# PPAR$\beta/\delta$ Activation Induces Enteroendocrine L Cell GLP-1 Production

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**BACKGROUND & AIMS:** Glucagon-like peptide (GLP)-1, an intestinal incretin produced by L cells through proglucagon processing, is secreted after nutrient ingestion and acts on endocrine pancreas beta cells to enhance insulin secretion. Peroxisome proliferator-activated receptor (PPAR) $\beta/\delta$ is a nuclear receptor that improves glucose homeostasis and pancreas islet function in diabetic animal models. Here, we investigated whether PPAR$\beta/\delta$ activation regulates L cell GLP-1 production. **METHODS:** Proglucagon regulation and GLP-1 release were evaluated in murine GLUTag and human NCI-H716 L cells and in vivo using wild-type, PPAR$\beta/\delta$-null, and *ob/ob* C57Bl/6 mice treated with the PPAR$\beta/\delta$ synthetic agonists GW501516 or GW0742. **RESULTS:** PPAR$\beta/\delta$ activation increased proglucagon expression and enhanced glucose- and bile acid–induced GLP-1 release by intestinal L cells in vitro and ex vivo in human jejunum. In vivo treatment with GW0742 increased proglucagon messenger RNA levels in the small intestine in wild-type but not in PPAR$\beta/\delta$-deficient mice. Treatment of wild-type and *ob/ob* mice with GW501516 enhanced the increase in plasma GLP-1 level after an oral glucose load and improved glucose tolerance. Concomitantly, proglucagon and GLP-1 receptor messenger RNA levels increased in the small intestine and pancreas, respectively. Finally, PPAR$\beta/\delta$ agonists activate the proglucagon gene transcription by interfering with the $\beta$-catenin/TCF-4 pathway. **CONCLUSIONS: Our data show that PPAR$\beta/\delta$ activation potentiates GLP-1 production by the small intestine. Pharmacologic targeting of PPAR$\beta/\delta$ is a promising approach in the treatment of patients with type 2 diabetes mellitus, especially in combination with dipeptidyl peptidase IV inhibitors.**

*Key words:* GLP-1; PPAR$\beta/\delta$; Incretin; Diabetes.

BASIC–ALIMENTARY TRACT

Glucagon-like peptides (GLPs) and glucagon are hormones encoded by the same gene, proglucagon, each having different physiologic activities.[1] Due to alternative posttranslational processing, glucagon is predominantly produced in endocrine pancreatic alpha cells by prohormone convertase (PC)-2, whereas GLPs are predominantly produced in the intestine and also the brain after cleavage by PC-1/3.[2–4] Glucagon stimulates hepatic glucose production to maintain blood glucose levels on fasting[5] and inhibits insulin gene expression in pancreatic beta cells.[6] Intestinal L cell GLP-1 is primarily produced during the postprandial state to promote beta cell insulin secretion to decrease alpha cell glucagon secretion.[7]

The role of GLP-1 in the metabolic response to glucose ingestion has been established by several studies both in human and animal models. Acute administration of a GLP-1 antagonist leads to increased blood glucose levels in humans,[8] whereas GLP-1 receptor gene disruption in mice results in glucose intolerance.[9–11] The insulinotropic activity of GLP-1 is preserved in type 2 diabetes mellitus[12–14] but is reduced compared with healthy subjects.[14,15] Treatment of diabetic patients with GLP-1 increases meal-stimulated insulin levels and suppresses postprandial hyperglycemia without causing hypoglycemia.[16,17] However, circulating GLP-1 has a very short half-life due to inactivation by the enzyme dipeptidyl peptidase IV (DPP-4). The potential of GLP-1 for the treatment of patients with diabetes has led to the development of long-acting DPP-4–resistant GLP-1 analogues and orally bioavailable DPP-4 inhibitors, both approaches having proven efficacy in lowering blood glucose levels and hemoglobin $A_{1c}$ in patients with type 2 diabetes mellitus.[14,18] However, an alternative approach may be to increase endogenous GLP-1 production through modulation of proglucagon gene transcription in enteroendocrine L cells.

The proglucagon promoter contains several transcriptional control elements localized in the 2.5-kilobase 5′-upstream sequence of the transcriptional initiation site.[19] A number of transcription factors control proglucagon gene expression in a tissue-specific manner, allowing physiologically appropriate regulation of the production

***Abbreviations used in this paper:*** **DCA, deoxycholic acid; DMSO, dimethyl sulfoxide; DPP-4, dipeptidyl peptidase 4; GLP, glucagon-like peptide; IPGTT, intraperitoneal glucose tolerance test; LCA, lithocholic acid; OGTT, oral glucose tolerance test; PC, prohormone convertase; PLN2, perilipin 2; PPAR, peroxisome proliferator-activated receptor; siRNA, small interfering RNA.**


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of the different active peptides.[20] The transcription factor Pax6 activates proglucagon gene transcription in alpha cells by binding the G1 response element in the proglucagon promoter.[21,22] Disruption of the murine Pax6 gene not only markedly disrupts islet development but also selectively eliminates enteroendocrine cell populations in the intestine, including the subpopulation of GLP-1–producing cells, indicating the importance of Pax6 also in the control of intestinal proglucagon gene expression.[23] By contrast, the G2 response element of the proglucagon promoter appears involved in the tissue-specific regulation of proglucagon transcription because protein kinase C activation enhances transcription in alpha cells via this site[24] without influencing intestinal expression.[25] Moreover, insulin and GSK-3β inhibitors such as lithium stimulate G2 response element activity specifically in enteroendocrine L cells through the β-catenin/TCF-4 signaling pathway, resulting in increased GLP-1 production.[26,27]

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily that regulate fatty acid, cholesterol, and carbohydrate metabolism.[28] Whereas PPARα and PPARγ are the targets of the fibrates and glitazones, respectively, the PPARβ/δ isoform appears to be an interesting pharmacologic target for the treatment of patients with disorders associated with the metabolic syndrome.[29,30] PPARβ/δ-deficient mice challenged with a high-fat diet exhibit obesity[31] and glucose intolerance.[32] Furthermore, in vivo PPARβ/δ activation reduces body weight and lipid accumulation in adipose tissue and improves insulin sensitivity through increased skeletal muscle fatty acid oxidation.[31,32]

In this study, we examined the hypothesis that PPARβ/δ activation enhances GLP-1 production by L cells, which would contribute to the improvement of glucose tolerance in vivo. Our data identify intestinal tissue–specific regulation of proglucagon gene expression as a new mechanism of action for PPARβ/δ in glucose metabolism, thus expanding its interest as a target for the treatment of patients with type 2 diabetes mellitus.

## Materials and Methods

### *Chemicals and Reagents*

See the Supplementary Materials and Methods.

### *In Vitro Studies*

**Cell culture and treatment.** The mouse GLUTag L cell model was kindly provided by D. J. Drucker (University of Toronto, Toronto, Ontario, Canada). The rat INS1-E beta cells were given by K. Ravnskjaer (University of Southern Denmark, Odense M, Denmark). The human NCI-H716 L cell, (Cat. CCL 251) and murine α-TC1/9 alpha cells, (Cat. CRL 2350) were purchased from American Type Culture Collection (ATCC, Manassas, VA). For details, see Supplementary Materials and Methods.

**Transient transfection assays.** GLUTag L cells ($14 \times 10^4$ cells/cm$^2$) were transfected for 12 hours using Lipofectamine 2000 reagent (Invitrogen, Cergy Pontoise, France) in serum-free culture medium. For details, see Supplementary Materials and Methods.

**Gene reporter assays.** See Supplementary Materials and Methods.

**GLP-1 secretion assays.** GLUTag L cells were starved for 30 minutes in glucose-free Krebs/phosphate-buffered medium (120 mmol/L NaCl, 5 mmol/L KCl, 0.25 mmol/L $MgCl_2$, 0.5 mmol/L $CaCl_2$, and 2.2 mmol/L $NaHCO_3$, pH 7.2) supplemented with diprotin A 100 μmol/L and bovine serum albumin 0.1%. Cells were subsequently stimulated for 30 minutes with Krebs buffer in the presence or absence of glucose 5 mmol/L or a bile acid mix (50 μmol/L each; lithocholic acid [LCA] and deoxycholic acid [DCA]). The cell supernatants were centrifuged at 500*g* at 4°C for 5 minutes, and GLP-1 was measured with an enzyme-linked immunosorbent assay kit (EGLP-35K; Millipore, Billerica, MA) using Mithras technology (Berthold, Bad Wildbad, Germany).

**Western blot analysis.** See Supplementary Materials and Methods.

### *Ex Vivo Studies*

**Human islet isolation.** Human islet isolation and culture conditions were previously described.[33,34]

**Human jejunum tissue isolation.** Fresh human jejunum tissue was obtained with informed consent from obese patients undergoing gastric bypass surgery (gastro-jejunal derivation) and enrolled in the A Biological Atlas of Severe Obesity (ABOS) study (ClinicalTrials.gov; NCT01129297). For details, see Supplementary Materials and Methods.

### *Animal Models and Experimental Protocols*

**PPARβ/δ-deficient and *ob/ob* mice.** Six- to 8-week-old male PPARβ/δ-deficient,[35] obese *ob/ob*, and wild-type mice (Charles River Laboratories, Wilmington, MA), fed a chow diet (A03; UAR, France) and maintained in a temperature-controlled room (22°C) on a 12-hour light-dark cycle, were treated by gavage with vehicle or the PPARβ/δ synthetic agonists (GW0742 or GW501516) at the indicated doses and times. Plasma and several tissues including different intestinal sections (duodenum, jejunum, ileum, and colon) and pancreas were collected after 6 hours of fasting. All tissues were snap frozen in liquid nitrogen and stored at −80°C until RNA isolation. Animal care and experimental procedures were performed according to approved institutional guidelines (CEEA 02/2008).

**Oral and intraperitoneal glucose tolerance tests.** Oral (OGTT) and intraperitoneal (IPGTT) glucose tolerance tests were performed on mice fasted overnight. Sitagliptin was given orally at a dose of 25 mg/kg 45 minutes

before the glucose challenge (3 g/kg body wt). For details, see Supplementary Materials and Methods.

### *RNA Isolation and Quantification by Real-Time Quantitative Polymerase Chain Reaction*

RNA was extracted from cells and intestinal mucosa using TRIzol reagent (Life Technologies, Gaithersburg, MD). Mouse and human intestinal tissues and pancreas RNA were extracted using guanidinium thiocyanate/phenol/chloroform (Sigma, St Louis, MO). RNA extracts were treated with deoxyribonuclease I to eliminate contaminating genomic DNA. For details on RNA quantification, see Supplementary Materials and Methods.

### *Statistical Analysis*

Statistical analyses were performed using the unpaired Student *t* test. All data are expressed as mean ± SE. Statistically significant differences between treatments are reported to dimethyl sulfoxide (DMSO; control) and carboxymethylcellulose (vehicle) for in vitro and in vivo experiments, respectively. *P* values less than .05 were considered statistically significant.

## Results

### *Glucose Is a Positive Modulator of Proglucagon and PC-1/3 Gene Expression in GLUTag L Cells*

Glucose is the primary stimulus for GLP-1 secretion by enteroendocrine L cells. However, the transcriptional impact of glucose on proglucagon expression has not yet been reported. Time course analysis of gene expression in GLUTag L cells shows that proglucagon (Figure 1*A*) and PC-1/3 (Figure 1*B*) messenger RNA (mRNA) levels significantly increased on incubation with glucose compared with lactate. By contrast, PC-2 mRNA was not regulated by glucose within 12 hours of incubation, while both glucose and lactate tended to increase its expression after 24 hours (Figure 1*C*). Expression of GPBAR1 (also known as TGR5), the Gs-coupled membrane receptor that binds bile acids to increase GLP-1 secretion,[36–38] was detected in GLUTag L cells. GPBAR1 mRNA was not regulated by glucose (Figure 1*D*). The expression of the 3 PPAR isoforms was analyzed in GLUTag L cells. PPARβ/δ and PPARγ were expressed at high levels (Ct = 24 and Ct = 26, respectively), whereas PPARα was barely detectable (Ct ≥ 34). Glucose had no major effect on PPARβ/δ mRNA expression (Figure 1*E*). By contrast, PPARγ mRNA levels were significantly lowered by glucose (Figure 1*F*).

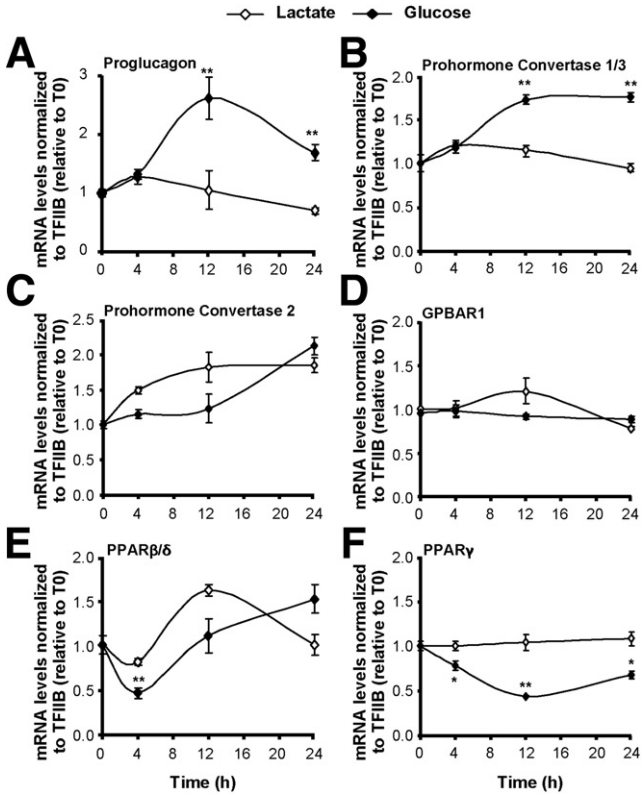


**Figure 1.** Glucose increases proglucagon and PC-1/3 and decreases PPARγ mRNA levels in GLUTag L cells. GLUTag L cells were starved for 12 hours in glucose-free medium and then stimulated with glucose (5 mmol/L) or lactate (10 mmol/L) as control for the indicated times. mRNA levels of (*A*) proglucagon, (*B*) PC-1/3, (*C*) PC-2, transmembrane bile acid receptor, (*D*) GPBAR1, (*E*) PPARβ/δ, and (*F*) PPARγ were quantified by real-time quantitative polymerase chain reaction using specific oligonucleotides. TFIIB expression was used as control. All real-time quantitative polymerase chain reaction experiments were performed in triplicate. Relative mRNA levels were calculated as fold induction relative to the corresponding control T0 time point, and statistically significant differences at each point are reported to lactate incubated GLUTag L cells. Student unpaired *t* test: $^{*}P < .05$; $^{**}P < .01$.

### *PPARβ/δ Activation in GLUTag L Cells Increases Proglucagon Expression and Improves Glucose- and Bile Acid–Induced GLP-1 Release*

Because PPARβ/δ and PPARγ are expressed in GLUTag L cells, it was next examined whether activation of these PPARs may regulate GLP-1 production. Surprisingly, PPARγ activation with rosiglitazone transiently decreased proglucagon mRNA levels (Supplementary Figure 1). By contrast, treatment with the PPARβ/δ agonist GW501516 increased proglucagon mRNA levels in a time-dependent manner, reaching a >2-fold increase after 24 hours (Figure 2*A*). Treatment of GLUTag L cells with GW0742, another PPARβ/δ agonist, also significantly increased proglucagon mRNA levels (Supplementary Figure 2*A*). Incubation of GLUTag L cells for 24 hours with increasing doses of GW501516 resulted in >4-fold elevated proglucagon mRNA levels at the highest tested dose (Figure 2*B*). To test whether this effect is mediated via PPARβ/δ, GLUTag L cells were transfected with PPARβ/δ small interfering RNA (siRNA) before incubation for 24 hours with GW501516. PPARβ/δ knockdown abolished the induction of proglucagon mRNA levels by GW501516 (Figure 2*C*). Western blot analysis

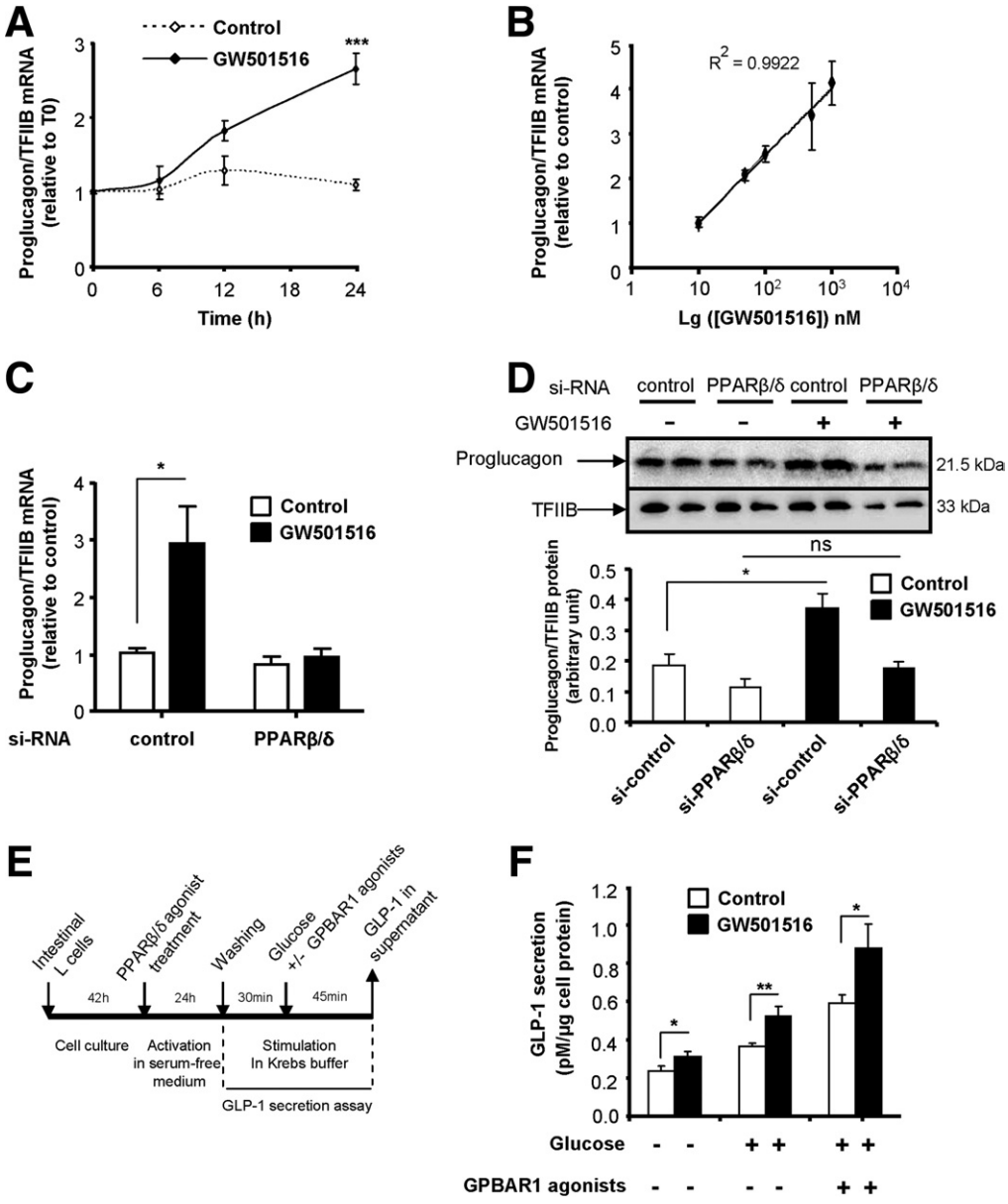


**Figure 2.** PPARβ/δ activation with GW501516 increases proglucagon mRNA levels and enhances glucose- and bile acid–induced GLP-1 release in GLUTag L cells. Proglucagon mRNA levels were quantified in cells treated with GW501516 (100 nmol/L) or DMSO (control) for (*A*) 6, 12, and 24 hours or for (*B*) 24 hours with increasing doses (10 to $10^3$ nmol/L). (*C* and *D*) The cells were transfected overnight with mouse PPARβ/δ siRNA or control and then treated or not for 24 hours with GW501516 (1 μmol/L). Proglucagon (*C*) mRNA and (*D*) protein levels were quantified by real-time quantitative polymerase chain reaction and Western blot analysis, respectively. (*E*) In vitro protocol to study the effects of PPARβ/δ activation on GLP-1 release by intestinal L cells. (*F*) GLUTag L cells were treated for 24 hours with GW501516 (1 μmol/L) or control (DMSO) and then stimulated for 30 minutes with glucose (5 mmol/L) or a combination of both glucose (5 mmol/L) and bile acid GPBAR1 agonists DCA and LCA (50 μmol/L). GLP-1 concentrations in supernatants were normalized to cell protein content.[53] Student unpaired *t* test: $^*P < .05$; $^{**}P < .01$; $^{***}P < .001$.

showed that proglucagon protein levels increased on treatment for 24 hours with GW501516, an effect blunted by PPARβ/δ silencing (Figure 2*D*). PC-1/3 mRNA levels were not influenced by PPARβ/δ agonist treatment (data not shown).

Next, it was determined whether the increased proglucagon expression due to PPARβ/δ activation influences GLP-1 production according to the protocol described in Figure 2*E*. Treatment for 24 hours with GW501516 enhanced GLP-1 secretion both in basal and on stimulation with glucose alone or in combination with GPBAR1 agonists (LCA and DCA) (Figure 2*F*). GLP-1 release also increased in response to glucose on treatment for 24 hours with the other PPARβ/δ agonist GW0742 (Supplementary Figure 2*B*).

### *PPARβ/δ Activation Increases Proglucagon mRNA Levels and Enhances GLP-1 Release by Human Enteroendocrine L Cells In Vitro and Ex Vivo*

Similar to GLUTag L cells, treatment of the human enteroendocrine NCI-H716 L cell line for 24 hours with GW501516 increased proglucagon mRNA (Figure 3*A*) and protein levels (Figure 3*B*) and enhanced GLP-1 secretion both in basal and on stimulation with glucose and GPBAR1 agonists (LCA and DCA) (Figure 3*C*). Ex vivo treatment for 24 hours with GW501516 of jejunal tissue obtained from obese patients after gastric bypass surgery significantly increased proglucagon mRNA levels (Figure 3*D*) and enhanced glucose- and bile acid–induced GLP-1 release (Figure 3*E*).

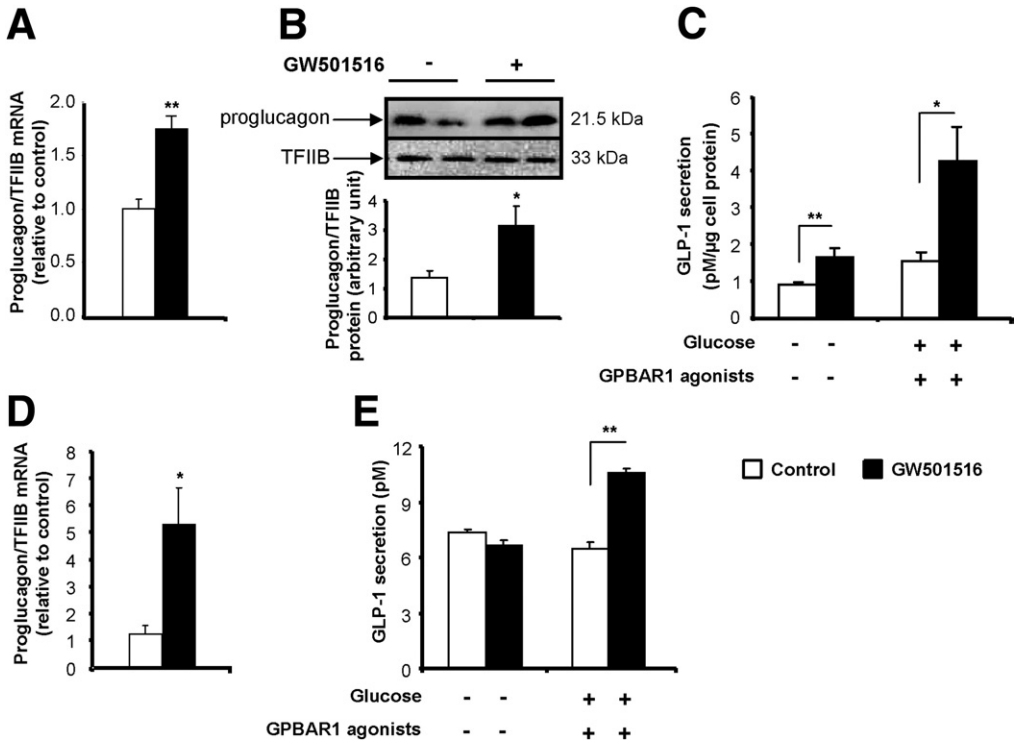


**Figure 3.** PPARβ/δ activation increases proglucagon expression and enhances glucose- and bile acid–induced GLP-1 release in human enteroendocrine L cells. Differentiated NCI-H716 L cells were treated for 24 hours with GW501516 (1 μmol/L) or DMSO (control). Proglucagon (*A*) mRNA and (*B*) protein levels were quantified by real-time quantitative polymerase chain reaction and Western blot analysis, respectively. (*C*) GLP-1 secretion assay was performed in supernatant from differentiated NCI-H716 L cells treated for 24 hours with GW501516 (1 μmol/L) or DMSO (control) and thereafter stimulated for 30 minutes with glucose (5 mmol/L) or a combination of both glucose (5 mmol/L) and bile acid GPBAR1 agonists DCA and LCA (50 μmol/L). (*D* and *E*) Fresh human jejunum tissue was exposed ex vivo for 24 hours to GW501516 (1 μmol/L) or DMSO (control). (*D*) Proglucagon mRNA levels and (*E*) GLP-1 glucose- and bile acid–induced GLP-1 release were measured by real-time quantitative polymerase chain reaction and GLP-1 secretion assay, respectively. Student unpaired *t* test: $^{*}P < .05$; $^{**}P < .01$.



### *PPARβ/δ Activation Induces Proglucagon Promoter Activity Through the β-Catenin/TCF-4 Signaling Pathway in GLUTag L cells*

To determine how PPARβ/δ regulates proglucagon transcription, the −350–base pair proglucagon-Luc reporter gene was transfected together with the PPARβ/δ expression vector in GLUTag L cells, which were subsequently treated with PPARβ/δ agonists. Treatment of transfected GLUTag L cells with GW501516 or GW0742 resulted in activation of proglucagon promoter activity, an effect enhanced by PPARβ/δ cotransfection (Figure 4*A*). Because lithium activates the −350–base pair proglucagon promoter in GLUTag L cells via the β-catenin/TCF-4 signaling pathway[26,27] and because PPARβ/δ activation has been shown to influence the β-catenin/TCF-4 signaling pathway,[39] it was determined whether the PPARβ/δ induction of proglucagon gene transcription in enteroendocrine L cells is mediated through the β-catenin/TCF/Lef signaling pathway. siRNA knockdown of TCF-4 abolished the GW501516-induced increase of proglucagon mRNA levels in GLUTag cells (Figure 4*B*). Moreover, siRNA knockdown of β-catenin in GLUTag L cells did not alter basal proglucagon mRNA levels but significantly reduced GW501516-induced proglucagon mRNA expression. In addition, overexpression of the constitutively active mutant β-catenin/S33Y significantly increased basal and GW501516-induced proglucagon mRNA expression (Figure 4*C*). Therefore, PPARβ/δ transcriptionally regulates enteroendocrine L cell proglucagon expression through stimulation of the β-catenin/TCF-4 pathway (Figure 4*D*).

### *Activation of PPARβ/δ Increases Intestinal Proglucagon mRNA Expression and GLP-1 Secretion in Response to an Oral Glucose Load in Mice*

To determine whether PPARβ/δ activation regulates proglucagon gene expression in vivo, C57Bl/6 mice were treated with synthetic PPARβ/δ agonists. GW0742 treatment resulted in a significant increase of proglucagon mRNA levels in the different parts of the small intestine (Figure 5 and Supplementary Figure 3, *top*). Measured as a marker of PPARβ/δ activity and a well-characterized PPARβ/δ target gene,[40,41] perilipin 2 (PLN2, also called ADRP) intestinal mRNA levels increased on GW0742 treatment measured (Supplementary

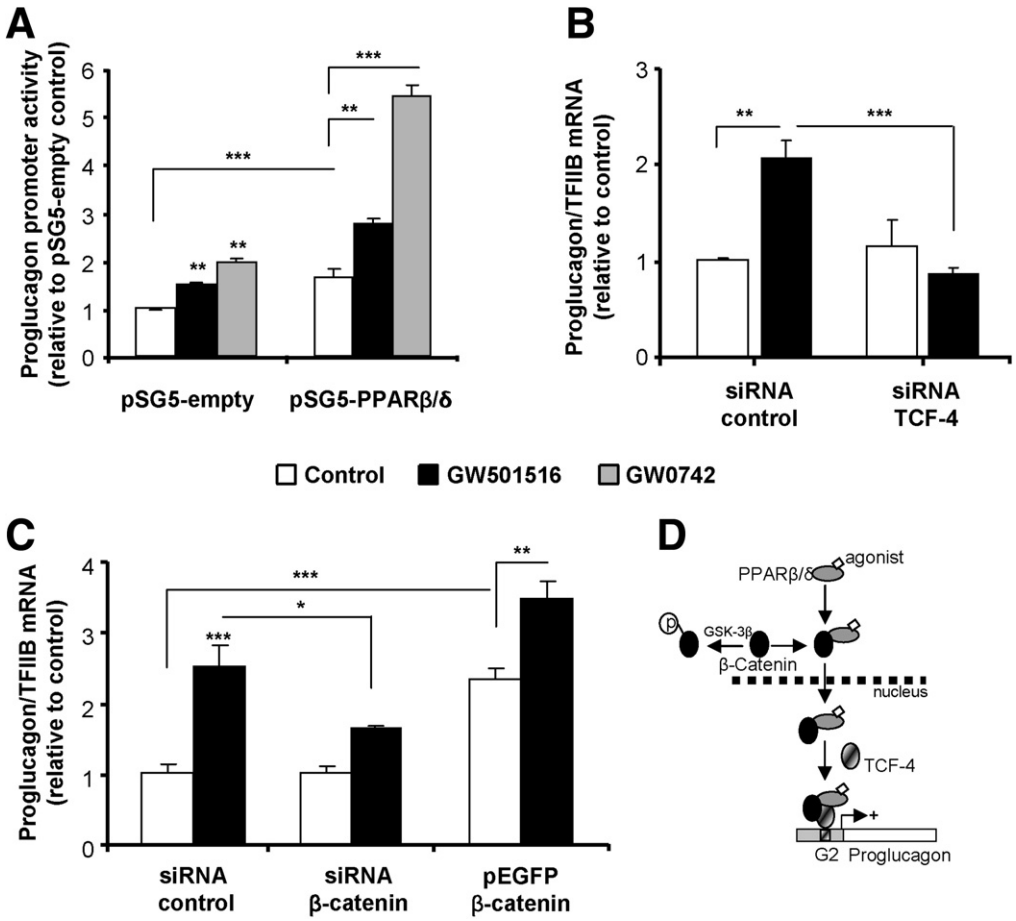


**Figure 4.** PPARβ/δ activation induces proglucagon promoter activity through the β-catenin/TCF-4 signaling pathway in GLUTag L cells. (*A*) Cells were cotransfected with −350–base pair proglucagon-Luc and pSG5-PPARβ/δ or empty pSG5 plasmids and then treated or not with GW501516 or GW0742 (1 μmol/L) for 24 hours. The values were normalized to pSG5-empty control, and the average of at least 3 independent experiments is presented. (*B* and *C*) Proglucagon mRNA levels were quantified in cells transfected with mouse TCF-4 siRNA vs control siRNA or with mouse β-catenin siRNA vs control siRNA or with the pEGFP/β-catenin/S33Y plasmid encoding nondegradable human β-catenin and then treated or not for 24 hours with GW501516 (1 μmol/L). All real-time quantitative polymerase chain reaction experiments were performed in triplicate. Statistically significant differences are reported to control. Student unpaired *t* test: $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. (*D*) Proposed mechanism of PPARβ/δ activation in positive regulation of GLP-1–producing L cell proglucagon gene expression.

Figure 3, *bottom*). This effect was mediated via PPARβ/δ, because proglucagon mRNA levels were not increased on GW0742 treatment of PPARβ/δ-deficient mice (Figure 5). A similar increase of proglucagon mRNA levels was observed in the jejunal mucosa on GW501516 treatment (Figure 6*A*, *left panel*). However, proglucagon mRNA levels were not regulated in the pancreas (Figure 6*A*, *center panel*) or in αTC-1/9 cells (Supplementary Figure 4*A*) or isolated human islets (Supplementary Figure 4*B*), whereas PLN2 or CTP-1α, analyzed as markers of PPARβ/δ activity, were induced. These data indicate that the proglucagon gene regulation by PPARβ/δ activation is restricted to the intestine. By contrast, GLP-1 receptor mRNA levels increased both in the pancreas of GW501516-treated mice (Figure 6*A*, *right panel*) and in isolated human islets (Supplementary Figure 4*B*). This up-regulation may enhance the response to the increased intestinal GLP-1 production. By contrast, treatment of rat INS1-E pancreatic beta cells with GW51516 did not influence RGLP-1 mRNA, whereas CPT-1 mRNA levels increased (Supplementary Figure 4*C*). This suggests that the effects of PPARβ/δ activation on RGLP-1 gene regulation in the pancreas are likely due to indirect mechanisms requiring paracrine signaling.

To investigate whether PPARβ/δ activation enhances the incretin effect in vivo, treated and control wild-type mice were challenged with a glucose load. GW501516-treated mice displayed an increase of plasma GLP-1 (Figure 6*B*) and insulin concentrations (Figure 6*C*) 15 minutes after the oral glucose gavage, associated with an improved glucose tolerance (Figure 6*D*). Indeed, the glycemic integrative area under the curve ($iAUC_{glucose}$) was reduced by 25% ($P < .05$) after PPARβ/δ activation (Figure 6*D*, *inset*). These results suggest that PPARβ/δ activation may improve glucose homeostasis, at least in part, through enhancing the GLP-1 pathway.

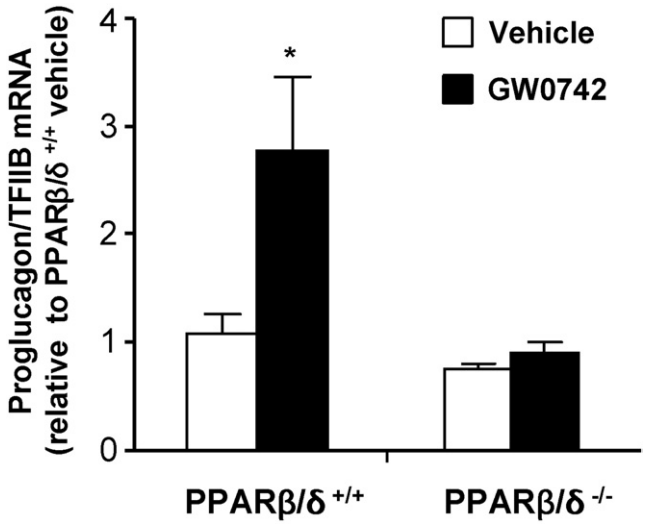


**Figure 5.** The increase of intestinal proglucagon mRNA levels by GW0742 is PPARβ/δ dependent. Wild-type (PPARβ/$\delta^{+/+}$) and PPARβ/δ-deficient (PPARβ/$\delta^{-/-}$) male mice (n = 4–5 animals per group) were treated or not (vehicle) during 5 days with GW0742 at 10 mg/kg per day. The ileal mucosa was scraped and proglucagon mRNA levels were quantified by real-time quantitative polymerase chain reaction. Student unpaired *t* test: *$P < .05$.

BASIC–ALIMENTARY TRACT

### *Activation of PPARβ/δ Enhances Intestinal GLP-1 Release, Restores Pancreas Responsiveness to Insulin Secretion, and Reduces Postprandial Glycemia in* ob/ob *Mice*

The pathophysiologic relevance of the enhanced oral glucose–induced GLP-1 secretion on PPARβ/δ activation was examined in insulin-resistant obese mice. Treatment of *ob/ob* mice with GW501516 resulted in a significant increase of proglucagon as well as PLN2 mRNA levels (Figure 7A). In line, plasma GLP-1 increased 15 minutes after oral, but not intraperitoneal, glucose loading in GW501516-treated *ob/ob* mice (Figure 7B), evidencing the contribution of the incretin axis. Moreover, fasting plasma insulin level was lower in GW501516-treated compared with control *ob/ob* mice (Figure 7C), likely a reflection of the previously reported peripheral insulin-sensitizing effects of PPARβ/δ activation.[31,32] However, the capacity of beta cells to secrete insulin on glucose challenge was also improved in GW501516-treated *ob/ob* mice. A significant increase in plasma insulin level was observed in GW501516-treated *ob/ob* mice 15 minutes after a glucose tolerance test, which was more pronounced on oral versus intraperitoneal glucose loading, thus illustrating the enhanced incretin effect after PPARβ/δ activation. By contrast, no significant changes in plasma insulin level were found in control *ob/ob* mice, likely due to the pronounced hyperinsulinemic insulin-resistant phenotype (Figure 7C). Finally, glucose excursion in the oral glucose tolerance test was improved in GW501516-treated *ob/ob* mice (Figure 7D) with a significant reduction of glycemic integrative area under the curve ($iAUC_{glucose}$) compared with untreated *ob/ob* mice (Figure 7D, inset).

## Discussion

Our results show that PPARβ/δ activation positively regulates enteroendocrine L cell GLP-1 production and enhances its response to glucose and bile acids. Proglucagon mRNA levels and GLP-1 production were markedly increased in PPARβ/δ agonist-treated mice, contributing to a significant improvement of glucose homeostasis. Likewise, activation of mouse and human enteroendocrine L cells in vitro or ex vivo with PPARβ/δ agonists significantly increased proglucagon mRNA levels and enhanced glucose- and bile acid–induced GLP-1 secretion.

As described by Drucker et al,[42,43] the murine GLUTag L cell line is a useful model for the analysis of the molecular determinants of enteroendocrine gene expression. We thus used this model and studied the transcriptional regulation of genes whose products participate in GLP-1 production. Our data show that glucose is not only a primary stimulus of GLP-1 release, but it is also a positive transcriptional modulator of proglucagon and PC-1/3 mRNA levels in GLUTag L cells. Moreover, we found that GLUTag L cells express PPARγ and PPARβ/δ at high levels, whereas PPARα is not expressed. A significant decrease of PPARγ was observed in glucose-stimulated GLUTag L cells, whereas no major change of PPARβ/δ expression was observed. Interestingly, proglucagon mRNA levels were significantly decreased in GLUTag L cells treated with the PPARγ agonist rosiglitazone. Additionally, in wild-type mice treated with rosiglitazone, a significant decrease of proglucagon mRNA levels in the small intestine was observed (data not shown). This observation is consistent with results obtained in pancreatic alpha cells in which proglucagon gene transcription is also decreased by rosiglitazone treatment through interfering with the Pax6/G1 enhancer element pathway.[44,45] We thus speculate that the negative rosiglitazone effect on proglucagon gene expression in enteroendocrine L cells may be due to inhibition of the Pax6 pathway, which has an important role in intestinal proglucagon gene expression.[23] In line with these observations, it is reasonable to speculate that PPARγ does not enhance GLP-1 biosynthesis.

Because previous reports have shown that GLP-1 receptor agonists inhibit beta cell apoptosis under conditions of glucolipotoxicity[46,47] and that PPARβ/δ activation also prevents palmitate-induced beta cell apoptosis,[28] we speculated that the protective effect of PPARβ/δ on beta cell apoptosis also may be indirectly modulated via GLP-1. Interestingly, GLP-1 receptor levels were induced in the pancreas of GW501516-treated mice, which may potentiate beta cell GLP-1 responsiveness. Our data show that PPARβ/δ activation enhances GLP-1 secretion both in response to an oral glucose load in vivo and in the presence of GLP-1

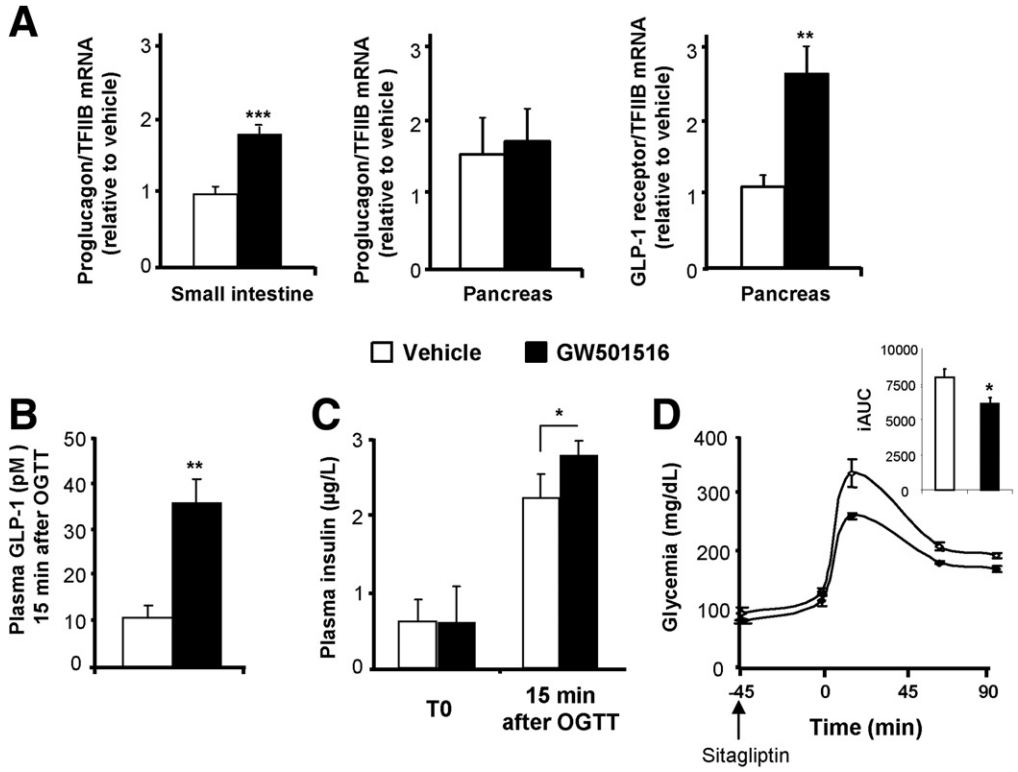


**Figure 6.** In vivo PPARβ/δ activation with GW501516 increases intestinal proglucagon mRNA levels, enhances GLP-1 secretion, and improves glucose homeostasis. Wild-type C57BL/6 male mice (10 animals per group) were treated or not (vehicle) with GW501516 at 10 mg/kg per day. (*A*) After 3 weeks of treatment, 6-hour–fasted treated mice were killed and mRNA levels of proglucagon in jejunal mucosa of small intestine (*left panel*) and pancreas (*center panel*) or GLP-1 receptor in pancreas (*right panel*) were quantified by real-time quantitative polymerase chain reaction. (*B–D*) OGTT was performed on overnight-fasted 2-week–treated mice receiving the DPP-4 inhibitor sitagliptin (25 mg/kg) 45 minutes before glucose loading. (*B*) Plasma GLP-1 levels were measured 15 minutes after glucose loading. (*C*) Plasma insulin levels were quantified before and 15 minutes after glucose loading. (*D*) Glucose excursion curves during OGTT. The *inset graph* represents the glycemic integrative area under the curve (iAUC). Student unpaired *t* test: $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.



secretagogues (glucose and bile acids) in enteroendocrine L cells in vitro and ex vivo. These effects correlated with higher proglucagon mRNA levels in both L cells and the small intestine, whereas proglucagon mRNA levels remained unaffected in the pancreas. The increase of proglucagon mRNA levels in L cells in vitro by PPARβ/δ activation was confirmed by Western blot analysis. Furthermore, these effects were absent both in vivo using PPARβ/δ-deficient mice and in vitro using siRNA PPARβ/δ in GLUTag L cells. These observations collectively show that the observed effects of GW501516 and GW0742 are mediated via PPARβ/δ. More importantly, GW501516-activated human NCI-H716 L cells and human intestinal tissue displayed a significant increase of proglucagon mRNA levels, supporting the notion that PPARβ/δ activation of this pathway is also relevant to humans. Together, our results show that PPARβ/δ activation likely potentiates GLP-1 action through the increase of both enteroendocrine L cell GLP-1 production and pancreatic GLP-1 receptor expression.

Further, it has been established that lithium, a GSK-3β inhibitor, and insulin increase proglucagon expression through stimulation of the β-catenin/TCF/Lef signaling pathway only in enteroendocrine GLP-1–producting L cells, not in pancreatic glucagon-producing alpha cells.[26,27] This is due to the fact that the transcription factor TCF-4 is expressed in small intestine epithelium[39] but not in islet alpha cells.[27] Han et al recently showed that the PPARβ/δ agonist GW501516 stimulates the Wnt/β-catenin/TCF/Lef signaling pathway in a human cholangiocarcinoma cell line model.[48] The role of the β-catenin/TCF-4 signaling pathway was thus investigated in the response of enteroendocrine L cells to PPARβ/δ activation. Our data strongly suggest that the mechanism through which PPARβ/δ activates proglucagon promoter activity involves the β-catenin/TCF-4 pathway. GW501516 induction of proglucagon gene expression was abolished by TCF-4 knockdown and significantly decreased by β-catenin knockdown. However, an increase of proglucagon mRNA levels was observed in GLUTag L cells overexpressing constitutively β-catenin, which was significantly much more important after PPARβ/δ activation. This result is consistent with a previous report showing that G2 element activity of the proglucagon was increased in β-catenin/S33Y transfected GLUTag L cells, whereas in the InR1-G9 alpha cell line, G2 response element activity

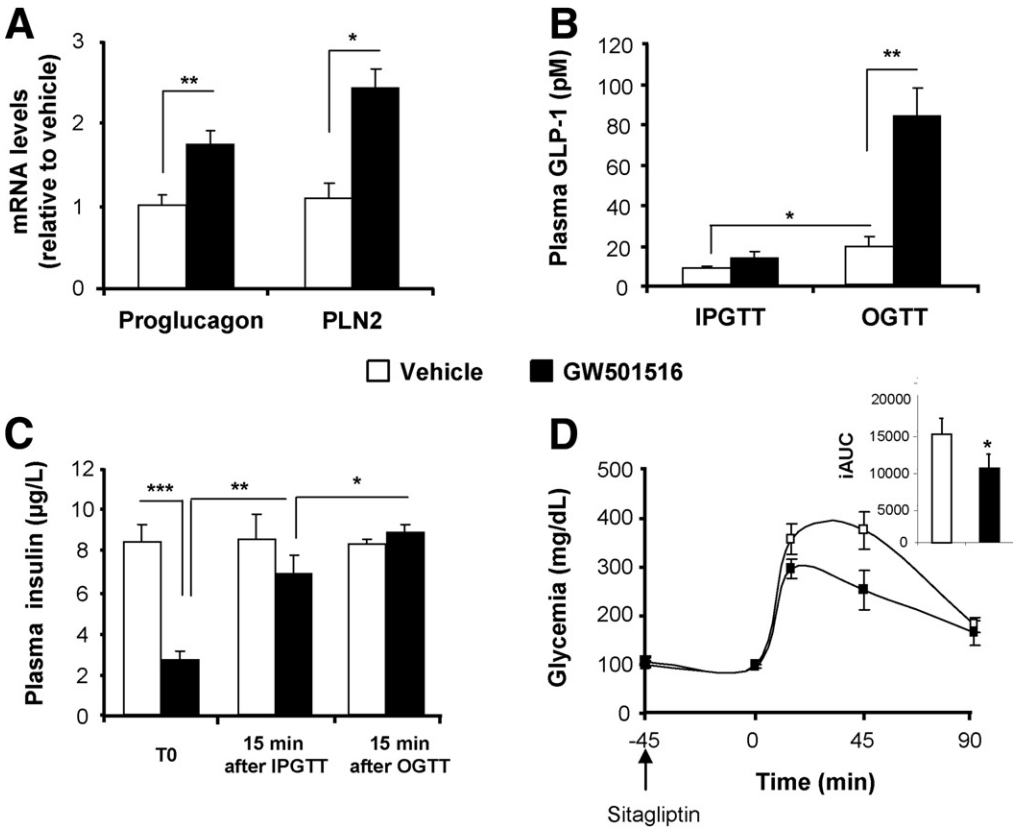


**Figure 7.** PPARβ/δ activation enhances GLP-1 secretion and restores insulin secretion in *ob/ob* mice. Obese (*ob/ob*) C57BL/6 male mice (8–10 animals per group) were treated or not with GW501516 at 10 mg/kg. (*A*) Proglucagon and PLN2 mRNA levels in jejunal mucosa from 6-hour–fasted 3-week *ob/ob* treated mice were quantified by real-time quantitative polymerase chain reaction. (*B–D*) IPGTT and OGTT were performed in 2-week–treated overnight-fasted *ob/ob* mice that received the DPP-4 inhibitor sitagliptin (25 mg/kg) 45 minutes before glucose challenge. (*B*) Plasma GLP-1 levels were measured 15 minutes after glucose challenge. (*C*) Plasma insulin levels were quantified before and 15 minutes after glucose loading. (*D*) Glucose excursion curves during OGTT. The *inset bar graph* represents the glycemic integrative area under the curve (iAUC). Student unpaired *t* test: $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.



remained unaffected.[27] This mechanism is consistent with the observation that PPARβ/δ activation does not influence proglucagon gene expression in pancreatic alpha cells.

Previous studies suggested that PPARβ/δ activation is similar to PPARγ activation in their effects on improving insulin sensitivity.[32,49,50] PPARβ/δ agonist treatment of diabetic *db/db*, *ob/ob*, or high-fat–fed mice reduces weight gain, increases skeletal muscle fatty acid oxidation, and decreases plasma triglyceride levels.[31,51,52] In line with these previous data, our results show that GW501516-treated *ob/ob* mice also displayed a 10% decrease of body weight in association with a reduction of epididymal adipocyte tissue mass ($2.7 \pm 0.1$ vs $3.1 \pm 0.1$ g; $P < .05$) and fasting glycemia ($167.8 \pm 11.5$ vs $205.8 \pm 7.1$ mg/dL; $P < .05$). Moreover, GW501516 treatment of *ob/ob* mice significantly decreased basal plasma insulin levels and restored insulin secretion after a glucose load. In addition to the peripheral effects of PPARβ/δ treatment, our results also show that GW501516 treatment of *ob/ob* mice leads to improved glucose homeostasis through stimulation of the GLP-1 signaling pathway, as shown by the increase of intestinal proglucagon expression associated with a higher GLP-1 release in response to oral glucose. As a result, a more pronounced increase in plasma insulin levels is observed when glucose is administered orally.

In conclusion, our study identifies a new role for PPARβ/δ as a positive regulator of GLP-1 signaling by increasing both proglucagon and GLP-1 receptor gene expression in enteroendocrine GLP-1–producing L cells and pancreas, respectively. Together, pharmacologic targeting of the GLP-1 pathway by PPARβ/δ agonists may prove to be a promising approach for the treatment of type 2 diabetes, especially in combination with DPP-4 inhibitors.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.01.045.

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*Conflicts of interest*

**The authors disclose no conflicts.**

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