Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-α in mice

A. Maida · B. J. Lamont · X. Cao · D. J. Drucker

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Abstract

Aims/hypothesis Metformin is widely used for the treatment of type 2 diabetes. Although it reduces hepatic glucose production, clinical studies show that metformin may reduce plasma dipeptidyl peptidase-4 activity and increase circulating levels of glucagon-like peptide 1 (GLP-1). We examined whether metformin exerts glucoregulatory actions via modulation of the incretin axis.

Methods Metformin action was assessed in Glp1r−/−, Gipr−/−, Glp1r:Gipr−/−, Pparα (also known as Ppara)−/− and hyperglycaemic obese wild-type mice with or without the GLP-1 receptor (GLP1R) antagonist exendin(9-39). Experimental endpoints included glucose tolerance, plasma insulin levels, gastric emptying and food intake. Incretin receptor expression was assessed in isolated islets from metformin-treated wild-type and Pparα−/− mice, and in INS-1 832/3 beta cells with or without peroxisome proliferator-activated receptor (PPAR)-α or AMPK-activated protein kinase (AMPK) antagonists.

Results In wild-type mice, metformin acutely increased plasma levels of GLP-1, but not those of gastric inhibitory polypeptide or peptide YY; it also improved oral glucose tolerance and reduced gastric emptying. Metformin significantly improved oral glucose tolerance despite loss of incretin action in Glp1r−/−, Gipr−/− and Glp1r−/−:Gipr−/− mice, and in wild-type mice fed a high-fat diet and treated with exendin(9-39). Levels of mRNA transcripts for Glp1r, Gipr and Pparα were significantly increased in islets from metformin-treated mice. Metformin directly increased Glp1r expression in INS-1 beta cells via a PPAR-α-dependent, AMPK-independent mechanism. Metformin failed to induce incretin receptor gene expression in islets from Pparα−/− mice.

Conclusions/interpretation As metformin modulates multiple components of the incretin axis, and enhances expression of the Glp1r and related insulinotropic islet receptors through a mechanism requiring PPAR-α, metformin may be mechanistically well suited for combination with incretin-based therapies.

Keywords Dipeptidyl peptidase-4 · GIP · GLP-1 · Islets

Abbreviations

AICAR Aminoimidazole carboxamide ribonucleotide
AMPK AMP-activated protein kinase
DPP-4 Dipeptidyl peptidase-4
GIP Gastric inhibitory polypeptide
GLP-1 Glucagon-like peptide 1
GLP1R GLP-1 receptor
HBSS Hanks’ balanced salt solution
LKB1 Liver kinase B1
PPAR Peroxisome proliferator-activated receptor
Introduction

The biguanide metformin is the most commonly used agent in initial management of type 2 diabetes mellitus [1]. Although the mechanisms through which metformin exerts glucose-lowering actions are incompletely understood, treatment of diabetic patients with metformin reduced hepatic glucose production, primarily via decreased gluconeogenesis [2]. The molecular mechanisms mediating the actions of metformin on hepatic glucose production remain incompletely understood and are thought to involve engagement of the AMP-activated protein kinase (AMPK) pathway and its upstream kinase, liver kinase B1 (LKB1) [3, 4]. Important roles for the cyclic AMP response element binding protein [5] and the transcription factor Kruppel-like factor 15 [6] have been proposed as molecular targets for the glucoregulatory actions of metformin. Understanding of metformin’s mechanism of action is further complicated by the fact that it inhibits hepatic glucose production independently of AMPK and LKB1 [7, 8].

Metformin and glucagon play opposing roles in the control of hepatic glucose production. As type 2 diabetes mellitus is characterised by increased levels of glucagon and enhanced hepatic gluconeogenesis [9], there is considerable interest in reducing glucagon action in type 2 diabetes mellitus. Incretin-based therapies, the glucagon-like peptide 1 (GLP-1) receptor agonists and the dipeptidyl peptidase-4 (DPP-4) inhibitors, exert their actions in part via suppression of glucagon secretion from islet alpha cells [10]. Both GLP-1 receptor (GLP1R) agonists and DPP-4 inhibitors are widely used in combination with metformin, further amplifying the effects of these combinations on suppression of hepatic glucose production.

Unexpectedly, assessment of DPP-4 activity in plasma or serum from rodents or humans treated with metformin often reveals reduced DPP-4 activity [11–14]. In contrast, metformin does not directly inhibit DPP-4 activity in vitro [15, 16]. Moreover, the biguanide compounds, metformin, phenformin and buformin, significantly increased plasma GLP-1 in normal rats and in F344/DuCrj rats with genetic DPP-4 deficiency, strongly suggesting that metformin increases GLP-1 levels independently of effects on DPP-4 activity [16]. These observations are consistent with human studies demonstrating that a single dose of metformin increases plasma levels of total, but not intact GLP-1 following oral glucose administration in non-diabetic patients [17]. Similarly, chronic therapy with metformin specifically increases plasma total GLP-1 levels in diabetic and non-diabetic participants [18].

Taken together, these observations suggest that metformin may exert some of its metabolic actions in part via amplification of incretin action. Accordingly, we examined the actions of metformin in wild-type and incretin receptor knockout mice. Our findings demonstrate that metformin increases plasma levels of GLP-1 and induces islet incretin receptor gene expression in vivo through a mechanism that is independent of AMPK, but dependent on peroxisome proliferator-activated receptor (PPAR)-α.

Methods

Peptides and reagents

Metformin was from MP Biomedicals (Solon, OH, USA). Aminoimidazole carboxamide ribonucleotide (AICAR) was from Toronto Research Chemicals (North York, ON, Canada), the AMPK activator 5-(3-(4-(2-(4-fluorophenyl)ethoxy)phenyl)propyl)furan-2-carboxylic acid and compound C were from Calbiochem (Gibbstown, NJ, USA). Acetaminophen, MK-886, pyruvate, Hanks’ balanced salt solution (HBSS), collagenase V, p-nitroaniline, aprotinin and diprotin A were from Sigma-Aldrich Canada (Oakville, ON, Canada). TRI reagent was from Molecular Research Center (Cincinnati, OH, USA). Exendin(9-39) was from Chi Scientific (Maynard, MA, USA).

Animals

All experiments were carried out with strict adherence to the Animal Protocols and Procedures approved by the Mount Sinai Hospital Animal Care Committee. C57BL/6 male mice (aged 10–14 weeks) were obtained from a colony originating from the Jackson Laboratory (Bar Harbor, ME, USA). For high-fat feeding studies, male C57BL/6 mice were fed a diet containing 58% of energy in fat, plus sucrose (D12331; Research Diets, New Brunswick, NJ, USA) for 10 to 12 weeks commencing at 8 weeks of age. Littermate Glp1r−/−, Gipr−/−, Glp1r−/−:Gipr−/− and corresponding wild-type mice on the C57BL/6 background were obtained by intercrossing Glp1r−/−:Gipr−/− mice and genotyped as described [19]. Pparaα (also known as Ppara)−/− mice were obtained from the Jackson Laboratory on the 129S4/SvJae background and backcrossed to the C57BL/6 background for seven generations before inter-breeding Pparaα−/− mice for experiments.

Assessment of metformin action in mice with disrupted incretin receptor signalling

Glucose tolerance and food intake experiments had a crossover design, where the same mice were given vehicle or test agents on separate occasions spaced at least 1 week apart. For acute administration, metformin was administered in methylcellulose (0.5% [wt/vol.] in distilled water). The AMPK activator 5-(3-(4-(2-(4-fluorophenyl)ethoxy)
Glucose and pyruvate tolerance in animals fed regular chow Mice were fasted for 6 h (07:30–13:30 hours) and then given metformin or vehicle (0.5% [wt/vol.] methylcellulose) 1 h prior to glucose or pyruvate administration. For OGTT, a solution containing glucose (2 g/kg for regular chow-fed mice, 1 g/kg for mice on high-fat diet) and acetaminophen (0.1 g/kg) was administered orally. For pyruvate tolerance tests, pyruvate, 2 g/kg, was dissolved in PBS and administered i.p. Blood glucose was sampled via tail vein using a glucometer (Bayer, Toronto, ON, Canada). For measurement of plasma hormones, blood was collected into EDTA-coated tubes and acetaminophen (0.1 g/kg) was administered orally. For pyruvate tolerance tests, pyruvate, 2 g/kg, was dissolved in PBS and administered i.p. Blood glucose was sampled via tail vein using a glucometer (Bayer, Toronto, ON, Canada). For measurement of plasma hormones, blood was collected into EDTA-coated tubes containing aprotinin and diprotin A at final concentrations of 500 kIU/ml and 1 μmol/l, respectively.

Food intake Singly housed, overnight-fasted mice were given metformin or vehicle orally 1 h prior to re-feeding and food weight was recorded at the indicated time-points.

Plasma hormones and acetaminophen Plasma insulin, peptide YY (both from Alpco Diagnostics, Salem, NH, USA) and gastric inhibitory polypeptide (GIP) (Millipore, Billerica, MA, USA) were determined by ELISA. Total GLP-1 was determined using an electrochemiluminescent sandwich immunoassay (Mesoscale Scale Discovery, Gaithersburg, MD, USA). Plasma acetaminophen was measured with an enzymatic assay (Genzyme Diagnostics, Charlottetown, PE, Canada). Plasma DPP-4 activity was determined by enzymatic assay [20].

The effect of metformin on islet incretin receptor expression

Subchronic metformin administration in mice Metformin was administered in the drinking water at a dose of 300 mg/kg daily for 48 h. Water consumption and food intake were monitored and did not differ between mice treated with metformin and those treated by control. At 4 h prior to killing by CO₂ asphyxiation mice were fasted and given an oral dose of metformin (75 mg/kg). Islets were isolated from a cohort of mice as described below. Pancreatic RNA was isolated from the splenic tail and stored at −80°C until further analysis. Duodenal or colonic segments were flushed with saline, snap-frozen and stored at −80°C until RNA isolation.

Islet isolation After mice were killed by CO₂ asphyxiation, the pancreas was inflated via the pancreatic duct with collagenase type V (0.7 mg/ml in HBSS), excised and digested at 37°C for 10 to 15 min. The digest was washed twice with cold HBSS containing 0.25% wt/vol BSA and islets were separated using a Histopaque density gradient. The interface containing islets was removed, washed with HBSS + BSA and islets further purified by hand-picking using a light microscope. Islets were washed once with PBS prior to lysis and RNA isolation with a kit (RNeasy Mini; Qiagen, Mississauga, ON, Canada).

INS-1 832/3 cell culture This clonal beta cell line was a kind gift from C. Newgard (Department of Pharmacology and Cancer Biology, Duke University, Raleigh, NC, USA). Cells were seeded in six-well plates at 2×10⁶ cells/well 48 h prior to experiments. After a 6 h serum starvation period in the presence of RPMI media containing 5 mmol/l glucose and 0.1% (wt/vol.) BSA, fresh medium was added containing 5 or 20 mmol/l glucose with or without metformin or AICAR. Inhibitors or vehicle (DMSO) were added 15 min prior to metformin or AICAR treatment. Cells were washed twice with PBS prior to lysis with TRI reagent. To determine the effect of chronic metformin treatment on incretin-stimulated insulin secretion, cells were seeded in 24-well plates at 0.5×10⁶ cells/well 48 h prior to experiments. Serum-free medium was added containing 5 mmol/l glucose and 0.1% (wt/vol.) BSA with or without metformin (1 mmol/l) and cells were incubated at 37°C for 24 h prior to assessment of insulin secretion as described previously [21]. Levels of secreted insulin were normalised to insulin content per well and expressed relative to high glucose.

RNA isolation and cDNA synthesis RNA was isolated from mouse tissues and INS-1 cells using TRI reagent. First-strand cDNA was synthesised from total RNA using a reverse transcriptase synthesis system (SuperScript III; Invitrogen, Carlsbad, CA, USA) and random hexamers. Real-time PCR was performed with a sequence detection system (ABI Prism 7900; Applied Biosystems, Foster City, CA, USA) using gene expression assays andUniversal PCR Master Mix (TaqMan; Applied Biosystems). Levels of mRNA transcripts were normalised to peptidyl-propyl isomerase A (cyclophilin) for murine tissues and general transcription factor II B for rat INS-1 832/3 cells.

Statistical analysis

Results are presented as mean ± SEM. Statistical significance was determined using one-way or two-way ANOVA with Bonferroni post hoc tests or t tests (as appropriate) and GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA). In experiments with a crossover design, paired or
repeated measures analysis was employed. Statistical significance was noted as given at \( p < 0.05 \).

**Results**

Assessment of metformin action in mice with disrupted incretin receptor signalling

As metformin increases plasma levels of GLP-1 in humans and rats [16, 17], we determined whether oral dosing of metformin without concomitant glucose administration influenced plasma incretin levels in mice. Metformin significantly increased plasma GLP-1 at all doses compared with vehicle alone (Fig. 1a), with levels of total GLP-1 after the 300 mg/kg metformin dose similar to that achieved with enteral glucose administration (Fig. 1a). In contrast, levels of plasma GIP were unchanged or significantly decreased after metformin (Fig. 1b). Similarly, plasma levels of peptide YY, co-secreted with GLP-1 from subsets of L cells, remained unchanged after metformin administration (Electronic supplementary material [ESM] Fig. 1a).

As metformin exerts multiple metabolic actions via AMPK [3], we assessed whether pharmacological AMPK ab

Fig. 1 Metformin regulates plasma incretin levels, glucose tolerance and gastric emptying in mice. a Levels of plasma GLP-1 and (b) GIP were measured in wild-type mice that had fasted for 6 h and received the following orally: vehicle (black squares; 0.5% [wt/vol.] methylcellulose), metformin (black circles, 75 mg/kg; white triangle, 150 mg/kg; black triangle, 300 mg/kg) or glucose at \( t = 0 \) (white diamonds, 2 g/kg). c Metformin or vehicle alone, given by gavage, as above (a, b) or exendin-4 (white diamonds, 3 \( \mu \)g i.p.) were administered 1 h prior to OGTT. d Mean AUC glucose for each treatment. V, vehicle; E, exendin-4. e Levels of plasma insulin and (f) plasma acetaminophen, a marker of gastric emptying, were quantified during the OGTT, with (g) AUC for acetaminophen. Grey bars (e), 0 min; black bars, 10 min. h Metformin or vehicle, key as above (a, b), was administered 1 h prior to administration of pyruvate (at \( t = 0 \)) and blood glucose levels were determined as an indirect assessment of hepatic glucose production, with AUC glucose (i). *\( p < 0.05 \), **\( p < 0.01 \) and ***\( p < 0.001 \) vs vehicle; \( n = 5 \) mice.
activation increased plasma GLP-1. Intraperitoneal injection of AICAR produced a robust elevation in plasma GLP-1 (15 vs 113 pg/ml, p<0.001) and a significant increase in plasma GIP (60 vs 116 pg/ml, p<0.05 for vehicle vs AICAR) (ESM Fig. 1b, c). The orally active AMPK activator 5-(3-(4-(2-(4-fluorophenyl)ethoxy)phenyl)propyl)furan-2-carboxylic acid [22] tended to increase levels of plasma total GLP-1 (p=0.07), but not of total GIP, to a lower degree relative to AICAR (ESM Fig. 1b, c). Doses of metformin (75–300 mg/kg) and AICAR that increased plasma levels of GLP-1 had no effect on plasma DPP-4 activity (ESM Fig. 1d).

To determine the extent to which metformin and GLP1R agonists share overlapping actions, we acutely administered metformin during an OGTT in wild-type mice. Metformin and the GLP1R agonist exendin-4 significantly reduced the AUC of glucose (Fig. 1c, d), without changes in plasma insulin levels (Fig. 1e). As GLP1R activation improves oral glucose tolerance via a mechanism independent of insulin and involving inhibition of gastric emptying [23], we assessed gastric emptying via measurement of plasma acetaminophen [24]. Metformin (75 and 300 mg/kg) significantly reduced the rate of gastric emptying (Fig. 1f, g). We next examined the effect of metformin on plasma glucose levels following pyruvate administration, an indirect assessment of hepatic glucose production. At the two lower doses that increased plasma GLP-1 and inhibited gastric emptying, metformin had no consistent effect on glucose appearance after pyruvate (Fig. 1h, i); however, at the highest dose (300 mg/kg), metformin significantly reduced pyruvate-induced glycaemic excursions (Fig. 1h, i).

We next ascertained whether the glucoregulatory actions of metformin required intact GLP-1 receptor signalling. The GLP1R antagonist exendin(9-39) impaired oral glucose tolerance in wild-type mice (ESM Fig. 2a–c). Furthermore, even the 75 mg/kg dose of metformin, which did not affect pyruvate tolerance, continued to lower blood glucose in the presence of exendin(9-39) (ESM Fig. 2b–c). Metformin did not affect OGTT plasma insulin levels in the presence or absence of exendin(9-39) (ESM Fig. 2d). In contrast, the GLP1R antagonist diminished the ability of metformin to reduce gastric emptying (ESM Fig. 2e).

As exendin(9-39) does not completely eliminate GLP-1 action, we tested the acute glucoregulatory actions of metformin in mice with genetic disruption of one or both incretin receptors. Metformin (75 mg/kg) significantly reduced glycaemic excursions following enteral glucose challenge; this happened independently of the GLP1R or GIP receptor (Fig. 2a–h). Levels of plasma insulin (Fig. 2i) and insulin:glucose ratios (ESM Fig. 3) were comparable in vehicle vs metformin-treated wild-type littermate, *Glp1r<sup>−/−</sup>, Gipr<sup>−/−</sup>* and *Glp1r<sup>−/−</sup>;Gipr<sup>−/−</sup>* mice. While gastric emptying was slightly increased in *Glp1r<sup>−/−</sup>* and *Glp1r<sup>−/−</sup>;Gipr<sup>−/−</sup>* mice vs corresponding wild-type controls, it was inhibited by metformin in these groups; unexpectedly, metformin failed to reduce gastric emptying in *Gipr<sup>−/−</sup>* mice (Fig. 2j). Plasma glucagon was reduced post-glucose administration independent of the presence of metformin or the GLP1R (ESM Fig. 4). Although metformin and GLP1R agonists such as exendin-4 are capable of reducing food intake (ESM Fig. 5), metformin significantly reduced food intake in a GLP1R-independent manner (Fig. 3).

To study the glucoregulatory actions of metformin under conditions of moderate insulin resistance and hyperglycaemia [25], we assessed glucose tolerance in wild-type C57BL/6 mice after 12 weeks on a high-fat diet (Fig. 4a). Fasting blood glucose was significantly increased in mice on a high-fat diet (12.3 vs 8.4 mmol/l, p<0.001 for high-fat diet vs regular chow-fed animals, respectively). In the same group, metformin significantly reduced glycaemic excursions (Fig. 4b, c), without changes in plasma insulin levels (Fig. 4d). Interruption of GLP1R signalling with exendin(9-39) alone increased the amplitude of glycaemic excursions; however, metformin continued to reduce glycaemic excursions in exendin(9-39)-treated mice on a high-fat diet (Fig. 4b, c). Metformin also tended to inhibit gastric emptying in the presence of the GLP1R antagonist; however, further experimentation is required to definitively determine whether GLP-1 contributes to the inhibitory effect of metformin on gastric emptying (Fig. 4c).

The effect of metformin on islet incretin receptor expression

As metformin upregulates the expression of incretin receptors in an islet cell line in vitro [26], we assessed whether metformin modulates pancreatic or islet incretin receptor expression in murine islets. Metformin-treated mice (Fig. 5a) had significantly increased levels of *Gipr* but no change in levels of *Glp1r, Pparα* or insulin mRNA transcripts in RNA from whole pancreas (Fig. 5b). To ascertain the effects of metformin on gene expression in the endocrine pancreas, we isolated islets from metformin-treated mice. *Glp1r*, *Gipr* and *Pparα*, but not insulin mRNA transcripts were significantly upregulated in islets from metformin-treated mice (Fig. 5c). To ascertain whether metformin exerted a direct effect on islet beta cells, we incubated INS-1 832/3 cells with or without metformin at 5 or 20 mmol/l glucose. Metformin had no direct effect on *Gipr* mRNA transcripts (Fig. 5d). However, a significant induction of *Glp1r* and *Pparα* mRNA transcripts was detected in metformin-treated INS-1 832/3 cells at both glucose concentrations (Fig. 5d). In contrast, the AMPK activator AICAR reduced levels of *Gipr* and *Glp1r* mRNA transcripts in INS-1 832/3 cells (Fig. 5e, f).
To delineate mechanism(s) mediating the actions of metformin on islet incretin receptors, we treated INS-1 832/3 cells with the AMPK inhibitor, compound C. Consistent with the inability of AICAR to induce Glp1r expression, inhibition of AMPK activity had no effect on the induction of Glp1r mRNA by metformin (Fig. 5e) and reduced basal levels of Gipr mRNA transcripts (Fig. 5f). Although AICAR increased expression of Pparα in an AMPK-dependent manner in INS-1 832/3 cells, the inducing effects of AICAR on Pparα were independent of PPAR-α activity (Fig. 5g). In contrast, the PPAR-α inhibitor MK-886 prevented induction of the Glp1r (Fig. 5e) and Pparα (Fig. 5g) mRNA transcripts by metformin. We next examined whether metformin treatment altered the sensitivity of INS-1 832/3 cells to incretin-stimulated insulin secretion. Interestingly, the relative effects of GIP and GLP-1 on insulin secretion were significantly increased by metformin treatment (Fig. 5h).

The demonstration that metformin induces islet Glp1r gene expression in a PPAR-α-dependent manner in islet cells in vitro prompted us to assess the importance of PPAR-α for the actions of metformin on islet gene
expression in vivo. Metformin treatment of mice (Fig. 6a) increased expression of Gipr, Gccr, Cckar and Insr, and tended to increase Glp1r mRNA transcripts in islets isolated from \( Ppara^{+/+} \) mice (Fig. 6b); this occurred independently of changes in food intake, blood glucose or body weight (data not shown). In contrast, metformin failed to increase islet Glp1r, Gipr, Gcgr, Cckar and Insr mRNA transcripts in \( Ppara^{-/-} \) islets (Fig. 6b). Although metformin upregulated islet gene expression (Fig. 6b), no significant changes in expression of Gip, Cck, Pcs1 (also known as Pcsk1), proglucagon and peptide YY mRNA transcripts were observed in the upper or lower gastrointestinal tract of metformin-treated \( Ppara^{+/+} \) and \( Ppara^{-/-} \) mice (Fig. 6c, d).

**Discussion**

Our data demonstrate that metformin rapidly increases plasma levels of GLP-1, but not GIP or peptide YY, even in the absence of oral glucose administration. Given the potent actions of metformin on [1] suppression of hepatic glucose production and [2] peripheral glucose disposal, it is not...
surprising that elimination of incretin receptor signalling fails to eliminate the salutary effects of metformin on oral glucose tolerance in normal mice or mice on a high-fat diet. These findings demonstrate that incretin receptors do not represent the dominant mechanism for metformin action on oral glucose tolerance in this acute experimental setting. Moreover, incretin receptor knockout mice may exhibit a number of compensatory mechanisms that further complicate the study of incretin action in vivo [27]. Intriguingly, in the presence of the GLP1R antagonist exendin(9-39), metformin did not significantly reduce gastric emptying in moderately diabetic wild-type mice. Hence, we cannot rule
Fig. 5 Metformin upregulates incretin receptor expression in islet cells in a PPAR-α-dependent manner. Male C57BL/6 mice were treated with or without metformin in the drinking water as depicted (a). Gene expression was quantified by real-time PCR in RNA from pancreas (b) and freshly isolated islets (c) of metformin- (M) or vehicle (Veh)-treated mice. Transcript levels were expressed relative to levels in vehicle-treated mice. *p<0.05 and **p<0.01 metformin vs corresponding vehicle-treated groups. n=11–12 mice (b) and n=7–8 islet preparations from individual mice (c).

Fig. 6 Metformin regulates islet gene expression through a mechanism requiring PPAR-α. Male Pparα−/− and littermate Pparα+/− mice were administered drinking water containing metformin at 300 mg/kg daily or standard drinking water for 48 h. At 4 h prior to killing of mice food was removed from cages and mice were given a low dose of metformin (75 mg/kg) or vehicle (water) by gavage. Islets were isolated for quantification of gene expression by real-time PCR. Data were generated from n=6–8 individual islet preparations. *p<0.05 vs Pparα−/− vehicle; †p<0.06. e mRNA levels were quantified for Gcg, Gip, Cck and Pc1 in duodenum, and (d) for Gcg, Pyy and Pc1 in colon of vehicle vs metformin-treated Pparα−/− and littermate Pparα+/− mice. Light grey bars, metformin-treated Pparα−/−; dark grey bars, metformin-treated Pparα+/−; white bars, vehicle-treated Pparα−/−; black bars, vehicle-treated Pparα+/−. n=9–10 mice per group.

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YY, another enteroendocrine-derived peptide that is co-secreted with GLP-1 from subsets of L cells [30]. Hence, the molecular mechanisms linking metformin exposure to increased circulating levels of GLP-1 require further study.

The effects of metformin on islets have been somewhat contradictory, with some reports demonstrating that metformin inhibits insulin secretion [31], impairs glucose-responsivity and enhances susceptibility to apoptosis [32]. In contrast, metformin was also reported to improve insulin secretion, increase insulin gene expression and insulin content, and reduce islet apoptosis in human islets exposed to high glucose [33]. These discrepancies possibly reflect variation in experimental models and culture conditions, species-specific differences, doses of metformin and duration of metformin exposure, and require further study. In the second section of our study, we demonstrated that subchronic treatment of mice with metformin leads to enhanced expression of the Glp1r and Gipr genes in murine islets. Pan and colleagues indirectly implicated a role for AMPK in the actions of metformin on islet gene expression via their demonstration that AICAR mimicked the actions of metformin on Glp1r and Gipr mRNA transcripts in INS-1 cells [26]. In contrast, our data demonstrate that AICAR downregulates Glp1r and Gipr mRNA transcripts in INS-1 832/3 beta cells. Furthermore, the actions of metformin in increasing Glp1r mRNA transcripts were not diminished by the AMPK inhibitor compound C. Taken together, these findings strongly suggest that the inductive effects of metformin on Glp1r expression are not mediated through an AMPK-dependent pathway.

A role for PPAR-α in the control of Gipr gene expression has previously been reported in studies of INS (832/13) cells. Thus Lynn and colleagues demonstrated that the PPAR-α agonist WY14643 increased Gipr gene expression in BRIN-D11 beta cells and in rat islets [34]. Conversely, the PPAR-α antagonist MK-886 and a dominant negative Pparα cDNA reduced Gipr expression under conditions of low glucose in transfected INS(832/13) cells [34]. Our data demonstrate that PPARα is essential for the upregulating effects of metformin on multiple receptor genes thought to play a role in islet function, including Glp1r, Gipr, Gcgr, Cckar and Insr. Consistent with the observed changes in incretin receptor expression, metformin treatment moderately enhanced the relative insulinotropic response to GLP-1 and GIP in islet cells. Intriguingly, the related nuclear receptor, PPAR-γ, has recently been implicated in control of islet Gipr expression [35]. The precise molecular mechanisms through which metformin recruits PPAR-α to control islet gene expression require further elucidation.

In summary, we show here that metformin acutely and differentially increases plasma levels of GLP-1 but not those of GIP in mice. Moreover, metformin produces robust induction of islet gene expression and incretin responsiveness. Although the glucoregulatory actions of metformin on glucose tolerance and inhibition of food intake are not completely eliminated in incretin receptor knockout mice, the extent to which metformin exerts its metabolic actions in part through incretin receptors in more severely diabetic models that are resistant to suppression of hepatic glucose production merits further exploration. To resolve the question of whether chronic metformin therapy is associated with greater dependence upon the incretin axis for glucoregulation in type 2 diabetic human patients, more effective incretin antagonists would need to be developed for human use, and so this question remains unanswered. Taken together, our findings provide new insights into the relationship between metformin, plasma levels of incretins and the expression of islet receptors transducing the actions of incretins in vivo.

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Duality of interest D. J. Drucker consults for several companies developing incretin-based therapeutics as outlined in detail at www.glucagon.com/druckerlab.html. All other authors declare that there is no duality of interest associated with this manuscript.

References

Supplementary Figure 1
Supplementary Figure 2

A
OGTT timeline

-60 -45 0 10 120 mins
i.p. PBS / Ex-9 Oral Veh / Met Oral Glucose Insulin measure Insulin measure

B

Blood glucose (mM)

-60 -5 0 25 50 75 100 125
Time (mins)

- Con
- Met
- Ex-9
- Ex-9 + Met

C

AUC glucose

Con Met Ex-9 Ex-9 + Met

D

Plasma insulin (pmol/l)

0 10 0 10 0 10 0 10
Time (min)

Con Met Ex-9 Ex-9 + Met

E

Plasma acetaminophen (μM)

0 50 100 150 200 250
Con Met Ex-9 Ex-9 + Met

Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
Supplementary Figure 5