β-Cell Inactivation of *Gpr119* Unmasks Incretin Dependence of GPR119-Mediated Glucoregulation

Brandon L. Panaro, Grace B. Flock, Jonathan E. Campbell, Jacqueline L. Beaudry, Xiemin Cao, and Daniel J. Drucker

Diabetes 2017;66:1626–1635 | https://doi.org/10.2337/db17-0017

GPR119 was originally identified as an orphan β-cell receptor; however, subsequent studies demonstrated that GPR119 also regulates β-cell function indirectly through incretin hormone secretion. We assessed the importance of GPR119 for β-cell function in *Gpr119*$^{-/-}$ mice and in newly generated *Gpr119*$^{\beta cell-/-}$ mice. *Gpr119*$^{-/-}$ mice displayed normal body weight and glucose tolerance on a regular chow (RC) diet. After high-fat feeding, *Gpr119*$^{-/-}$ mice exhibited reduced fat mass, decreased levels of circulating adipokines, improved insulin sensitivity, and better glucose tolerance. Unexpectedly, oral and intraperitoneal glucose tolerance and the insulin response to glycemic challenge were not perturbed in *Gpr119*$^{\beta cell-/-}$ mice on RC and high-fat diets. Moreover, islets from *Gpr119*$^{-/-}$ and *Gpr119*$^{\beta cell-/-}$ mice exhibited normal insulin responses to glucose and β-cell secretagogues. Furthermore, the selective GPR119 agonist AR231453 failed to directly enhance insulin secretion from perifused islets. In contrast, AR231453 increased plasma glucagon-like peptide 1 (GLP-1) and insulin levels and improved glucose tolerance in wild-type and *Gpr119*$^{\beta cell-/-}$ mice. These findings demonstrate that β-cell GPR119 expression is dispensable for the physiological control of insulin secretion and the pharmacological response to GPR119 agonism, findings that may inform the lack of robust efficacy in clinical programs assessing GPR119 agonists for the therapy of type 2 diabetes.

The prevalence of type 2 diabetes (T2D) has been rising steadily, with several hundred million individuals worldwide at risk for developing diabetes-associated complications (1). These complications include cardiovascular disease, neuropathy, nephropathy, and retinopathy and result in extraordinary human and economic costs (2). Although considerable progress has been made toward development of new drugs for the management of hyperglycemia, many subjects still experience difficulty in attaining treatment goals with existing therapies (3). It seems evident that improved understanding of the pathophysiology of diabetes, β-cell failure, and insulin resistance is central to the development of new more effective agents for the treatment of T2D.

The importance of the β-cell response for maintaining normoglycemia in the face of chronic nutrient excess and obesity-associated insulin resistance has refocused attention on how nutrient-derived signals augment insulin secretion. Indeed physiological characterization of the incretin effect led to the discovery of the glucoregulatory roles of glucose-dependent insulinotropic polypeptide (GIP), and later, glucagon-like peptide 1 (GLP-1). Both these peptides act directly through their cognate receptors to augment glucose-dependent insulin secretion from islet β-cells, supporting the development of GLP-1 receptor agonists and dipeptidyl peptidase 4 inhibitors for the treatment of T2D (4,5).

The validation of two incretin receptors as feasible drug targets has energized scientific efforts directed at understanding how β-cells sense and respond to nutritional cues to amplify insulin secretion. Although several hundred G-protein–coupled receptors (GPCRs) are expressed in islets and many are coupled to control of insulin secretion, meaningful progress in identification of new antidiabetic drug candidates based on functional characterization of islet GPCR expression and activity has been limited (6,7). Nevertheless, there remains intense interest in understanding the signals that couple the sensing of

Lunenfeld-Tanenbaum Research Institute, Department of Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

Corresponding author: Daniel J. Drucker, drucker@lunenfeld.ca.

Received 4 January 2017 and accepted 28 February 2017.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db17-0017/-/DC1.

nutrients after meal ingestion to rapid augmentation of insulin secretion.

Incretin hormones represent prototypical examples of how ingested energy indirectly transmits signals to the endocrine pancreas via a gut–β-cell endocrine axis (8,9). Nevertheless, the digestion of nutrients yields a complex mixture of amino acids, carbohydrates, fatty acids, and metabolites, which in turn function as ligands that directly or indirectly promote insulin secretion (10). For example, GPR40, encoded by *FFAR1*, is a fatty acid–sensing receptor that increases insulin secretion in rodent and human islets, spurring development of investigational GPR40 agonists for the treatment of human T2D (11). Other nutrients, exemplified by the amino acid leucine, enhance insulin secretion through interaction with intracellular protein sensors and β-cell pathways, coupling nutrient sensing and fuel metabolism to control of ion channel activity, intracellular ion flux, and stimulation of exocytosis.

The complexity of nutrient sensing and control of the gut-islet axis is highlighted by demonstration that nutrient and chemosensing receptors may be expressed on both enteroendocrine cells (EECs) and β-cells, enabling overlapping mechanisms for nutrient regulation of insulin secretion. For example, GPR40 and the G protein–coupled bile acid receptor 1 (also known as TGR5) agonists promote GLP-1 secretion from EECs (12), and both GPR40 agonists and bile acids also directly enhance insulin secretion from rodent and human islets (13). Bile acids also signal through the farnesoid X receptor (FXR) to control GLP-1 secretion in EECs, and FXR agonists directly augment glucose-stimulated insulin secretion (GSIS) in isolated islets (14). Similarly, cannabinoid receptors control the secretion of gut hormones (8) and some cannabinoid receptor ligands directly augment insulin secretion from β-cells, depending on the species studied and receptor specificity (15).

Among the most extensively studied nutrient response receptors is GPR119, a $G_{\alpha s}$-linked class A GPCR that recognizes oleoylethanolamide, lysophosphatidylcholine, and structurally related lipids. Activation of GPR119 leads to stimulation of cAMP accumulation and increased insulin secretion directly from islet β-cells (16,17). GPR119 agonists also indirectly augment glucose-dependent β-cell function through stimulation of incretin secretion from EECs (16,18–20). These functional properties of GPR119, coupled with its suitability as a target for development of orally available small molecule agonists, led to the development and clinical testing of multiple GPR119 agonists for the treatment of T2D. Unexpectedly, the efficacy of these drugs in human subjects with diabetes was substantially less than predicted from the results of preclinical studies (21). Here we reexamine the importance of GPR119 for β-cell function in mice, demonstrating that GPR119 activity in β-cells is dispensable for the physiological control of glucose homeostasis and the acute pharmacological glucoregulatory response to AR231453, a potent and selective GPR119 agonist. These findings prompt reevaluation of the functional importance of GPR119 within β-cells, with implications for understanding the pharmaceutical potential of the GPR119 axis for the treatment of metabolic disorders.

RESEARCH DESIGN AND METHODS

Animal Experiments

Animal experiments were performed according to protocols approved by the Mount Sinai Hospital and The Centre for Phenogenomics (TCP). Male C57BL/6 mice were purchased from the in-house colony at TCP. *Gpr119*$^{-/-}$ mice were obtained from Arena Pharmaceuticals (San Diego, CA) (22) and maintained on a C57BL/6 background. Construction of targeting vector and generation of a floxed *Gpr119* mouse was performed by InGenious Targeting Laboratory (Ronkonkoma, NY) using homologous recombination of mouse embryonic stem cells, and subsequent injection into blastocysts. The resulting mice were rederived at TCP on a mixed 129/B6 background. The mice were bred with FLP mice to remove the FRT-flanked Neo selection cassette and then backcrossed a minimum of seven times onto a C57BL/6J background. The floxed *Gpr119* mice were subsequently crossed with *Mip*-CreERT mice (23) to generate mice with β-cell–specific inactivation of *Gpr119* (*Gpr119*$^{\beta cell-/-}$). All mice were housed under specific pathogen-free conditions in ventilated microisolator cages and maintained on a 12-h light/dark cycle with free access to food and water unless otherwise noted. For a list of genotyping primers used, see Supplementary Table 1.

Histological Analysis of Islet Morphometry and Apoptosis

For analysis of β-cell apoptosis, 8–10-week-old age-matched *Gpr119*$^{-/-}$ and littermate controls were fasted for 4 h before streptozotocin (STZ; 50 mg/kg/day) or vehicle (0.1 mol/L sodium citrate, pH 5) injection once daily for five consecutive days as previously described (24,25). Histology sections from formalin-fixed, paraffin-embedded whole pancreas were generated and analyzed as previously described (26–28). Serial sections were immunostained for insulin and either cleaved caspase-3 or TUNEL to identify apoptotic β-cells, or glucagon to identify α-cells. Slides were scanned and analyzed using the ScanScope CS system and software (Aperio Technologies, Vista, CA) and α-cell/β-cell area quantified as previously described (27,28).

Chronic High-Fat Diet Studies

Male mice were randomized to receive a regular chow (RC) diet (18% kcal from fat, Teklad Global Rodent Diet; Harlan Laboratories) or high-fat diet (HFD) (45% kcal from fat; Research Diets, Inc.) for a minimum of 12 weeks prior to experimentation.

Glucose or Insulin Tolerance Tests and Hormone Analyses

After a 5-h daytime fast, glucose tolerance tests were performed as previously described (28,29). When specified, AR231453 (Ab141627; Abcam, Toronto, ON, Canada) or vehicle (80% PEG-400, 10% Tween-80, 10% ethanol) was

provided by oral gavage 30 min prior to testing. Blood samples (50–100 μL) were collected and mixed with 5–10 μL of a chilled solution containing 5,000 kIU/mL Trasylol (Bayer, Toronto, ON, Canada), 32 mmol/L EDTA, and 0.01 mmol/L diprotin A (Sigma-Aldrich, St. Louis, MO), and plasma collected for subsequent assessment of total GLP-1 (mouse/rat total GLP-1 assay kit; Mesoscale Discovery, Gaithersburg, MD), total or active GIP (rat/mouse GIP [total] ELISA kit from Millipore, Billerica, MA; or mouse GIP ELISA kit from CrystalChem, Downer's Grove, IL), insulin (Ultrasensitive Mouse Insulin ELISA; Alpco Diagnostics, Salem, NH), and glucagon (Milliplex endocrine assay from Millipore; or Glucagon ELISA from Mercodia, Uppsala, Sweden). Insulin tolerance tests were similarly performed in mice fasted for 5 h using a single intraperitoneal dose of insulin (lean mice, 1.2 units/kg; obese mice, 1.7 units/kg) (Humulin R; Eli Lilly and Company, Scarborough, ON).

MRI, Indirect Calorimetry, and Locomotor Activity

For assessment of fat and lean mass body composition, a whole-body magnetic resonance analyzer was used (EchoMRI-100; Echo Medical Systems). Measures of O_2 consumption, CO_2 production, respiratory quotient, and physical activity were obtained using an Oxymax system (Columbus Instruments) (28).

Gene Expression Analysis

First-strand complementary DNA was synthesized according to the manufacturer's protocol (RNeasy Kit from Qiagen, Mississauga, ON, Canada; or Tri-Reagent from Molecular Research Center, Inc., Cincinnati, OH) using the SuperScript III reverse transcriptase synthesis system (Invitrogen, Carlsbad, CA) and random hexamers. Quantitative PCR was performed with the ABI Prism 7900 Sequence Detection System using the TaqMan Gene Expression Assays and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA).

Pancreatic Insulin and Glucagon Content

Pancreatic fragments, isolated from the same anatomical portion of each mouse pancreas, were homogenized in ice-cold acid-ethanol solution (0.18 mol/L HCl, 70% ethanol). Insulin and glucagon levels were determined in pancreatic extracts using the rat insulin RIA kit (Millipore, St. Charles, MO) and the mouse Glucagon ELISA (Mercodia).

Islet Perifusion and Hormone Secretion

Isolated islets were allowed to recover overnight at 37°C in RPMI containing 10% FBS. Medium-sized islets were then handpicked into a 0.275-mL chamber containing Krebs-Ringer bicarbonate HEPES buffer (KRBH) solution containing 0.1% (weight for volume) BSA and perifused as previously described (28). Perifusion flow rate was 200 μL/min for insulin measurements and 100 μL/min for glucagon measurements. Insulin concentrations are expressed as percent of total islet insulin for each sample and measured using a Rat Insulin RIA (Millipore). Glucagon concentrations are expressed as fold-change from baseline and measured by Glucagon ELISA.

Static Insulin Release

After overnight recovery, groups of five islets were incubated in low-glucose KRBH medium, and then high-glucose KRBH medium for 1 h each, with or without AR231453, in triplicate. The supernatant from each 1-h incubation (2 mL) was collected for insulin measurement by RIA. Insulin values were normalized to total islet insulin levels from each group of islets.

Statistical Analysis

All results are expressed as mean ± SD. Statistical significance was assessed by ANOVA and, where appropriate, two-tailed, unpaired Student *t* test unless otherwise noted using GraphPad Prism (version 5; GraphPad Software). A P value <0.05 was considered to be statistically significant.

RESULTS

Metabolic Characterization of Whole-Body *Gpr119*$^{-/-}$ Mice

To determine the importance of GPR119 for β-cell function, we assessed the metabolic phenotype(s) of whole-body *Gpr119*$^{-/-}$ mice (22) under basal conditions (18% fat RC diet). No differences in body weight or fat or lean mass were observed in *Gpr119*$^{-/-}$ versus littermate control *Gpr119*$^{+/+}$ mice (Fig. 1*A* and *B*). Moreover, oral glucose tolerance (Fig. 1*C*) and plasma insulin and incretin levels were similar in *Gpr119*$^{-/-}$ versus *Gpr119*$^{+/+}$ mice (Fig. 1*D–F*). Conversely, intraperitoneal glucose tolerance was slightly improved but not significantly different in *Gpr119*$^{-/-}$ mice (Fig. 1*G*), associated with increased circulating insulin levels (Fig. 1*H*). Insulin tolerance and the glucagon response to insulin-induced hypoglycemia was not different in *Gpr119*$^{-/-}$ versus *Gpr119*$^{+/+}$ mice (Fig. 1*I* and *J*). To determine the susceptibility of *Gpr119*$^{-/-}$ β-cells to apoptotic injury, we analyzed islets from mice after STZ administration. More cleaved caspase-3–immunopositive cells were detected in islets from *Gpr119*$^{-/-}$ mice (Supplementary Fig. 1*A* and *B*); however, pancreatic insulin area and β-cell size were not different (Supplementary Fig. 1*C* and *D*). Collectively, these findings are consistent with previous reports demonstrating no major metabolic perturbations in RC-fed *Gpr119*$^{-/-}$ mice (16,30).

Improved Glucose Tolerance and Insulin Sensitivity in HFD-Fed *Gpr119*$^{-/-}$ Mice

We next examined whether germline loss of *Gpr119* disrupts the adaptive metabolic response to HFD feeding. Although weight gain was comparable in *Gpr119*$^{-/-}$ versus *Gpr119*$^{+/+}$ mice (Fig. 2*A*), fat mass (Fig. 2*B*) and circulating levels of leptin (Fig. 2*C*) and resistin (Fig. 2*D*) were reduced in *Gpr119*$^{-/-}$ mice. Histological analysis of the pancreas revealed reduced β-cell (but not α-cell) area in HFD-fed mice (Fig. 2*E*). Food intake (Supplementary Fig. 2*A*) and the nocturnal respiratory exchange ratio (Supplementary Fig. 2*B*) were increased in HFD-fed *Gpr119*$^{-/-}$ mice, without changes in oxygen consumption (Supplementary Fig. 2*C*) or locomotor activity (Supplementary Fig. 2*D*).

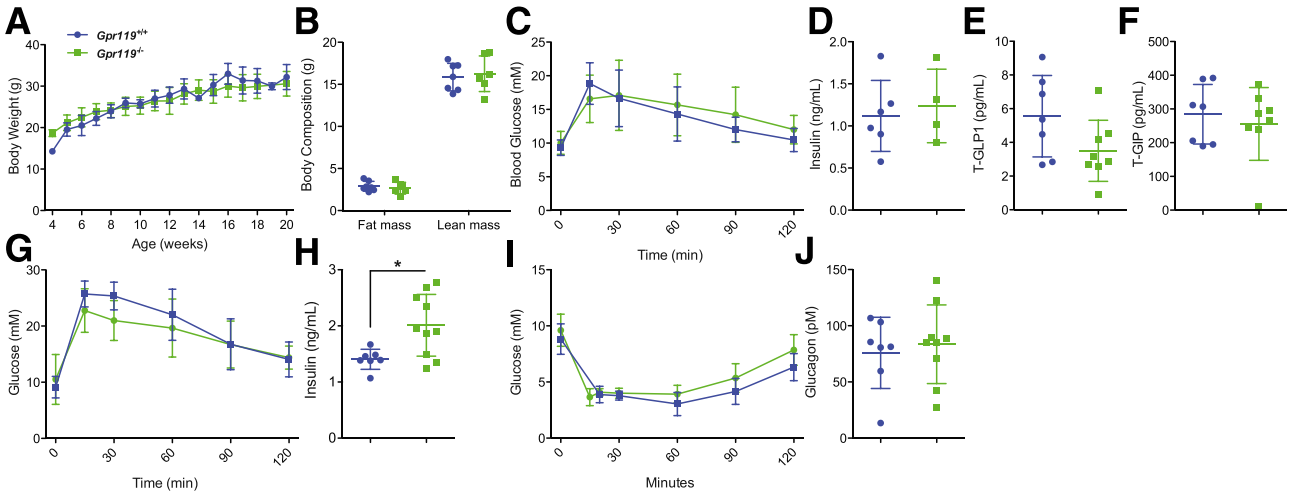

Figure 1—Basal metabolic characterization of *Gpr119*$^{-/-}$ mice. *Gpr119*$^{-/-}$ and littermate control (*Gpr119*$^{+/+}$) mice were weaned at 4 weeks of age and started on RC (18% kcal from fat). *A*: Weekly body weight. *B*: Body composition determined by MRI (5-week-old mice). *C*: Glucose excursions during oral glucose tolerance test (7-week-old mice). *D*: Plasma insulin determined 10 min after oral glucose administration. *E*: Glucose-stimulated total GLP-1 (T-GLP1) measured 10 min after glucose. *F*: Circulating total GIP (T-GIP) levels 10 min after glucose load. *G*: Glucose excursions during intraperitoneal glucose tolerance test (8-week-old mice). *H*: Plasma insulin measured 15 min after intraperitoneal glucose. *I*: Plasma glucose during insulin tolerance test (9-week-old mice). *J*: Circulating glucagon levels measured 20 min after insulin administration. *Gpr119*$^{+/+}$, n = 6–7; *Gpr119*$^{-/-}$, n = 8–10. All data are displayed as mean ± SD and were analyzed using Student t test. *P < 0.05.

Both oral and intraperitoneal glucose tolerance were impaired in HFD-fed mice, to a lesser extent in *Gpr119*$^{-/-}$ mice (Fig. 2*F* and *K*), associated with lower levels of glucose-stimulated insulin (Fig. 2*G* and *L*). Glucagon levels were not different after oral or intraperitoneal glucose administration (Fig. 2*H* and *M*). Circulating levels of total GLP-1 (Fig. 2*I*) were not different; however, total GIP levels (Fig. 2*J*) were lower in HFD-fed *Gpr119*$^{-/-}$ mice. Moreover, basal rates of gastric emptying and motility were reduced, and fecal triglycerides trended lower in *Gpr119*$^{-/-}$ mice (Supplementary Fig. 3*A*–*D*).

Insulin sensitivity was not different in RC-fed *Gpr119*$^{-/-}$ mice but significantly improved in HFD-fed *Gpr119*$^{-/-}$ mice (Fig. 2*N*). Consistent with findings of improved insulin sensitivity, fasting insulin levels were reduced in HFD-fed *Gpr119*$^{-/-}$ mice (Fig. 2*O*); however, plasma glucagon levels were similar after insulin administration (Fig. 2*P*). Moreover, pancreas weight was lower and pancreatic insulin content was reduced in HFD-fed *Gpr119*$^{-/-}$ mice (Supplementary Fig. 4*A* and *B*). Furthermore, pancreatic levels of *Irs2*, *Gcgr*, *Glp1r*, *Gpr40*, *Gck*, and *Sp1* mRNA transcripts were lower in *Gpr119*$^{-/-}$ mice (Supplementary Fig. 4*C*).

Generation and Characterization of *Gpr119*$^{\beta cell-/-}$ Mice

Mechanistic attribution of the modest differences in metabolic and islet phenotypes arising in whole-body *Gpr119*$^{-/-}$ mice is challenging due to pleiotropic roles for GPR119 in control of β-cell function, gut hormone secretion, gastrointestinal motility, food intake, and lipid absorption (16,19,22,27,31,32). As GPR119 was originally described as an orphan β-cell receptor (33) coupled to direct stimulation of insulin secretion (16,18), we mated floxed *Gpr119* mice with *Mip*-CreERT mice (23,34,35) to generate *Gpr119*$^{\beta cell-/-}$ mice, enabling selective conditional tamoxifen-mediated reduction of *Gpr119* expression in adult β-cells (Fig. 3*A*). *Mip*-CreERT littermates were used as controls (denoted as *Gpr119*$^{\beta cell+/+}$) for all studies analyzing *Gpr119*$^{\beta cell-/-}$ mice (35). Levels of *Gpr119* mRNA transcripts in *Gpr119*$^{\beta cell-/-}$ islets and total pancreas were markedly reduced, whereas no decrease in *Gpr119* mRNA expression was detected in mRNA from colon in *Gpr119*$^{\beta cell-/-}$ mice (Fig. 3*B*).

No difference in body weight or composition was observed in *Gpr119*$^{\beta cell-/-}$ mice (Fig. 3*C* and *D*). Furthermore, glucose tolerance, levels of plasma insulin, GLP-1, and GIP, insulin sensitivity, and the glucagon response to hypoglycemia were not different in *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$ mice (Fig. 3*E*–*L*). Unlike findings in mice with germline disruption of *Gpr119* (Supplementary Fig. 1), *Gpr119*$^{\beta cell-/-}$ mice did not exhibit enhanced islet apoptosis after STZ administration (Supplementary Fig. 5*A* and *B*). Moreover, in contrast to the dysregulated expression of islet genes observed in *Gpr119*$^{-/-}$ mice (Supplementary Fig. 4*C*), levels of *Glp1r* mRNA were increased but mRNA transcripts for *Ins2*, *Gcg*, *Gcgr*, *Irs2*, *Gipr*, and *Gpr40* were not different in islet RNA from *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$ mice (Supplementary Fig. 5*C*).

Loss of GPR119 From β-Cells Does Not Alter the Response to High-Fat Feeding

To determine whether loss of β-cell GPR119 impairs the adaptation to metabolic stress, we assessed *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$ mice after HFD feeding. Body weight (Fig. 4*A*) and VO_2, VCO_2, respiratory exchange ratio, heat production, and activity were similar (Supplementary

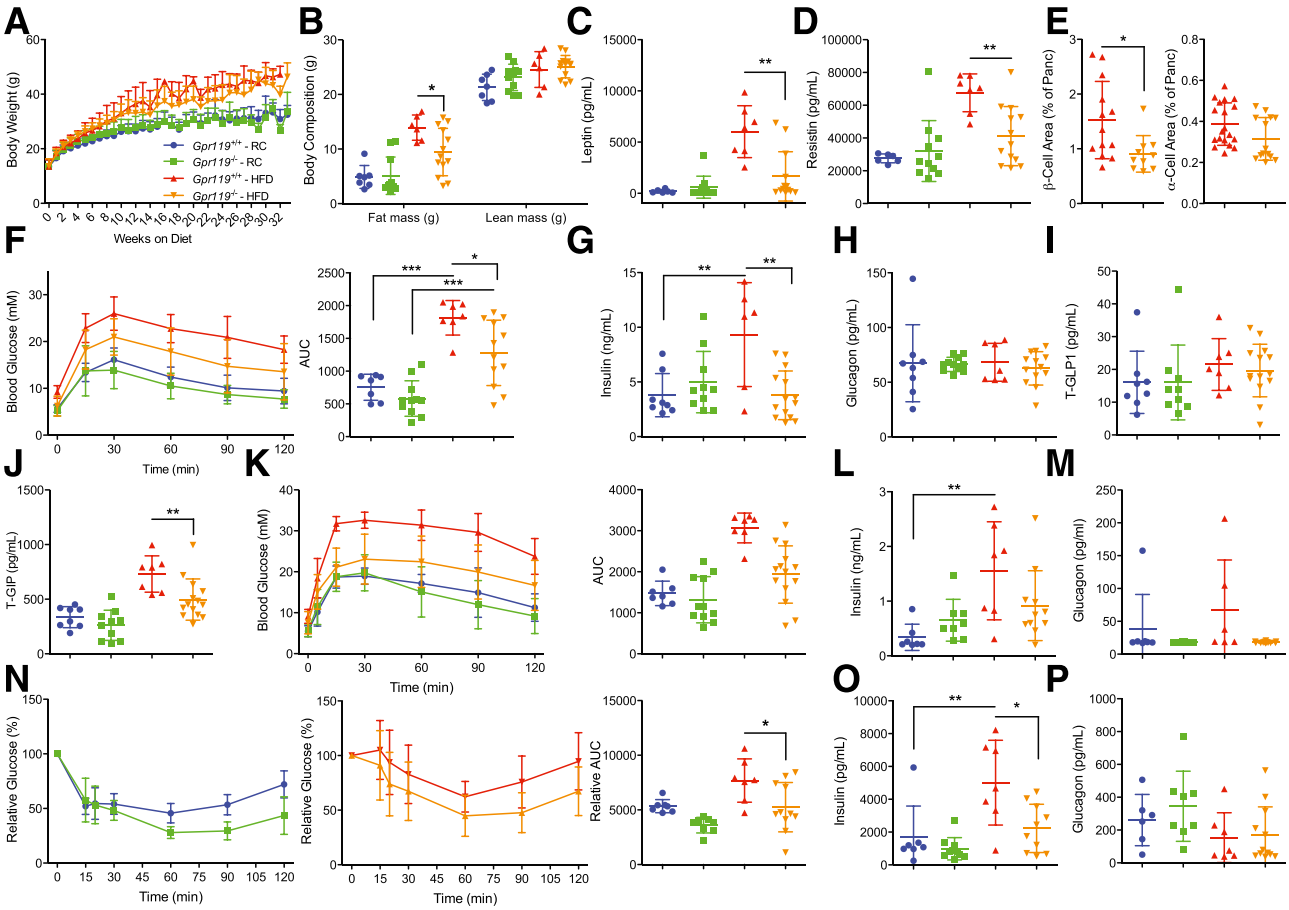

Figure 2—*Gpr119*$^{-/-}$ mice fed a 45% HFD exhibit improved glucose tolerance and reduced fat mass. *A*: Body weight over time on RC or HFD (45% kcal from fat). *B*: Body composition after 18 weeks on diet. *C* and *D*: Plasma leptin and resistin after 19 weeks on diet. *E*: Histological analysis of β-cell (insulin positive, left) and α-cell (glucagon positive, right) area in pancreata. *F–M*: Age-matched *Gpr119*$^{+/+}$ and *Gpr119*$^{-/-}$ male mice maintained on 18% RC or 45% HFD for 19–20 weeks prior to oral glucose tolerance test (OGTT) and intraperitoneal glucose tolerance test (IPGTT), respectively. *F*: Glucose excursion during OGTT and area under the curve (AUC) of glucose excursion on right. *G*: Plasma insulin assessed 5 min after glucose administration. *H*: Plasma glucagon levels, assessed 5 min after glucose administration. Total GLP-1 (T-GLP1) (*I*) and GIP (T-GIP) (*J*), determined 5 min after glucose load. *K*: Glucose excursion during IPGTT and AUC on right. *L*: Plasma insulin during IPGTT 15 min after glucose administration. *M*: Plasma glucagon levels 15 min after intraperitoneal glucose administration. *N*: Glucose levels during insulin tolerance test (ITT) of RC- and HFD-fed mice (left and middle) and AUC of relative glucose levels during ITT (right). *O*: Fasting insulin prior to start of ITT. *P*: Plasma glucagon levels 20 min after insulin injection during ITT. *Gpr119*$^{+/+}$ (RC), $n = 7$; *Gpr119*$^{-/-}$ (RC), $n = 11$; *Gpr119*$^{+/+}$ (HFD), $n = 7$; *Gpr119*$^{-/-}$ (HFD), $n = 14$. All data are displayed as mean ± SD and analyzed using ANOVA with Bonferroni posttest. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Fig. 6*A–F*); however, fat mass trended lower and lean mass was increased in HFD-fed *Gpr119*$^{\beta cell-/-}$ mice (Fig. 4*B*). β-Cell and α-cell area were similar (Fig. 4*C*) and both oral and intraperitoneal glucose tolerance and plasma levels of insulin and glucagon were comparable in HFD-fed *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$ mice (Fig. 4*D–I*). Insulin sensitivity was improved (Fig. 4*J*), with a trend toward higher glucagon levels (Fig. 4*K*), likely reflecting the lower relative glucose levels achieved after insulin administration in HFD-fed *Gpr119*$^{\beta cell-/-}$ mice.

Preservation of Insulin Secretion in Mice With Global or Selective Loss of GPR119 in β-Cells

The surprising preservation of glucose tolerance in HFD-fed *Gpr119*$^{\beta cell-/-}$ mice prompted us to further scrutinize insulin secretion in islets lacking GPR119. We first tested insulin secretory responses in islets isolated from lean 12-week-old *Gpr119*$^{-/-}$ and *Gpr119*$^{+/+}$ mice. No genotype-dependent differences in GSIS were detected (Fig. 5*A*). Furthermore, both *Gpr119*$^{-/-}$ and *Gpr119*$^{+/+}$ islets responded similarly to the GLP-1R agonist exendin-4 (1 nmol/L) and to 30 mmol/L KCl (Fig. 5*A*). To eliminate potential confounding effects arising in whole-body *Gpr119*$^{-/-}$ mice, we assessed insulin secretion in perifused islets from *Gpr119*$^{\beta cell-/-}$ mice. Insulin responses to glucose, exendin-4, and 30 mmol/L KCl were similar in islets from *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$ mice (Fig. 5*B*). Moreover, to verify persistent knockdown of *Gpr119* in islets ex vivo, we reassessed *Gpr119* expression. *Gpr119* mRNA transcripts were undetectable in RNA from *Gpr119*$^{-/-}$ islets and markedly reduced in *Gpr119*$^{\beta cell-/-}$ versus *Gpr119*$^{\beta cell+/+}$

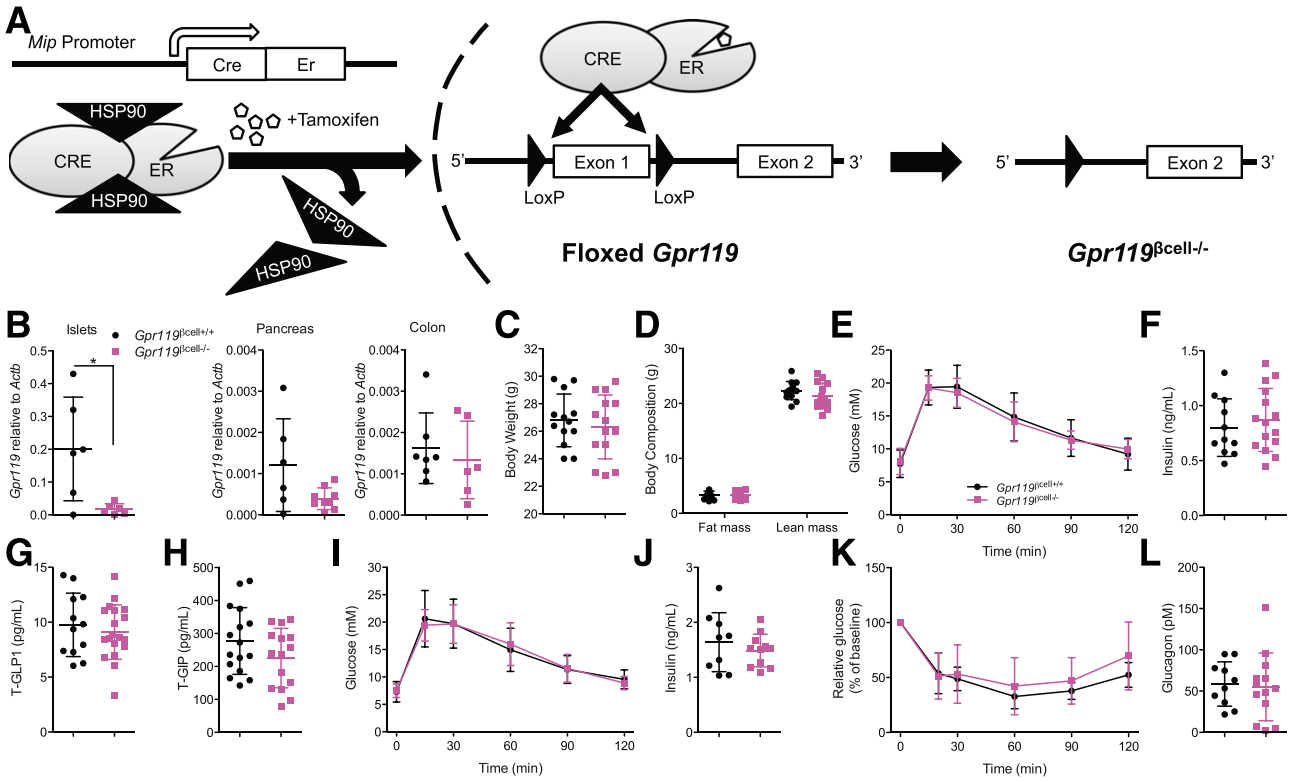

Figure 3—Characterization of *Gpr119*$^{\beta cell-/-}$ mice on RC. *A*: Generation of *Gpr119*$^{\beta cell-/-}$ mice containing a floxed *Gpr119* exon 1, bred with *Mip*-CreERT mice. *B*: Assessment of *Gpr119* mRNA levels by quantitative PCR in islets, pancreas, and colon from *Gpr119*$^{\beta cell-/-}$ and *Gpr119*$^{\beta cell+/+}$ mice. Values are expressed relative to transcript levels of β-actin (*Actb*). Islets: $n = 6$ per group. Pancreas: *Gpr119*$^{\beta cell+/+}$, $n = 6$; *Gpr119*$^{\beta cell-/-}$, $n = 9$. Colon: *Gpr119*$^{\beta cell+/+}$, $n = 7$; *Gpr119*$^{\beta cell-/-}$, $n = 6$. *C*: Body weight. *D*: Body composition. *E*: Glucose excursion from oral glucose tolerance test (OGTT) performed 2 weeks after last tamoxifen (TMX) dose; $n = 15–18$ per group. *F*: Glucose-stimulated insulin during OGTT (15 min after glucose load). *G*: Circulating total GLP-1 (T-GLP1) (*G*) and GIP (T-GIP) (*H*) levels assessed 15 min after glucose load. *I*: Intraperitoneal glucose tolerance test 1 week after last TMX dose; $n = 9–11$ per group *J*: Plasma insulin 15 min after intraperitoneal glucose. *K*: Glucose profile during insulin tolerance test. Values are expressed relative to fasting glucose; $n = 13–18$ per group. *L*: Circulating glucagon levels measured 20 min after exogenous insulin administration. *$P < 0.05$.

islet mRNA, whereas *Glp1r* expression was unaffected (Fig. 5*C* and *D*).

As some but not all studies have demonstrated that GPR119 agonists directly stimulate insulin secretion from rodent islets (16,27,36), we assessed insulin release from perifused mouse islets in the presence or absence of AR231453, a highly selective GPR119 agonist that fails to lower glucose or stimulate insulin levels in *Gpr119*$^{-/-}$ mice (16). No AR231453-dependent enhancement of insulin secretion was observed in wild-type (WT) islets (Fig. 6*A*), irrespective of the timing and dose of AR231453 exposure during the perifusion period (Supplementary Fig. 7A and *B*). Moreover, insulin release was not consistently increased after AR231453 treatment of static cultures of mouse islets (Fig. 6*B*). Furthermore, in separate experiments, AR231453 did not modify glucagon secretion from perifused islets; in contrast, glucagon levels rose robustly after exposure to arginine (Fig. 6*C*).

GPR119 Agonism Enhances Insulin Secretion in Mice

As AR231453 did not directly regulate insulin or glucagon secretion in islets, we reassessed its bioactivity in vivo. Administration of an oral dose (20 mg/kg) of the same batch of AR231453 used in islet studies (Fig. 6*D*) produced a rapid reduction in glycemic excursion and increase in plasma insulin levels after an oral glucose challenge in WT mice (Fig. 6*E* and *F*). Furthermore, AR231453 robustly lowered glycemic excursions (Fig. 7*A*) and increased plasma levels of insulin and GLP-1 (Fig. 7*B* and *C*), but not GIP (Fig. 7*D*), in *Gpr119*$^{\beta cell-/-}$ as well as in *Gpr119*$^{\beta cell+/+}$ mice (data not shown). Taken together, these findings demonstrate that β-cell GPR119 is not required for the physiological control of glucose homeostasis or the acute insulinotropic response to pharmacological GPR119 agonism.

DISCUSSION

The expression of GPR119 in β-cells and EECs, together with the robust glucoregulatory actions of GPR119 agonists in preclinical studies, raised considerable enthusiasm for GPR119 as a target for T2D drug development. To better understand the physiology of GPR119, we first reevaluated the metabolic phenotype of mice with whole-body germline disruption of *Gpr119*. Consistent with previous reports (22,30), islet morphology and glucose homeostasis were not dysregulated in RC-fed *Gpr119*$^{-/-}$ mice (16,30). Lan et al.

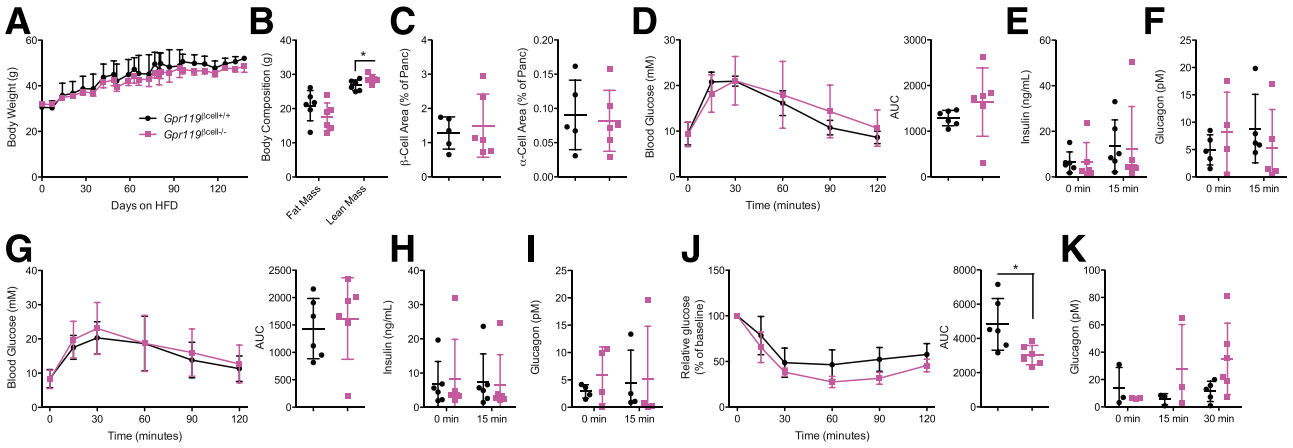

Figure 4—*Gpr119*$^{\beta cell-/-}$ mice exhibit normal glucose tolerance. All tests were performed at least 1 week apart and after a minimum of 12 weeks of HFD feeding. *A*: Body weight over time during chronic HFD feeding. *B*: Body composition at end of study. *C*: Histological analysis of β-cell (insulin positive, left) and α-cell (glucagon positive, right) area in pancreata. *D*: Glucose excursion during oral glucose tolerance test and area under the curve (AUC) of glucose excursion. *E*: Plasma insulin at the indicated time points. *F*: Plasma glucagon at the indicated time points. *G*: Glucose excursion during intraperitoneal glucose tolerance test and AUC of glucose excursion. *H*: Plasma insulin at the indicated time points. *I*: Plasma glucagon at the indicated time points. *J*: Glucose levels relative to baseline during an insulin tolerance test (ITT) and AUC of relative glucose levels. *K*: Plasma glucagon during ITT. *Gpr119*$^{\beta cell+/+}$, $n = 6$; *Gpr119*$^{\beta cell-/-}$, $n = 6$. *$P < 0.05$.

(30) demonstrated that plasma insulin levels and lean body mass trended lower in *Gpr119*$^{-/-}$ mice, consistent with our current findings. Moreover, we observed improved insulin and glucose tolerance with lower insulin levels in HFD-fed *Gpr119*$^{-/-}$ mice. Our findings of increased lean body mass and improved insulin sensitivity (and hence reduced β-cell secretory demand) in *Gpr119*$^{-/-}$ mice complicates ascertainment of the importance of GPR119 activity in β-cells (31).

The essential role(s) of GPR119 in control of the incretin axis further challenges interpretation of β-cell phenotypes arising in *Gpr119*$^{-/-}$ mice. Several studies have

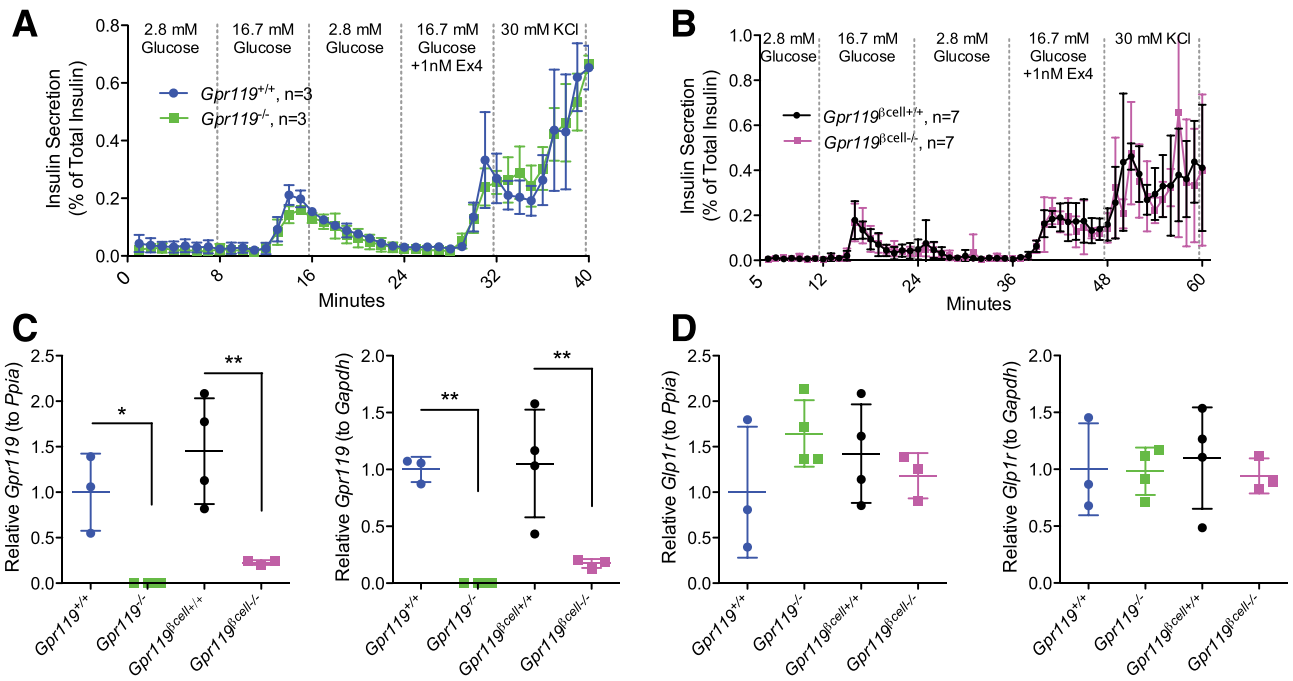

Figure 5—*Gpr119* inactivation does not impair GSIS. *A* and *B*: Insulin secretion data during islet perifusion in *Gpr119*$^{+/+}$ and *Gpr119*$^{-/-}$ islets isolated at 12 weeks of age (*A*) and *Gpr119*$^{\beta cell+/+}$ and *Gpr119*$^{\beta cell-/-}$ islets isolated from mice 1 week after tamoxifen treatment at 12 weeks of age (*B*). Treatment conditions, indicated on graphs, include low glucose, high glucose, high glucose + 1 nmol/L exendin-4 (Ex4), and 30 mmol/L KCl. *C*: *Gpr119* mRNA expression in islets relative to *Ppia* (left) and *Gapdh* (right). *D*: Islet *Glp1r* mRNA expression relative to *Ppia* (left) and *Gapdh* (right); $n = 3$–4 per group unless otherwise stated on graph. Data are expressed as mean ± SD and analyzed using ANOVA with Bonferroni posttest. *$P < 0.05$; **$P < 0.01$.

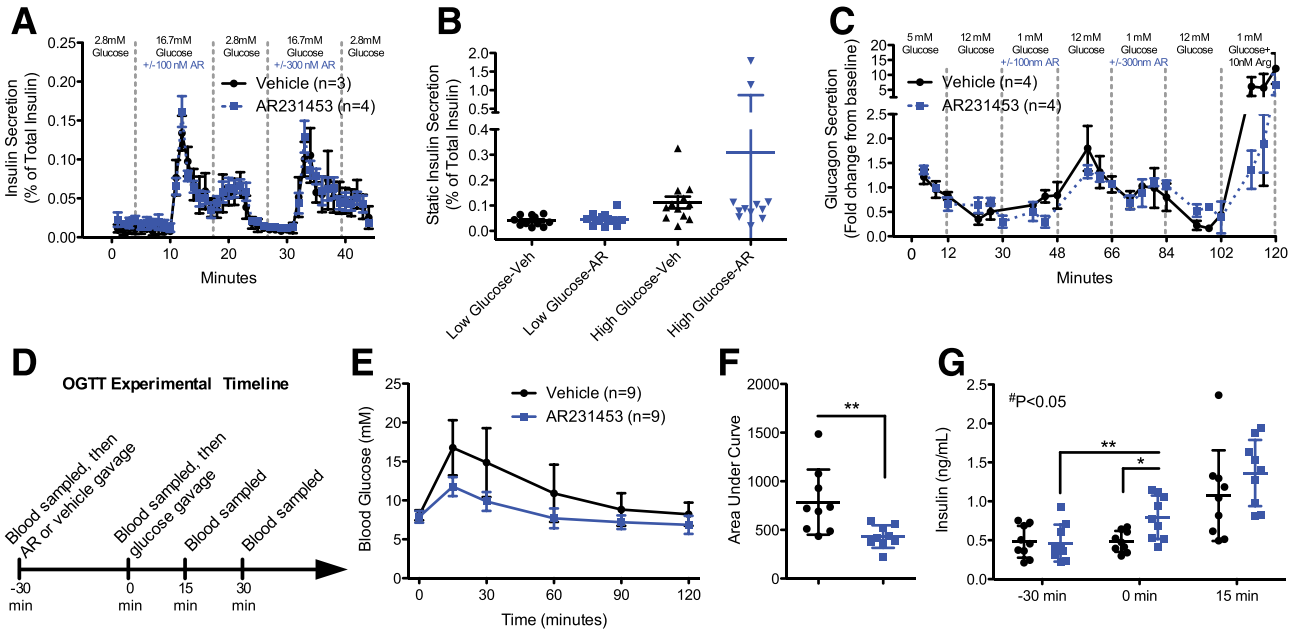

Figure 6—GPR119 agonism does not directly stimulate insulin secretion. *A*: Insulin secretion data during perifusion in islets isolated from C57BL/6J mice, with transitions from low to high glucose in conjunction with 100 and 300 nmol/L of GPR119 agonist AR231453. *B*: Individual data points from groups of five islets from a static insulin release assay from 1-h incubation of islets and listed treatment conditions; n = 11–12 per group. *C*: Glucagon secretion from perifusion of islets under the listed conditions in the presence of either AR231453 or vehicle. *D*: Experimental timeline of oral glucose tolerance test (OGTT) and AR231453 administration. *E*: Glucose excursion during OGTT. *F*: Area under the curve of glucose excursion in mice treated with vehicle or AR231453. All mice received both vehicle and AR231453 treatment in random order, 1 week apart. *G*: Insulin levels from OGTT. AR, AR231453; Arg, arginine. Data analyzed in *F* and *G* using paired Student t test. $^*P < 0.05$; $^{**}P < 0.01$. #, significant effect of drug treatment on insulin levels by two-way ANOVA.

demonstrated that the glucoregulatory actions arising from GPR119 activation are dependent in part on enhanced GIP and GLP-1 secretion (19,22,27). Moreover, whole-body loss of GPR119 leads to lower circulating levels of GLP-1 in both RC- and HFD-fed *Gpr119*$^{-/-}$ mice, and levels of GIP also trended lower (30). Consistent with these findings, plasma levels of GLP-1 and GIP were lower after acute olive oil challenge in *Gpr119*$^{-/-}$ mice (32), and plasma GIP levels were lower in HFD-fed *Gpr119*$^{-/-}$ mice studied herein. Moreover, analysis of islet mRNA from HFD-fed *Gpr119*$^{-/-}$ mice revealed reduced levels of *Glp1r*, *Gcgr*, and *Gpr40* mRNA transcripts, and *Gipr* mRNA levels trended lower (Supplementary Fig. 4). Hence, interpreting changes in β-cell function arising in whole-body *Gpr119*$^{-/-}$ mice is complex due to potential compensation arising from changes in plasma incretin hormones and altered islet expression of functionally related receptors.

To circumvent the potential confounding importance of GPR119 for organ formation and cell differentiation that might produce adaptive compensation in metabolically sensitive tissues, and to avoid perturbations within the gut GPR119 EEC system, we generated *Gpr119*$^{\beta cell-/-}$

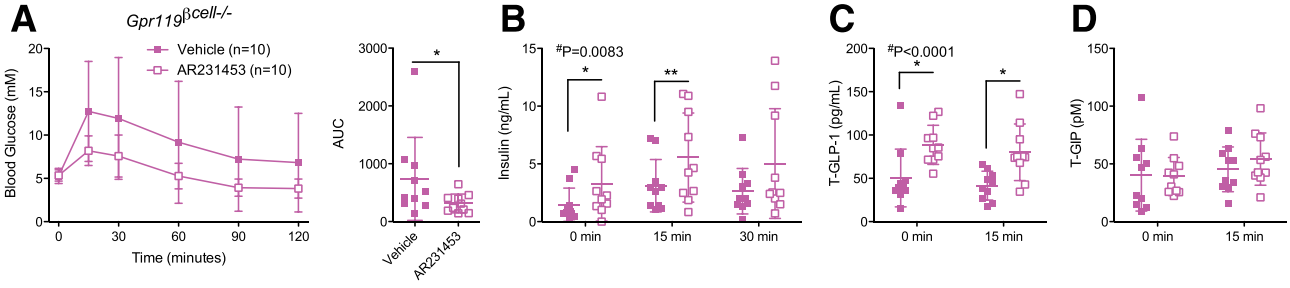

Figure 7—AR231453 improves glucose tolerance in *Gpr119*$^{\beta cell-/-}$ mice. *A*: Glucose excursion during oral glucose tolerance test (OGTT) in *Gpr119*$^{\beta cell-/-}$ mice treated with AR231453 or vehicle and area under the curve (AUC) of glucose excursion. *B*: Plasma insulin at indicated time points during OGTT. Total GLP-1 (T-GLP1) (*C*) and total GIP (T-GIP) (*D*) in plasma. Data were analyzed by paired Student t test to determine the statistical significance of drug treatments in each group. $^*P < 0.05$; $^{**}P < 0.01$. #, significant effect of drug treatment on insulin levels by two-way ANOVA.

mice. We detected ~80–90% knockdown of *Gpr119* expression in islets from *Gpr119*$^{\beta cell-/-}$ mice (Fig. 3*A* and Fig. 5*C*). This level of knockdown in total islets using the *Mip*-CreERT mouse to achieve recombination selectively in β-cells is consistent with substantial expression of islet *Gpr119* in β-cells, and roughly correlates with the relative proportion of β-cells within mouse islets. The 10–20% residual *Gpr119* mRNA expression in *Gpr119*$^{\beta cell-/-}$ islets could be attributed to inefficiency of knockdown in β-cells, or more likely to residual *Gpr119* expression in the non–β-cell islet population.

Surprisingly, *Gpr119*$^{\beta cell-/-}$ mice did not exhibit overt metabolic defects when studied on RC or after prolonged HFD feeding. A modest reduction in fat mass, increased lean body mass, and improved insulin sensitivity in *Gpr119*$^{\beta cell-/-}$ mice resembles phenotypes we described for *Gipr*$^{\beta cell-/-}$ mice (28), and likely reflects a role for β-cell GPR119 as a component of the β-cell response to lipid ingestion, as recently highlighted in studies of EECs (32). Furthermore, the β-cell insulin secretory response to glucose was completely normal in *Gpr119*$^{-/-}$ and *Gpr119*$^{\beta cell-/-}$ islets. Equally surprisingly, we were unable to directly elicit insulin secretion using AR231453 in WT islets, at doses as high as 300 nmol/L (16), under perifused or static incubation conditions. Similarly, despite rare reports of GPR119 agonists regulating glucagon secretion in mice (36), we did not detect alteration of glucagon secretion using AR231453 in perifused islets. In contrast, the same batch of AR231453 robustly lowered glucose and increased GLP-1 and insulin levels after acute administration to *Gpr119*$^{\beta cell-/-}$ mice. These findings, together with our previous studies elucidating the essential requirements for both incretin receptors in GPR119-dependent glucose control (27), strongly suggest that the β-cell GPR119 is not an essential target for the glucoregulatory activity of GPR119 agonists.

Our data reveal that GPR119 expression within murine β-cells is not functionally important for the β-cell response to hyperglycemia, or for transduction of a direct insulinotropic response to pharmacological GPR119 agonism (36). Despite an extensive literature demonstrating robust GPR119-dependent incretin and insulin secretion, associated with reduction of glycemia in preclinical studies, successful translation of pharmacological GPR119 agonism for the treatment of T2D in human subjects has not been realized (21,37). GPR119 agonists increase incretin secretion from human EECs ex vivo (12); however, these observations have not been as robustly recapitulated in clinical studies of human subjects. Although multiple GPR119 agonists have been tested in clinical trials, reported increases in gut hormones have been modest, and usually not associated with robust changes in insulin levels or glycemic excursion (21,37,38).

Another potential explanation for the failure of GPR119 agonists to effectively lower glycemia and HbA_{1c} in clinical studies may be related to species differences in islet cell expression of *GPR119*. Notably, single-cell transcriptomic analysis of individual human islet cells revealed a 10-fold higher level of *GPR119* expression in isolated α-cells, relative to β- or δ-cells (39). Nevertheless, analysis of individual islet cell transcriptomes consistently detects *GPR119* mRNA transcripts in both mouse and human β-cells, at levels approximating those obtained for the functionally related fatty acid receptor FFAR1 (40,41). Our findings, using isolated islets and *Gpr119*$^{\beta cell-/-}$ mice, strongly suggest that β-cell GPR119 is dispensable for *1*) the insulin response to glucose, *2*) the adaptive islet response to HFD feeding, and *3*) the acute glucoregulatory response to pharmacological GPR119 agonism. Collectively, these results support the evolving view that GPR119 functions primarily as an enteroendocrine lipid sensor (32,42) and may be much less important as a direct β-cell target for the stimulation of insulin secretion.

Funding. B.L.P. was supported by fellowships from TD Bank, the Canadian Diabetes Association (grant PF-3-15-4737-BP), and the National Institutes of Health (fellowship grant F32DK107050). This work was supported in part by grants from the Canadian Diabetes Association (OG-3-11-3270-DD), Canadian Institutes of Health Research (CIHR) (Institute of Nutrition, Metabolism and Diabetes grants 82700 and 123391), the Canada Research Chairs Program, and the Banting & Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology (D.J.D.). J.E.C. was supported by a CIHR fellowship, and J.L.B. was supported by a Canadian Diabetes Association fellowship.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. B.L.P. and G.B.F. designed and performed the experiments and wrote the manuscript. J.E.C., J.L.B., and X.C. performed the experiments. D.J.D. designed the experiments and wrote the manuscript. D.J.D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Zimmet PZ, Magliano DJ, Herman WH, Shaw JE. Diabetes: a 21st century challenge. Lancet Diabetes Endocrinol 2014;2:56–64

2. Association AD; American Diabetes Association. Economic costs of diabetes in the U.S. in 2012. Diabetes Care 2013;36:1033–1046

3. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 2014;383:1068–1083

4. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 2013;17:819–837

5. Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. Physiol Rev 2015;95:513–548

6. Ahrén B. Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. Nat Rev Drug Discov 2009;8:369–385

7. Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ. An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. Pharmacol Ther 2013;139:359–391

8. Psichas A, Reimann F, Gribble FM. Gut chemosensing mechanisms. J Clin Invest 2015;125:908–917

9. Drucker DJ. Evolving concepts and translational relevance of enteroendocrine cell biology. J Clin Endocrinol Metab 2016;101:778–786

10. Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. Nature 2015;517:302–310

11. Mancini AD, Poitout V. GPR40 agonists for the treatment of type 2 diabetes: life after 'TAKing' a hit. Diabetes Obes Metab 2015;17:622–629

12. Habib AM, Richards P, Rogers GJ, Reimann F, Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. Diabetologia 2013;56:1413–1416
13. Kumar DP, Rajagopal S, Mahavadi S, et al. Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β cells. Biochem Biophys Res Commun 2012;427:600–605
14. Düfer M, Hörth K, Wagner R, et al. Bile acids acutely stimulate insulin secretion of mouse β-cells via farnesoid X receptor activation and K(ATP) channel inhibition. Diabetes 2012;61:1479–1489
15. Picone RP, Kendall DA. Minireview: From the bench, toward the clinic: therapeutic opportunities for cannabinoid receptor modulation. Mol Endocrinol 2015;29:801–813
16. Chu ZL, Jones RM, He H, et al. A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. Endocrinology 2007;148:2601–2609
17. Moran BM, Abdel-Wahab YH, Flatt PR, McKillop AM. Activation of GPR119 by fatty acid agonists augments insulin release from clonal β-cells and isolated pancreatic islets and improves glucose tolerance in mice. Biol Chem 2014;395: 453–464
18. Soga T, Ohishi T, Matsui T, et al. Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan G-protein-coupled receptor. Biochem Biophys Res Commun 2005;326:744–751
19. Lauffer LM, Iakoubov R, Brubaker PL. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes 2009;58:1058–1066
20. Overton HA, Fyfe MC, Reynet C. GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity. Br J Pharmacol 2008; 153(Suppl. 1):S76–S81
21. Ritter K, Buning C, Halland N, Pöverlein C, Schwink L. G protein-coupled receptor 119 (GPR119) agonists for the treatment of diabetes: recent progress and prevailing challenges. J Med Chem 2016;59:3579–3592
22. Chu ZL, Carroll C, Alfonso J, et al. A role for intestinal endocrine cell-expressed GPR119 in glycemic control by enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. Endocrinology 2008;149: 2038–2047
23. Tamarina NA, Roe MW, Philipson L. Characterization of mice expressing Ins1 gene promoter driven CreERT recombinase for conditional gene deletion in pancreatic β-cells. Islets 2014;6:e27685
24. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. J Biol Chem 2003; 278:471–478
25. Maida A, Hansotia T, Longuet C, Seino Y, Drucker DJ. Differential importance of glucose-dependent insulinotropic polypeptide vs glucagon-like peptide 1 receptor signaling for beta cell survival in mice. Gastroenterology 2009;137: 2146–2157
26. Flock G, Baggio LL, Longuet C, Drucker DJ. Incretin receptors for glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide are essential for the sustained metabolic actions of vildagliptin in mice. Diabetes 2007;56:3006–3013
27. Flock G, Holland D, Seino Y, Drucker DJ. GPR119 regulates murine glucose homeostasis through incretin receptor-dependent and independent mechanisms. Endocrinology 2011;152:374–383
28. Campbell JE, Ussher JR, Mulvihill EE, et al. TCF1 links GIPR signaling to the control of beta cell function and survival. Nat Med 2016;22:84–90
29. Mulvihill EE, Varin EM, Gladanac B, et al. Cellular sites and mechanisms linking reduction of dipeptidyl peptidase-4 activity to control of incretin hormone action and glucose homeostasis. Cell Metab 2017;25:152–165
30. Lan H, Vassileva G, Corona A, et al. GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis. J Endocrinol 2009;201:219–230
31. Overton HA, Babbs AJ, Doel SM, et al. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metab 2006;3:167–175
32. Ekberg JH, Hauge M, Kristensen LV, et al. GPR119, a major enteroendocrine sensor of dietary triglyceride metabolites coacting in synergy With FFA1 (GPR40). Endocrinology 2016;157:4561–4569
33. Fredriksson R, Höglund PJ, Gloriam DE, Lagerström MC, Schiöth HB. Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. FEBS Lett 2003;554:381–388
34. Wicksteed B, Brissova M, Yan W, et al. Conditional gene targeting in mouse pancreatic β-cells: analysis of ectopic Cre transgene expression in the brain. Diabetes 2010;59:3090–3098
35. Oropeza D, Jouvet N, Budry L, et al. Phenotypic characterization of MIP-CreERT1Lphi mice with transgene-driven islet expression of human growth hormone. Diabetes 2015;64:3798–3807
36. Ahlkvist L, Brown K, Ahrén B. Upregulated insulin secretion in insulin-resistant mice: evidence of increased islet GLP1 receptor levels and GPR119-activated GLP1 secretion. Endocr Connect 2013;2:69–78
37. Nunez DJ, Bush MA, Collins DA, et al. Gut hormone pharmacology of a novel GPR119 agonist (GSK1292263), metformin, and sitagliptin in type 2 diabetes mellitus: results from two randomized studies. PLoS One 2014;9:e92494
38. Katz LB, Gambale JJ, Rothenberg PL, et al. Pharmacokinetics, pharmacodynamics, safety, and tolerability of JNJ-38431055, a novel GPR119 receptor agonist and potential antidiabetes agent, in healthy male subjects. Clin Pharmacol Ther 2011;90:685–692
39. Segerstolpe Å, Palasantza A, Eliasson P, et al. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metab 2016;24:593–607
40. Benner C, van der Meulen T, Cacéres E, Tigyi K, Donaldson CJ, Huising MO. The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression. BMC Genomics 2014;15:620
41. Ku GM, Kim H, Vaughn IW, et al. Research resource: RNA-Seq reveals unique features of the pancreatic β-cell transcriptome. Mol Endocrinol 2012;26: 1783–1792
42. Hansen KB, Rosenkilde MM, Knop FK, et al. 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. J Clin Endocrinol Metab 2011;96: E1409–E1417