free access to food and water, unless otherwise noted. The HFHC diet has 0.2% per weight cholesterol, 42% kcal from fat, and 42.7 % kcal from carbohydrate (Teklad, TD88137). For metabolic tests, food was removed at 8 am for a five hour fast. Cre activity was induced by administering tamoxifen (Sigma-Aldrich, #T5648) in corn oil (50 mg/mL, Sigma-Aldrich) by oral gavage in all *Dpp4*$^{\beta\text{-cell-/-}}$ and control genotypes (5 mg/mouse/day) for 5 consecutive days.[63] Tamoxifen was not administered to PDX-Cre mice.[62]

METHOD DETAILS

Glucose, insulin, meal, and arginine tolerance tests

Oral glucose (2 g/kg), intraperitoneal (i.p.) glucose (1.2 g/kg females, 2 g/kg males), intraperitoneal arginine (2 g/kg), and insulin (0.6 IU/kg) tolerance tests were performed as previously described where glucose is measured for 90 minutes after gavage or i.p. injection.[14] For the meal tolerance test, mice were bled before and after 200 μL Ensure meal replacement gavage. Blood for active GLP-1 (K1526LK, Mesoscale), GIP (CrystalChem, USA), insulin (Mouse Ultrasensitive Insulin ELISA, Alpco, 80-INSMSU-E01), and glucagon (CrystalChem, USA) measurements was collected in K^{2}EDTA-coated tubes and blood was mixed with 10% TED (5000 KIU/mL Trasylol, 1.2 mg/mL EDTA, 0.1 nmol/L Diprotin A). Plasma was stored at -80°C until analysis. Terminal blood samples were collected via cardiac puncture. Tissues were flash frozen in liquid nitrogen and stored at -80°C. Tissues for histological analyses were fixed in 4% paraformaldehyde for 24 hours prior to transferring to 70% ethanol, processing, and embedding in paraffin wax.

Gene Expression

RNA was isolated using TRIzol reagent (Life Technologies, Canada) and cDNA was synthesized from total RNA using a high-capacity cDNA reverse transcription kit (Applied biosystems). Specific mRNA levels were measured by quantitative real-time PCR on a QuantStudio 5. mRNA abundance was determined using the standard curve method and expression was normalized to housekeeping gene *Tbp* or *Hprt*.

Islet isolation and perifusion

The pancreas was inflated through the pancreatic duct with collagenase (7.5 mg/mL, Sigma C7657) in HBSS (5 mM glucose, 1 mM $MgCl_2$), excised and incubated at 37°C for 12 minutes. The digestion was quenched with ice-cold HBSS (5 mM glucose, 1 mM $MgCl_2$, 1 mM $CaCl_2$). The digested pancreas was washed with HBSS, and islets were separated using a Histopaque gradient (Sigma, 10771). Islets were handpicked to achieve final purity of 95-99% and incubated in complete RPMI (10% FBS, 1% penicillin/streptomycin) overnight at 37°C and 5% O_2 before perifusion.

A Biorep perifusion system (Biorep Technologies) was used to stimulate islet hormone secretion *in vitro*. Solutions were prepared in KRBH buffer (115 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 24 mM $NaHCO_3$, 10 mM HEPES, and 1% BSA; pH = 7.4). Islets were equilibrated for 48 minutes with 2.8 mM glucose and then perifused in intervals in the indicated experimental conditions. Flow rate (100 μl/min) and temperature (37°C) remained constant, and the type of treatment is indicated at the top of each figure (Arginine, Sigma, #A5006; GLP-1 7-36, Bachem, 4030663; GIP-(D-Ala^2)-GIP, Bachem #4054476; Exendin 9-39, Bachem #4017799). Effluent fractions collected at 2-minute intervals were stored at -80°C for further assays. Insulin concentration was measured using a Mouse Ultrasensitive or High Range Insulin ELISA (ALPCO, 80-INSMSU-E01 or 80-INSMSH).

DPP4 activity and protein measurements

Mouse DPP4 ELISA (R&D Systems) was used to measure plasma DPP4 concentrations following the manufacturer's protocol. DPP4 activity was measured by a fluorometric assay as previously described.[14] Tissue

DPP4 activity was normalized to total protein concentration measured by BCA assay (Pierce Rapid Gold BCA Protein Assay Kit, Thermo Fisher).

Immunofluorescent analysis

Five μm thick sections of paraffin embedded pancreas and liver tissues were deparaffinized in toluene and rehydrated. Antigen retrieval was done in the microwave with sodium citrate buffer (0.1 M, pH 6, with 0.05% Tween-20) and slides were washed with water and PBS before blocking with 10% donkey serum in PBS for 30 minutes at room temperature. Sections were incubated with polyclonal goat anti-mouse DPP4 (1/40, R&D Systems, AF954), recombinant rabbit monoclonal anti-insulin (1/250; Abcam ab181547, clone number EPR17359), and recombinant anti-glucagon (1/500; Abcam ab92517, clone number EP3070) primary antibodies overnight at 4°C. Sections were incubated with Alexa Fluor488 donkey anti-goat (1/500; Thermo Fisher Scientific A32814) and Alexa Fluor Plus 647 donkey anti-rabbit (1/500; Thermo Fisher Scientific A32795) secondary antibodies for 45 minutes and with DAPI for 5 minutes protected from light at room temperature. Images were captured with a Zeiss Axioscope AxioImagerZ1 fluorescent microscope using the 20X objective or 63X oil objective.

Flow cytometry

Following islet isolation from pancreas tissue, islets from 3 mice were pooled and dissociated in 0.25% trypsin EDTA for 15 minutes at 37°C. Trypsin was quenched with FBS and PBS. Dispersed islet cells were centrifuged at 400 x g for 5 min and resuspended in 1% FBS in PBS. Cells were incubated with biotin-conjugated lineage (TER-119, CD45, CD31; BioLegend) and surface antibodies (EpCAM-PerCP/Cy5.5, CD49f-PE, CD71-FITC, CD24-APC/Fire750, DPP4-APC) for 20 min at room temperature and washed with 1% FBS in PBS. Cells were then incubated with streptavidin-BV421 for 20 min at room temperature and washed with 1% FBS in PBS. Cells were resuspended in 1% FBS/2mM EDTA/0.5 μg/mL DAPI in PBS and acquired on an LSRFortessa cytometer (BD). Analysis was done in FlowJo v10.7 (BD).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are expressed as the mean ± SEM. Comparisons between groups were performed using one-way ANOVA or a t-test, depending on the number of variables (GraphPad Prism9), with a post-hoc Tukey test as specified in figure legends. All time course and perifusion glycemic curves were analyzed using 2-way ANOVA with mixed model analysis and Sidak's or Tukey's multiple comparisons and AUC calculations were performed. Statistically significant differences are indicated as *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.