

## GPR119 Regulates Murine Glucose Homeostasis Through Incretin Receptor-Dependent and Independent Mechanisms

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G protein-coupled receptor 119 (GPR119) was originally identified as a  $\beta$ -cell receptor. However, GPR119 activation also promotes incretin secretion and enhances peptide YY action. We examined whether GPR119-dependent control of glucose homeostasis requires preservation of peptidergic pathways *in vivo*. Insulin secretion was assessed directly in islets, and glucoregulation was examined in wild-type (WT), single incretin receptor (IR) and dual IR knockout (DIRKO) mice. Experimental endpoints included plasma glucose, insulin, glucagon, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), and peptide YY. Gastric emptying was assessed in WT, *Glp1r*<sup>−/−</sup>, DIRKO, *Glp2r*<sup>−/−</sup>, and GPR119<sup>−/−</sup> mice treated with the GPR119 agonist AR231453. AR231453 stimulated insulin secretion from WT and DIRKO islets in a glucose-dependent manner, improved glucose homeostasis, and augmented plasma levels of GLP-1, GIP, and insulin in WT and *Gipr*<sup>−/−</sup> mice. In contrast, although AR231453 increased levels of GLP-1, GIP, and insulin, it failed to lower glucose in *Glp1r*<sup>−/−</sup> and DIRKO mice. Furthermore, AR231453 did not improve ip glucose tolerance and had no effect on insulin action in WT and DIRKO mice. Acute GPR119 activation with AR231453 inhibited gastric emptying in *Glp1r*<sup>−/−</sup>, DIRKO, *Glp2r*<sup>−/−</sup>, and in WT mice independent of the Y2 receptor (Y2R); however, AR231453 did not control gastric emptying in GPR119<sup>−/−</sup> mice. Our findings demonstrate that GPR119 activation directly stimulates insulin secretion from islets *in vitro*, yet requires intact IR signaling and enteral glucose exposure for optimal control of glucose tolerance *in vivo*. In contrast, AR231453 inhibits gastric emptying independent of incretin, Y2R, or Glp2 receptors through GPR119-dependent pathways. Hence, GPR119 engages multiple complementary pathways for control of glucose homeostasis. (*Endocrinology* 152: 0000–0000, 2011)

Enteroendocrine cells and islet  $\beta$ -cells share a number of physiological regulatory mechanisms. Most notably, basal hormone secretion is maintained at a constant but low rate in the absence of nutrient ingestion; however, food intake rapidly augments hormone secretion. Enteroendocrine cells communicate with and amplify  $\beta$ -cell function through secretion of peptide hormones. For example, glucose-dependent insulinotropic peptide (GIP) secreted by K cells in the duodenum and proximal jejunum acts as a potent incretin to enhance glucose-dependent insulin secretion (1). Similarly, a second incretin, glucagon-like peptide-1 (GLP-1), secreted from gut L cells, exerts pleiotropic actions on islet  $\beta$ -cells,

including stimulation of insulin biosynthesis and secretion and cell proliferation, promotion of cell survival, and restoration of  $\beta$ -cell glucose sensing (2).

The ability of incretin hormones to enhance  $\beta$ -cell survival, restore glucose sensing to poorly responsive diabetic  $\beta$ -cells, and control glycemia in a glucose-dependent manner has fostered the development of drugs that potentiate incretin action (3). Two new classes of therapeutic agents based on incretin action, GLP-1 receptor (GLP-1R) agonists and dipeptidyl peptidase-4 inhibitors, are now used to treat type 2 diabetes, (4). The success of these agents has engendered interest in discovery and characterization of

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Abbreviations: AUC, Area under the curve; Arg, arginine; DIRKO, dual IR knockout; DMSO, dimethyl sulfoxide; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GPCR, G protein-coupled receptor; GPR119, GPCR 119; IPGTT, ip glucose tolerance test; IR, incretin receptor; OEA, oleoylethanolamide; PACAP, pituitary adenylate cyclase-activating polypeptide; PEG, polyethylene glycol; PYY, peptide YY; WT, wild type; Y2R, Y2 receptor.

$\beta$ -cell G protein-coupled receptors (GPCRs) that function in an incretin receptor (IR)-like manner.

A large number of GPCRs expressed in islet  $\beta$ -cells are coupled to stimulation of glucose-dependent insulin secretion (5). One of these receptors, designated GPCR 119 (GPR119) or glucose-dependent insulinotropic receptor, has been independently isolated by multiple groups and characterized in different species (6–8). GPR119 is expressed in rodent islets and  $\beta$ -cell lines, and GPR119 activation enhances cAMP formation and stimulation of insulin secretion (7, 9–12). These findings established the initial concept that GPR119, expressed on islet  $\beta$ -cells, may be a promising target for diabetes drug development through mechanisms involving direct potentiation of insulin secretion (8).

More recent findings have expanded the spectrum of GPR119 action to encompass stimulation of gut hormone secretion. The synthetic GPR119 agonist AR231453 (13) increased circulating levels of GLP-1 and GIP in wild-type (WT) mice but not in GPR119 $^{-/-}$  mice (14). Moreover, GPR119 expression has been detected in normal L and K cells (15, 16) and in the GLUTag enteroendocrine L cell line (14, 16), and direct GPR119 activation in L cells stimulates cAMP formation and GLP-1 secretion (11, 14). Furthermore, GPR119 $^{-/-}$  mice exhibit attenuated nutrient-stimulated GLP-1 release (17). Hence, these observations imply that GPR119 activation may control glucose through multiple mechanisms, including direct activation of the  $\beta$ -cell, or indirectly, through potentiation of incretin secretion. Consistent with this hypothesis, partial blockade of GLP-1R signaling with exendin(9-39) attenuates GPR119-dependent improvement in oral glucose tolerance in normal mice (14).

More recent evidence has deepened the complexity of GPR119 mechanisms of action by implicating an essential role for peptide YY (PYY) as a downstream target required for the gastrointestinal and glucoregulatory actions of GPR119 (18). Accordingly, to determine the relative importance of gut hormones for the glucoregulatory and gastrointestinal actions of GPR119, we examined GPR119 activation using a selective GPR119 agonist in normal mice, isolated islets, and in mice with inactivation of gut hormone receptors. We also defined a potent effect of and specific pathways required for GPR119-dependent inhibition of gastric emptying. Our findings invoke a role for both direct  $\beta$ -cell action and the incretin axis in the transduction of glucoregulatory mechanisms pursuant to GPR119 activation.

## Research Design and Methods

### Animal experiments

Animal experiments were carried out according to protocols approved by the Mt. Sinai Hospital and the Toronto Centre for Phenogenomics Animal Care Committees. Male, age-matched,

C57BL/6 mice were purchased from Taconic (Hudson, NY) and were allowed to acclimatize to the animal facility for 1 wk before experimentation. Dual IR knockout (DIRKO), Glp2r $^{-/-}$ , Gpr $^{-/-}$ , and Glp1r $^{-/-}$  and littermate control male mice were on a C57BL/6 genetic background (19, 20). Mice were maintained on a 12-h light, 12-h dark cycle. GPR119 $^{-/-}$  mice were obtained from Arena Pharmaceuticals (San Diego, CA) (7).

### Glucose tolerance tests

Mice were fasted overnight, weighed, and fasting blood glucose levels were measured by tail vein sampling using a glucometer (Bayer, Toronto, Ontario, Canada). A single oral dose of 20 mg/kg AR231453 (GPR119 agonist; Arena Pharmaceuticals) or vehicle [80% polyethylene glycol (PEG)400, 10% Tween 80, and 10% ethanol] was given 30 min before oral or ip glucose (1.5 g/kg body weight). Blood glucose levels were measured by sampling from the tail vein of gently held conscious mice, from 5 to 120 min after glucose administration. At the 5-min time point, a blood sample (150  $\mu$ l) was collected and immediately mixed with 15  $\mu$ l of a chilled solution containing 5000 KIU/ml trasylol (Bayer), 32 mM EDTA, and 0.01 mM Diprotin A (Sigma, St. Louis, MO) and kept on ice for assessment of total GLP-1, total GIP, insulin, and PYY levels in plasma. At the 15-min time point, a second blood sample (50  $\mu$ l) was collected from each mouse in EDTA-coated tubes (Sarstedt, Montreal, Quebec, Canada) for measurement of glucagon. Plasma was obtained by centrifugation at 4 C and stored at  $-80$  C until determination of insulin (ultrasensitive mouse insulin ELISA; Alpco Diagnostics, Salem, NH), total GLP-1 (mouse/rat total GLP-1 assay kit; Mesoscale Discovery, Gaithersburg, MD), total GIP [rat/mouse GIP (total) ELISA kit; Millipore, Billerica, MA], PYY (ELISA kit; Alpco Diagnostics) (21), and glucagon (Millipore) levels.

### Insulin tolerance test

Ten-week-old age-matched C57BL/6 and DIRKO male mice were fasted for 5 h. A single dose of 1.2 U/kg of insulin (Humulin R; Eli Lilly, Toronto, Ontario, Canada) was administered by ip injection, and blood glucose was determined at 0, 15, 30, 60, and 90 min.

### Arginine (Arg) stimulation test

Ten-week-old age-matched C57BL/6 and DIRKO male mice were fasted overnight. A fasting blood sample was collected, and mice were given a single ip dose of Arg 1 mg/g (Sigma). A second blood sample was collected 5 min after Arg administration. Insulin was measured at fasting and 5 min after Arg administration (ultrasensitive mouse insulin ELISA; Alpco Diagnostics).

### Insulin secretion in mouse islets

Islets were prepared as described (22). After 2 h of incubation at 37 C, islets were handpicked into fresh medium and allowed to recover overnight. Islets with preserved architectural integrity were used for insulin secretion experiments. Batches of 10 islets were distributed into tubes, in triplicates, containing 0.5 ml Krebs-Ringer buffer containing 2.8 or 16.7 mM glucose, with or without vehicle [dimethyl sulfoxide (DMSO)], or the GPR119 agonist AR231453, a 300 nM solution prepared in DMSO (Arena Pharmaceuticals), exendin-4 (10 nM), forskolin (10  $\mu$ M), and pituitary adenylate cyclase-activating polypeptide (PACAP) (10 nM; Sigma). After incubation for 1 h at 37 C, medium was collected and stored at  $-20$  C for assessment of insulin secretion.

Islet insulin content was extracted by transfer of islets to cold acid-ethanol solution (70% ethanol and 0.18 M HCl). Extracts were briefly sonicated (10 sec), and insulin was measured by RIA (Linco rat insulin RIA catalog no. RI-13K; Linco, St. Louis, MO).

### Analysis of GPR119 expression in islets

First-strand cDNA was synthesized from total islet RNA using the SuperScript III reverse transcriptase synthesis system (Invitrogen, Carlsbad, CA) and random hexamers. Real-time PCR was performed with the ABI Prism 7900 Sequence Detection System using TaqMan Gene Expression Assay GPR119 (Mm00731497\_s1) and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). IRS2 (Mm03038438\_m1) was used as a control, and levels of mRNA transcripts were normalized to levels of 18S RNA in the same samples (Hs99999901\_s1).

### Gastric emptying

Liquid-phase gastric emptying was assessed using the acetaminophen absorption test (23, 24). C57BL/6, *Glp1r*<sup>−/−</sup>, DIRKO, GPR119<sup>−/−</sup>, and *Glp2r*<sup>−/−</sup> male mice, 10–12 wk of age, were fasted overnight and given a single dose of either AR231453 (20 mg/kg) or vehicle (80% PEG400, 10% Tween 80, and 10% ethanol) 30 min before oral administration of a solution of glucose 15% and acetaminophen 1% (Sigma) at a dose of 1.5 g/kg glucose–0.1 g/kg acetaminophen. Exendin-4 (1 μg, ip) was used as a positive control to demonstrate inhibition of gastric emptying. Tail vein blood (50 μl) was collected into heparinized tubes at 15 and 30 min after glucose/acetaminophen administration. Plasma was separated by centrifugation at 4°C and stored at −20°C until measurement of acetaminophen levels using an enzymatic-spectrophotometric assay (Diagnostic Chemicals Ltd., Oxford, CT).

### Statistical analysis

Results are presented as mean ± SD; area under the curve (AUC) values are ± SEM. Statistical significance was determined using ANOVA with Bonferroni *post hoc* tests or *t* tests (as appropriate) using GraphPad Prism 4.0 (GraphPad Software, Inc., La Jolla, CA). Statistical significance was noted when *P* < 0.05.

## Results

### GPR119 activation and glucose tolerance

Because plasma levels of GLP-1 and GIP increase after GPR119 activation (14), we assessed whether complete loss of incretin action diminishes the glucoregulatory actions of GPR119. The GPR119 agonist AR231453 and oral glucose were administered to WT, *Gipr*<sup>−/−</sup>, *Glp1r*<sup>−/−</sup>, and DIRKO mice (Fig. 1). AR231453 significantly reduced glucose excursions after oral glucose loading in WT and *Gipr*<sup>−/−</sup> mice (Fig. 1, A and B). In contrast, a detectable but nonsignificant reduction in glycemic excursion was observed after oral glucose loading and AR231453 treatment of *Glp1r*<sup>−/−</sup> and DIRKO mice (Fig. 1, C and D). To elucidate the importance of enteral *vs.* parenteral glucose exposure for the glucoregulatory actions of GPR119 agonists, we tested whether AR231453 improved ip glucose tolerance. Oral

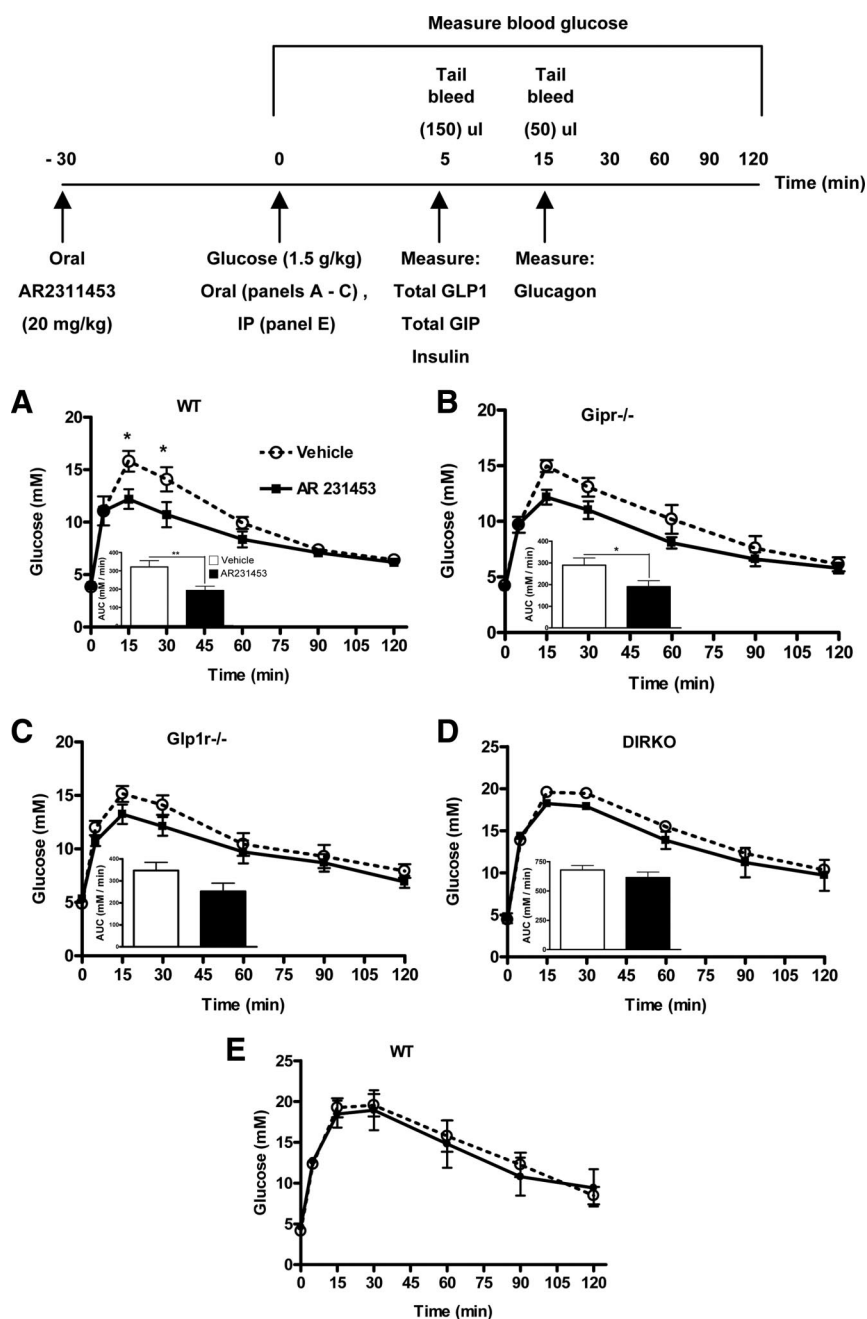
AR231453 administration failed to control glucose excursions in WT mice after ip glucose challenge (Fig. 1E). Plasma hormones assessed after ip glucose challenge revealed an increase in GLP-1 but no significant changes in circulating GIP, insulin, or glucagon (Supplementary Fig. 1, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>). Hence, GPR119 activation requires GLP-1R signaling for optimal control of oral glucose tolerance, and GPR119 activation in the absence of enteral glucose exposure is not sufficient for optimal control of glucose homeostasis.

### GPR119, incretin and islet hormones and insulin tolerance

To interpret the findings observed with WT and KO mice in Fig. 1, we assessed whether AR231453 regulates circulating levels of incretin and islet hormones to a similar extent in WT and IRKO mice. Plasma levels of total GIP were modestly increased by AR231453 in WT and *Gipr*<sup>−/−</sup> mice (Fig. 2A) and significantly increased in *Glp1r*<sup>−/−</sup> and DIRKO mice (Fig. 2A). In contrast, plasma levels of total GLP-1 were significantly increased by AR231453 in WT, *Gipr*<sup>−/−</sup>, *Glp1r*<sup>−/−</sup>, and DIRKO mice (Fig. 2B). Similarly, plasma insulin levels were significantly increased in WT, *Gipr*<sup>−/−</sup>, and *Glp1r*<sup>−/−</sup> mice after oral glucose and AR231453 administration (Fig. 2C). Although insulin levels were significantly lower in DIRKO compared with WT mice after oral glucose alone, reflecting defective β-cell function in DIRKO islets (19, 25, 26), AR231453 significantly increased plasma insulin levels in DIRKO mice, although to levels lower than those observed in WT or single IRKO mice (Fig. 2C). AR231453 had no effect on plasma glucagon levels in WT mice (Fig. 2D). However, basal glucagon levels were modestly lower in IRKO mice and increased significantly after oral glucose and AR231453 administration in *Gipr*<sup>−/−</sup>, *Glp1r*<sup>−/−</sup>, and DIRKO mice (Fig. 2D). Insulin sensitivity assessed by insulin tolerance testing was comparable in WT and DIRKO mice in the presence or absence of AR231453 (Fig. 3). Hence, GPR119 activation significantly increases GLP-1 levels in both single IRKO and DIRKO mice, and acute GPR119 activation increases plasma insulin levels despite the complete absence of IR action.

### GPR119 directly increases insulin secretion from isolated islets

We first assessed whether the diminished response of DIRKO mice to AR231453 reflects a selective inability of murine β-cells to respond to direct GPR119 activation and/or a generalized defect in secretagogue-regulated insulin secretion from DIRKO β-cells (19, 25, 26). Acute



**FIG. 1.** GPR119 activation and control of oral glucose tolerance in WT and IRKO mice. Age-matched male mice were fasted overnight, and AR231453 (20 mg/kg) or vehicle was administered orally 30 min before an oral glucose tolerance test (OGTT) (A–D) or ip glucose tolerance test (IPGTT) (E) (1.5 g/kg). Blood was collected from the tail vein at 5 min (150  $\mu$ l) and at 15 min (50  $\mu$ l) after glucose administration. Blood glucose values and AUC analysis during OGTT for (A) WT, (B) *Gipr*<sup>-/-</sup>, (C) *Gip1r*<sup>-/-</sup>, and (D) DIRKO mice treated with or without AR231453. Blood glucose values during IPGTT for WT mice treated with or without AR231453 (E). Statistical analysis was assessed by ANOVA. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . For vehicle-treated mice:  $n = 12$  (A),  $n = 8$  (B),  $n = 10$  (C), and  $n = 12$  (D); for AR231453-treated mice:  $n = 11$  (A),  $n = 8$  (B),  $n = 10$  (C),  $n = 12$  (D), and  $n = 10$ –12 (E).

administration of Arg significantly increased plasma insulin levels in both WT and DIRKO mice (Fig. 4A), demonstrating that DIRKO mice retain the ability to robustly respond to non-GPCR-mediated amino acid insulin secretagogues. One possibility that might explain altered glucoregulatory responses to AR231453 could be a change in

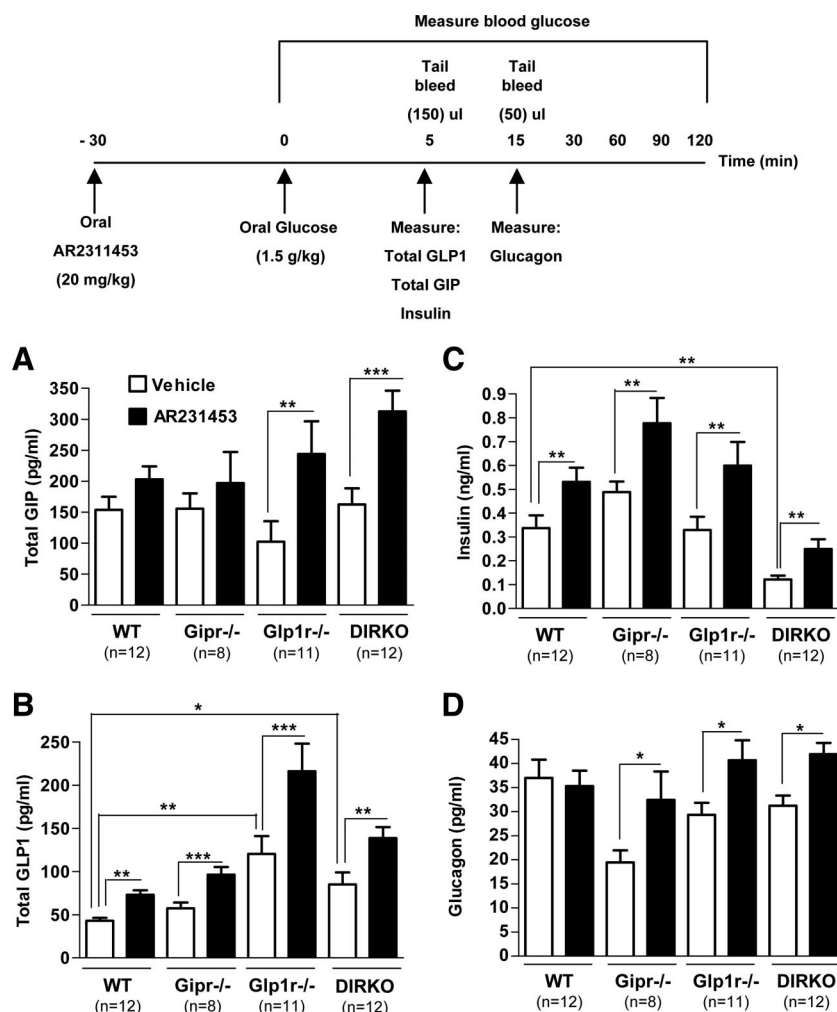
the relative level of GPR119 expression in single IR or DIRKO islets. However, no significant differences in the relative levels of GPR119 mRNA transcripts were observed in RNA from WT, *Glp1r*<sup>-/-</sup>, and *Gipr*<sup>-/-</sup> vs. DIRKO islets (Fig. 4B). As a control for assessment of gene expression in murine islets, we examined *Irs2*. In keeping with previous findings (22), levels of *Irs2* mRNA transcripts were significantly reduced in islet RNA from *Glp1r*<sup>-/-</sup> relative to *Gipr*<sup>-/-</sup> or WT mice (Fig. 4B). Similarly, consistent with the loss of GLP-1R activity, *Irs2* mRNA transcripts were also reduced in RNA from DIRKO islets (Fig. 4B).

We next investigated whether AR231453 directly stimulates insulin secretion from cultured murine islets *in vitro*. Glucose and PACAP significantly increased insulin secretion in both WT and DIRKO islets (Fig. 4C), whereas the GLP-1R agonist exendin-4 significantly increased insulin secretion in WT but not in DIRKO islets (Fig. 4C). AR231453 significantly increased insulin secretion in a glucose-dependent manner in both WT and DIRKO islets (Fig. 4C). Hence, these observations demonstrate that the diminished glucoregulatory response of DIRKO mice to GPR119 activation *in vivo* is not simply due to a generalized defect in the responsiveness of DIRKO  $\beta$ -cells to insulin secretagogues but likely reflects loss of incretin action in the context of GPR119 activation. Moreover, the inability of AR231453 to reduce glucose excursion during an ip glucose tolerance test (IPGTT) in WT mice is not explained by an inability of murine  $\beta$ -cells to directly respond to this GPR119 agonist.

#### GPR119 controls gut motility through mechanisms independent of the GLP-1R, GLP-2R, or Y2 receptor (Y2R)

Because GLP-1 regulates glucose homeostasis through control of gut motility (27, 28), and GPR119 activation robustly increases circulating levels of GLP-1 (Fig. 2), we examined gastric emptying after administration of





**FIG. 2.** GPR119 activation and plasma levels of GIP, GLP-1, insulin, and glucagon in WT and IRKO mice. Age-matched male mice were fasted overnight, and oral AR231453 (20 mg/kg) or vehicle was administered 30 min before an oral glucose tolerance test (OGTT). Plasma was obtained 5 min after oral glucose administration. This sample was used to simultaneously measure the levels of (A) total GIP immunoreactivity, (B) total GLP-1 immunoreactivity, (C) and insulin. A second plasma sample was collected from each mouse at 15 min after glucose for the measurement of glucagon levels (D) in WT, *Gipr*<sup>-/-</sup>, *Glp1r*<sup>-/-</sup>, and DIRKO mice. Statistical analysis was assessed by ANOVA. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

AR231453 in WT mice. AR231453 significantly reduced gastric emptying in WT mice (Fig. 5A). To determine whether the effects of AR231453 on gastric emptying were mediated through a GLP-1R-dependent mechanism, we repeated these studies in *Glp1r*<sup>-/-</sup> mice. Surprisingly, GPR119 activation continued to reduce gastric emptying in both *Glp1r*<sup>-/-</sup> and in DIRKO mice (Fig. 5A), suggesting that AR231453 controls gastric emptying through a mechanism that does not require the GLP-1R or GIP receptor.

Previous studies of AR231453 demonstrated that this molecule is a highly selective agonist that required the GPR119 receptor for control of glucose tolerance and insulin secretion (7). To assess whether the inhibitory actions of AR231453 on gastric emptying were also me-

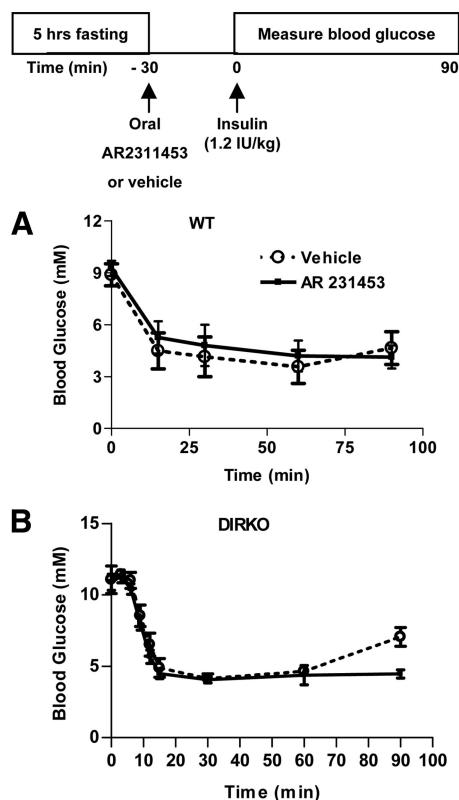
diated through GPR119, or reflected GPR119-independent mechanism(s) of action, we examined gastric emptying in *GPR119*<sup>-/-</sup> mice. The GLP-1R agonist exendin-4 potently reduced gastric emptying in both *GPR119*<sup>+/+</sup> and *GPR119*<sup>-/-</sup> mice (Fig. 5B). In contrast, AR231453 reduced gastric emptying in *GPR119*<sup>+/+</sup> but not in *GPR119*<sup>-/-</sup> mice (Fig. 5B). Hence, GPR119 transduces the actions of AR231453 on gastric emptying.

Because AR231453 likely increases plasma levels of the related L cell-derived peptide GLP-2, and GLP-2R activation may also inhibit the rate of gastric emptying (29), we assessed gastric emptying in *Glp2r*<sup>+/+</sup> and *Glp2r*<sup>-/-</sup> mice. AR231452 reduced gastric emptying in both *Glp2r*<sup>+/+</sup> and *Glp2r*<sup>-/-</sup> mice (Fig. 5C), demonstrating that the GLP-2R is not the sole dominant mechanism transducing the AR231453-dependent inhibition of gastric emptying (Fig. 5C).

GPR119 activation stimulates local release of PYY from isolated gut mucosa (18), and PYY also inhibits gastric emptying (30); hence, we examined the effect of acute GPR119 activation on plasma PYY. AR231453 administration before oral glucose loading significantly increased plasma PYY in WT mice (Fig. 6A). Accordingly, we assessed whether the actions of AR231453 on gastric emptying were diminished by concomitant administration of the Y2R antagonist, BIIE0246, a reagent previously shown to abrogate the effects of PYY on gut motility (30). AR231453 significantly inhibited gastric emptying despite concomitant administration of BIIE0246 (Fig. 6B), suggesting that the GPR119-mediated control of gut motility is not mediated through the Y2R.

## Discussion

Studies of the biological activity of GPR119 using synthetic receptor agonists initially focused on activity of this receptor in islet cells, demonstrating potent stimulation of insulin release from  $\beta$ -cells *in vitro*, and in rodents *in vivo* (7). The complexity of GPR119 biology was further expanded to encompass gut peptide action, because phar-



**FIG. 3.** GPR119 activation does not modify glucose profiles in an insulin tolerance test (ITT) in WT and DIRKO mice. WT ( $n = 10$ ) and DIRKO ( $n = 8$ ) age-matched mice were fasted for 5 h, and oral AR231453 (20 mg/kg) or vehicle was administered 30 min before ip insulin administration (1.2 U/kg). Blood glucose values during ITT for (A) WT and (B) DIRKO mice.

macological GPR119 activation also increased levels of both GIP and GLP-1 in mice and rats (11, 12, 14, 17). Furthermore, endogenous GPR119 appears essential for maximal GLP-1 secretion, because *GPR119*<sup>−/−</sup> mice exhibited reduced levels of circulating GLP-1 after nutrient or glucose ingestion (17). These findings raised the possibility that one or both incretin peptides contribute to the glucose lowering actions of GPR119 in the postprandial state.

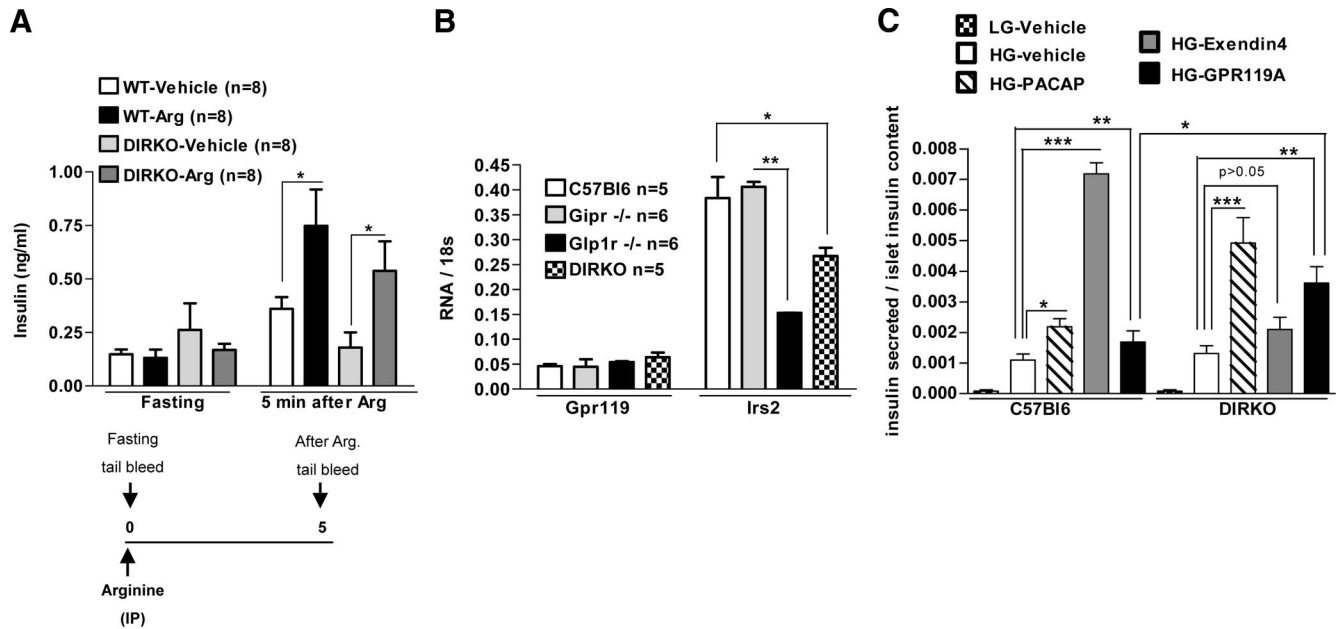
We have now examined the relative importance of three distinct components of GPR119 action that might contribute to the glucoregulatory actions of GPR119 agonists, namely 1) incretin peptides, 2) a direct insulinotropic role for GPR119 in islets, and 3) the control of gastric emptying. Our data are consistent with a model invoking all three mechanisms as contributing to the totality of GPR119 action *in vivo*. We demonstrate that although the glucoregulatory actions of AR231453 are diminished in DIRKO mice, AR231453 continues to significantly increase plasma insulin levels concomitant with oral glucose loading in DIRKO mice. Hence, an intact incretin axis is not required for the insulinotropic actions of GPR119 agonists *in vivo*. The failure of AR231453 to lower gly-

cemic excursion in DIRKO mice despite increasing insulin levels may be explained in part by the lack of GIP and GLP-1 action together with increased plasma levels of glucagon observed in the same experiments.

DIRKO mice exhibit modest defects in  $\beta$ -cell proliferation and impaired up-regulation of insulin gene expression and insulin secretion in response to high fat feeding (19); hence, one explanation for the relatively reduced insulinotropic response to AR231453 in DIRKO mice invokes a potential defect rendering DIRKO islets unable to respond adequately to GPR119 or other insulin secretagogues (19). Several lines of evidence argue against this interpretation of the data. First, GPR119 expression was comparable in RNA isolated from WT, single IRKO, and DIRKO islets. Furthermore, Arg briskly increased plasma insulin levels in both WT and DIRKO mice *in vivo*. Moreover, DIRKO islets exhibited a significant insulin secretory response to 1) AR231453 and 2) PACAP *in vitro*. Hence, the available data clearly demonstrate that GPR119 activation likely promotes insulin secretion via two complementary mechanisms: a direct  $\beta$ -cell effect and by activation of the incretin axis.

Interpretation of data from IRKO mice must be also tempered by the realization that these mice exhibit compensatory adaptations in levels of circulating incretins and in incretin responsivity (31, 32). Indeed, we observed a more robust induction of plasma GIP levels with AR231453 in the absence of a functional GLP-1R and increased plasma levels of GLP-1 in *Glp1r*<sup>−/−</sup> and DIRKO mice. These findings are consistent with previous studies suggesting that enteroendocrine cells regulate peptide hormone secretion in response to ambient levels of circulating gut hormones (33). Nevertheless, as previous (34) and more recent (35) studies have demonstrated that classical antagonists used to disrupt GLP-1 action, such as exendin(9-39), may not be completely specific for the GLP-1R, the use of mice with genetic disruption of IR genes provides a valuable complementary model for assessing the importance of IR signaling for glucoregulatory mechanisms activated by engagement of the GPR119 receptor.

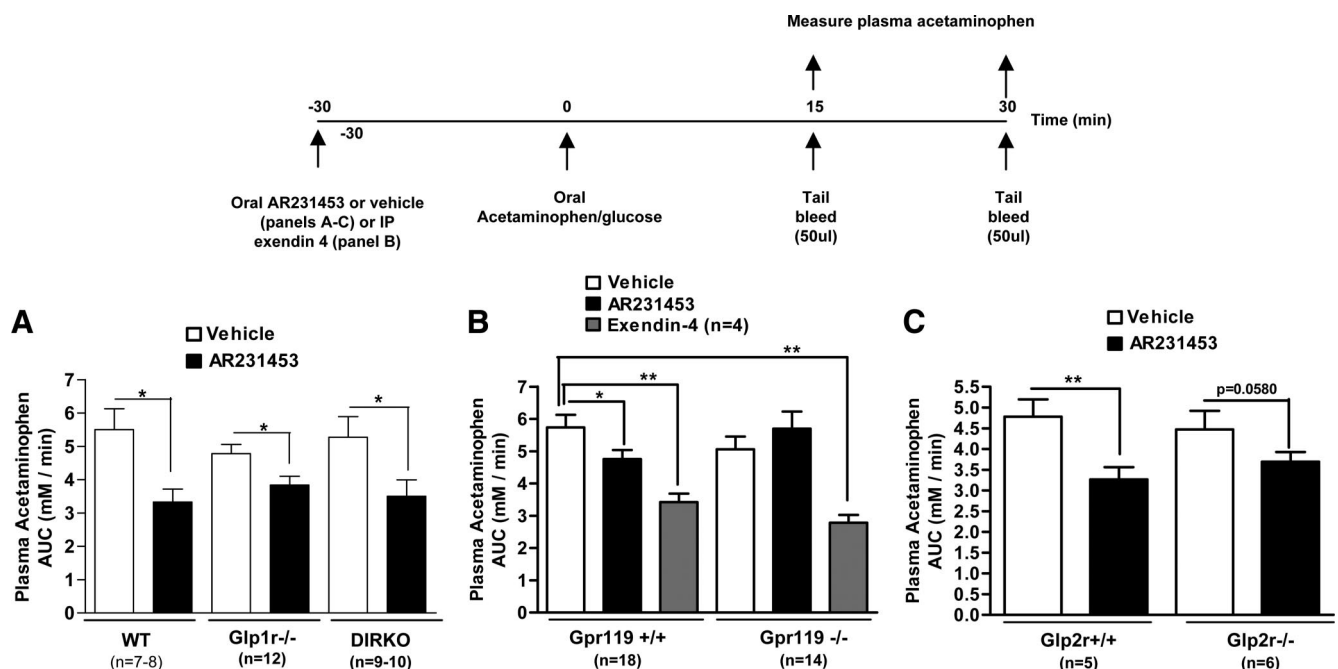
Previous studies of GPR119 action have not addressed whether glucagon contributes to the glucoregulatory properties observed with GPR119 agonists. GPR119 has been predominantly localized to  $\beta$ -cells, and in one report to PP cells (36), but not  $\alpha$ -cells. Although AR231453 had no effect on levels of plasma glucagon in WT mice, circulating glucagon levels were surprisingly increased after AR231453 administration in single IRKO and DIRKO mice. Basal GLP-1R signaling is known to tonically inhibit glucagon secretion, and even transient blockade of the GLP-1R using the antagonist exendin(9-39) increases lev-



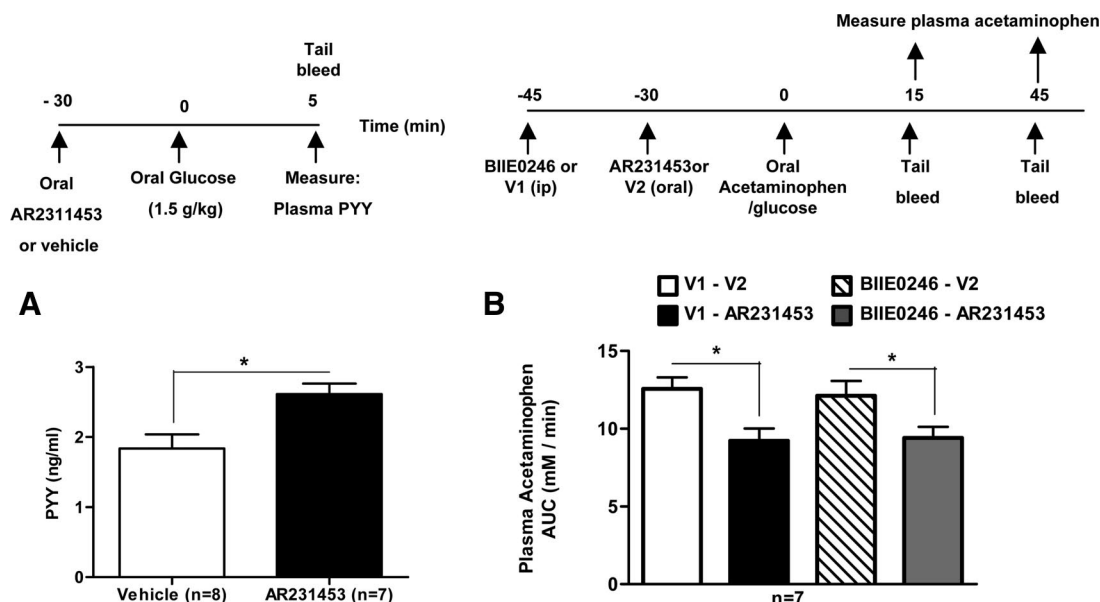
**FIG. 4.** GPR119 activation increases insulin secretion from WT and DIRKO islets. **A**, Arg stimulation increases plasma insulin levels in C57BL/6 and DIRKO mice *in vivo*. Mice were fasted overnight, plasma was collected to assess fasting insulin levels, and a single ip dose of either Arg (1 mg/g) or vehicle was administered at time zero. Blood was collected from the tail vein 5 min later for determination of plasma insulin levels. **B**, GPR119 and *Irs2* gene expression in WT and IRKO islets. GPR119 and *Irs2* mRNA levels were measured by real-time PCR in islets isolated from age-matched WT, *Gipr*<sup>-/-</sup>, *Glp1r*<sup>-/-</sup>, and DIRKO mice and normalized to levels of 18s RNA in the same samples. **C**, Insulin secretion from WT and DIRKO islets. Islets were isolated from 10-wk-old C57BL/6 and DIRKO mice and incubated under conditions of low glucose (LG) (2.5 mM) for 1 h. Islets were then incubated in low glucose or high glucose (HG) (16.6 mM) and treated for 1 h with either vehicle (DMSO), PACAP, exendin-4, or AR231453. Statistical analysis was assessed by ANOVA. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

els of plasma glucagon after oral glucose challenge in normal and diabetic subjects (37, 38). Hence, it is possible that loss of the suppressive component of GLP-1 action on

$\alpha$ -cells in *Glp1r*<sup>-/-</sup> and DIRKO mice unmasks a previously undetected action of GPR119, directly or indirectly, on the  $\alpha$ -cell.



**FIG. 5.** Gastric emptying in WT, *Glp1r*<sup>-/-</sup>, DIRKO, *Gpr119*<sup>-/-</sup>, and *Glp2r*<sup>-/-</sup> mice. Age-matched littermate WT and KO male mice were fasted overnight and given a single dose of AR231453 (20 mg/kg) or (A–C) vehicle, or (B) exendin-4 (1  $\mu$ g) before oral administration of a solution of glucose 15% and acetaminophen 1% at a dose of 1.5 g/kg glucose–0.1 g/kg acetaminophen. Gastric emptying rate was determined as described in *Research Design and Methods*. AUC for plasma acetaminophen levels in (A) WT, *Glp1r*<sup>-/-</sup>, and DIRKO mice, (B) *Gpr119*<sup>+/+</sup> and *Gpr119*<sup>-/-</sup> mice, and (C) *Glp2r*<sup>+/+</sup> and *Glp2r*<sup>-/-</sup> mice. Statistical analysis was assessed by ANOVA and paired *t* test (as appropriate); \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .



**FIG. 6.** GPR119 activation increases plasma PYY but inhibits gastric emptying independent of the Y2R. Age-matched male mice were fasted overnight and oral AR231453 (20 mg/kg) or vehicle was administered 30 min before an oral glucose tolerance test (OGTT). Blood samples were collected 5 min after glucose for the measurement PYY plasma levels (A). For gastric emptying (B), 10-wk-old C57Bl6 male mice were fasted overnight. At time zero, a single ip dose of either vehicle (V1 = 1.2% DMSO in water) or BIIE0246 (2 mg/kg) was administered 15 min before a single oral dose of either vehicle (V2 = 80% PEG400, 10% Tween 80, and 10% ethanol) or AR231453 (20 mg/Kg). A solution of glucose (15%)-acetaminophen (1%) was administered orally 30 min later at a dose of 1.5 g/kg glucose-0.1 g/kg acetaminophen, and blood samples were collected at 15 and 45 min for assessment of plasma acetaminophen levels as described in *Research Design and Methods*. Rate of appearance of acetaminophen in plasma as determined by the AUC (B). Statistical analysis was assessed by *t* test and ANOVA; \*, *P* < 0.05.

Nevertheless, there is very little data demonstrating that endogenous GIP action is critical for control of glucagon secretion, and it remains unclear how loss of the *Gipr* also leads to enhanced glucagon secretion after GPR119 activation in the *Gipr*<sup>−/−</sup> mouse. Hence, further exploration of the connection between GPR119 signal transduction and mechanisms regulating  $\alpha$ -cell secretion appears warranted.

Considerable previous experimentation supports the hypothesis that oleoylethanolamide (OEA), an endogenous fatty acid derivative, functions as an endogenous agonist at the GPR119 receptor (8, 11). Intriguingly, GPR119 expression has been detected in the central nervous system, and OEA reduces food intake (8), raising the possibility that GPR119 functions as a component of a satiety circuit controlling body weight. Similarly, a small molecule GPR119 agonist, PSN632408, also suppressed food intake and reduced body weight in rodents (8). More recent findings illustrate that the satiety effects of OEA are complex and require cluster of differentiation 36 and peroxisome proliferator-activated receptor  $\alpha$  (39). Moreover, OEA retains its anorectic actions in *Gpr119*<sup>−/−</sup> mice (17). In contrast, we found no effect of GPR119 activation on food intake using a selective GPR119 agonist in WT, single IRKO, or DIRKO mice (data not shown). These findings are consistent with the normal body weight previously reported for *Gpr119*<sup>−/−</sup> mice (7, 17) and suggest that pharmacological activation or genetic disruption of

GPR119 does not produce a phenotype linked to disordered control of energy homeostasis.

Because GPR119 activation significantly increases proglucagon-derived peptide secretion from the gut L cell and GLP-1, oxyntomodulin and GLP-2 all inhibit gastric emptying (2), we hypothesized that enhanced L-cell secretion might be associated with reduced gastric emptying. Surprisingly, however, there is little previous data examining whether selective GPR119 activation controls gastric emptying. We found that AR231453 significantly inhibited gastric emptying in WT mice. Unexpectedly, AR231453 also inhibited gastric emptying in *Glp1r*<sup>−/−</sup>, DIRKO, and *Glp2r*<sup>−/−</sup> mice, demonstrating that the inhibitory effect of GPR119 agonists on gut motility is not strictly dependent on the GLP-1, GIP, or GLP-2 receptors. Nevertheless, the ability of AR231453 to reduce gastric emptying was lost in *Gpr119*<sup>−/−</sup> mice, emphasizing that the inhibition of gastric emptying does not reflect an “off target” mechanisms of action of AR231453 and clearly requires a functional GPR119 signaling system. Consistent with our findings, the GPR119 ligand OEA also inhibits gastric emptying in mice, through poorly understood mechanisms independent of peroxisome proliferator-activated receptor  $\alpha$  or cannabinoid receptors (40). Moreover, consistent with our findings using AR231453 in *Glp1r*<sup>−/−</sup> mice, the effects of OEA on gut motility were not diminished by coadministration of the GLP-1R antagonist exendin(9-39) (40).



More recent experimentation has delineated a role for PYY as a downstream target for GPR119 regulation in the gastrointestinal mucosa. GPR119 activation using the agonist PSN632408 inhibited epithelial electrolyte secretion in a PYY-dependent and Y1 receptor-dependent manner (18). Unexpectedly, the glucoregulatory and insulinotropic actions of PSN632408 were also attenuated in *Pyy*<sup>−/−</sup> mice. Our data extend these findings by demonstrating that AR231453 increased plasma levels of PYY in association with enteral glucose loading *in vivo*. Nevertheless, although exogenous PYY is known to inhibit gastric emptying (41), the inhibitory effects of AR231453 on gastric emptying were not diminished by coadministration of the Y2R antagonist BIIE0246. Because multiple gut peptides inhibit gastric emptying, and many of these (GLP-1, GLP-2, oxyntomodulin, PYY, *etc.*) are downstream targets of GPR119, simply inhibiting the action of a single peptide is unlikely to completely reverse the inhibition of gastric emptying observed with GPR119 agonists. Hence, the precise mediators and mechanisms coupling GPR119 activation to control of gut motility require further study.

In summary our data illustrate that GPR119 controls enteral glucose tolerance through at least three distinct yet complementary mechanisms. First, activation of the  $\beta$ -cell GPR119 directly enhances insulin secretion in isolated murine islets independent of IRs. Moreover, we identify a role for GPR119 in the control of gastric emptying that is not completely dependent on the GLP-1R, GLP-2R, or Y2R. Finally, we demonstrate that functional IRs are required for maximal control of oral glucose tolerance after GPR119 activation. Taken together, it seems likely that multiple enteroendocrine cell-derived peptides simultaneously contribute to the glucoregulatory signals and control of gastric emptying emanating from GPR119 activation in the gut. These findings may have implications for optimization of therapeutic strategies and the safety of employing GPR119 agonists for the treatment of type 2 diabetes.

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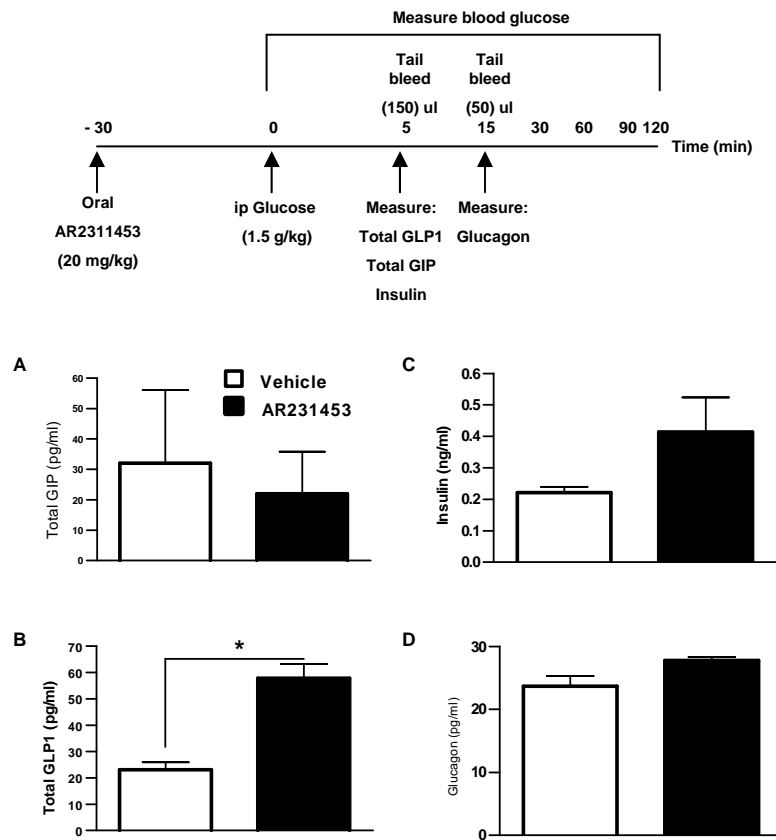
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## Supplemental Figure



Supplemental Figure 1 GPR119 activation and plasma levels of GIP, GLP-1, insulin, and glucagon in WT mice during an intraperitoneal glucose tolerance test (IPGTT)

Age matched WT mice were fasted over night and oral AR231453 (20 mg/kg) or vehicle was administered 30 min before ip glucose load (OGTT) (1.5g/kg). Plasma was obtained 5 minutes after glucose administration. This sample was used to simultaneously measure the levels of total GIP immunoreactivity (A), total GLP-1 immunoreactivity (B) and insulin (C). A second plasma sample was collected from each mouse at 15 min after glucose for the measurement of glucagon levels (D). Statistical analysis was assessed by ANOVA. \*p<0.05;